Small molecule modulators of histone acetyltransferase p300

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Abbreviations: PCAF-p300/CBP Associated Factor, HAT- Histone acetyltransferase, HDAC-Histone deacetylase, CNSL- Cashew Nut Shell Liquid, AA: Anacardic acid, CTPB: N-(4-Chloro-3-trifluoromethyl-phenyl)-2-ethoxy-6-pentadecyl-benzamide.

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Supporting Information Available: The structure of 6 has been deposited at Cambridge Crystallographic Data Centre under the number CCDC 182386.
Summary

Histone acetyltransferases (HATs) are a group of enzymes, which play a significant role in the regulation of gene expression. These enzymes covalently modify the N-terminal lysine residues of histones by the addition of acetyl groups from acetyl-CoA. Dysfunction of these enzymes is often associated with the manifestation of several diseases, predominantly cancer. Here we report that anacardic acid (AA) from Cashew Nut Shell Liquid (CNSL) is a potent inhibitor of p300 and PCAF histone acetyltransferase activities. Though it does not affect DNA transcription, HAT-dependent transcription from a chromatin template was strongly inhibited by AA. Furthermore, we describe the design and synthesis of an amide derivative N- (4-Chloro-3-trifluoromethyl-phenyl)-2-ethoxy-6-pentadecyl-benzamide (CTPB) using anacardic acid as a synthon, which remarkably activates p300 HAT activity but not that of PCAF. Though CTPB does not affect DNA-transcription, it enhances the p300 HAT dependent transcriptional activation from in vitro assembled chromatin template. However, it has no effect on histone deacetylase activity. These compounds would be useful as biological switching molecules for probing into the role of p300 in transcriptional studies and may also be useful as new chemical entities for the development of anticancer drugs.
Introduction

Eukaryotic genome is organized as a highly complex nucleo-protein structure called chromatin, the unit of which is the nucleosome. The nucleosome is composed of two copies each of four different histones, H3, H2B, H2A, and H4, which is wrapped around by 146 base pairs of DNA. Therefore, for any process that requires access to the DNA (e.g. transcription, replication, recombination and repair), the chromatin needs to be opened by the remodeling systems. There are two different biochemical processes to modify chromatin structure, namely, the covalent modifications of histones tails and the ATP dependent chromatin remodeling. Among the several covalent modifications of histones known, the reversible acetylation of key lysine residues in histones, holds a pivotal position in transcriptional regulation (1, 2). Acetylation of histones is a distinctive feature of the transcriptionally active genes, whereas deacetylation indicates the repressed state of a gene (1, 2). A balance between the acetylation and deacetylation states of histones regulates transcription. Dysfunction of the enzymes involved in these events, the histone acetyltransferases (HATs) and histone deacetylases (HDACs) is often associated with the manifestation of cancer (3). These enzymes thus become potential new targets for antineoplastic therapy (4).

A wide repertoire of transcriptional co-activator proteins is now recognized to possess histone acetyltransferase activity (1, 2). These include p300/CBP-associated factor (PCAF), which is similar to GCN5, nuclear hormone receptor cofactors such as steroid receptor cofactor 1 (SRC1) and activator of thyroid and retinoid receptor (ACTR) and the multifunctional p300/CBP. The p300/CBP is a global transcriptional coactivator, which plays a critical role in a variety of cellular process including cell cycle control, differentiation and apoptosis. Mutations in p300/CBP are associated with different human cancers and other human diseases (5, 6). It is one of the most potent histone acetyltransferase, which can acetylate all four-core histones within nucleosomes as well as free histone forms. The HAT activity of p300 is regulated by several other factors. For example, the viral oncoprotein E1A binds to p300 and inhibits its activity whereas phosphorylation of CBP by cyclin E/Cdk2 kinase activates its HAT activity (7). During the process of transcription, p300 is recruited on to the chromatin template through the direct interaction with the activator and enhances the transcription by acetylation of promoter proximal nucleosomal histones (8).

Though a significant progress has been made in the field of histone deacetylase inhibitors as antineoplastic therapeutics, some of the compounds are already in human trials (4); the reports
of HAT inhibitors/activators are scanty. Prior to the molecular characterization of HAT enzymes, several polyamines–CoA conjugates were found to block HAT activity in cell extracts. However, the target enzyme(s) for these conjugates was not known (9). Recently, two peptide-CoA conjugates, namely Lysyl CoA (Lys-CoA) and H3-CoA-20 were synthesized which specifically inhibit the HAT activity of p300 and PCAF respectively (10, 11).

Here we report that a small molecule compound anacardic acid from cashew nut shell liquid (CNSL), known to have anti-tumor activity (12), inhibits HAT activity of p300 and PCAF. Surprisingly, the amide derivative of the same compound shows an enhancement of p300 HAT activity with human core histones and positive transcriptional coactivator, PC4, as substrates. Furthermore, since this compound does not induce the HAT activity of PCAF, it is p300 specific. It had no effect in HDAC1 activity. As expected it enhanced the HAT dependent transcriptional activation from chromatin template, but did not show any effect on in vitro DNA transcription. This selective activator should be a very useful biological tool to understand the mechanisms of p300 function and may also introduce a novel group of compounds for anti-neoplastic therapeutics.
EXPERIMENTAL PROCEDURES

Purification of Human Core Histones and Recombinant proteins:
Human core histones were purified from HeLa nuclear pellet as described previously (13). The FLAG epitope tagged Human Topoisomerase I, histone deacetylase 1 (HDAC1) and PCAF, were purified from the recombinant baculovirus infected insect cell line, Sf21, by the immunoaffinity purification using M2-agarose (SIGMA) (14). Full-length p300 was also purified from the recombinant baculovirus infected Sf21 cells as a His6-tagged protein through the Ni-NTA affinity column (Qiagen) as described previously (13). The His6-tagged nucleosome assembly protein 1 (NAP1), used for the in vitro chromatin assembly was purified from E.coli cells as previously reported (13) and the FLAG-tagged chimeric activator Gal4-VP16, expressed in E. coli and purified by immunoaffinity purification with M2 agarose. Human positive transcriptional coactivator, PC4, was expressed in E.coli and purified as described earlier (15). The peptide substrate, a 45 residue core histone H3 N-terminal peptide (N-CARTKQTARKSTGGKAPRQLASKAARKSAPSTGGVKKPHRYKPG-C) was synthesized and purified (by HPLC), in the Peptide Research Laboratory, Cancer Research, London.

Synthesis of CTPB (6) from Anacardic acid (2):
Anacardic acid 2 (M.W 348) was treated with diethyl sulphate (16) in presence of K₂CO₃ in acetone, which yielded diethyl ester 3. Diethyl ester on treatment with potassium t-butoxide (17) in DMSO yielded O-ethyl anacardic acid 4. The compound 4 on treatment with thionyl chloride in presence of catalytic amount of DMF yielded corresponding acid chloride 5. The resultant acid chloride was taken for condensation step without further purification. Compound 5 was condensed with 5-amino-2-chloro benzenetriflouride in dichloromethane in presence of triethylamine as acid scavenger to yield compound (CTPB) 6 (M.W 554) (Figure 3A). The formation of the derivatives was confirmed by IR and NMR spectroscopy (data not shown).

X-ray structure determination of N- (4-Chloro-3-trifluoromethyl-phenyl)-2-ethoxy-6-pentadecyl-benzamide (CTPB):
Good-quality, needle-shaped crystals of the CTPB (0.1mM, 0.055g), grown from a mixture of hexane and dichloromethane (2:1) by slow evaporation at room temperature, were chosen after examination under an optical microscope and coated with epoxy before
mounting. X-ray diffraction intensities were measured by ω scans using a Siemens three-circle diffractometer attached with a CCD area detector and a graphite monochromator for the MoKα radiation (50 kV, 40 mA). A hemisphere of reciprocal space was collected using the SMART software with 2θ setting of the detector at 28°. Data reduction was performed using the SAINT program (Siemens, USA, 1995). The phase problem was solved by direct methods and the non-hydrogen atoms were refined anisotropically, by means of the full-matrix least-squares procedure using the SHELXTL program (Siemens, USA, 1995). All the hydrogen atoms were located using the difference Fourier method. The absolute structure of CTPB is shown in Figure 3B.

**HAT Assay:** HAT assays were performed as described elsewhere (13). Briefly, indicated amounts of proteins/peptide (see Figure Legends) were incubated in HAT-assay buffer containing 50 mM Tris-HCl, pH 8.0, 10% (v/v) glycerol, 1 mM dithiothreitol, 1 mM phenylmethyl sulfonyl fluoride, 0.1 mM EDTA, pH 8.0, 10 mM sodium butyrate at 30 °C for 10 min in presence or absence of compound followed by the addition of 1 µl of 6.2 Ci/mmol [3H]-acetyl Coenzyme A (acetyl-CoA) and were further incubated for another 10 min. The final reaction volume was 30µl. The reaction mixture was then blotted onto P-81 (Whatman) filter papers and radioactive counts were recorded on a Wallac 1409 liquid scintillation counter. In order to characterize the inhibition kinetics of anacardic acid, filter-binding assays were done using constant amount of HeLa core histones in the presence or absence of AA with increasing concentrations of [3H]-acetyl CoA (see Figure Legends, 2E). To visualize the radiolabeled acetylated histones, the reaction mixtures were resolved on 15% SDS-PAGE and processed for fluorography as described elsewhere (15).

**Histone deacetylase assay:** Deacetylation assays were performed in the HAT assay buffer without sodium butyrate. 2 µg of core histones were incubated with 20 ng of p300 and 1 µl of 6.2 Ci/mmol [3H]-acetyl CoA for 15 min at 30°C. The activity of p300 was inhibited by incubating the reaction mixture with 10 nM p300-HAT specific inhibitor, Lysyl-CoA (10), for 10 min after which 50 ng of HDAC1 was added, in the presence or absence of the compound, and incubated further for 45 min. The samples were analysed by fluorography.
**In Vitro Chromatin assembly**: Chromatin template for *in vitro* transcription experiments was assembled and characterized as described earlier (8).

**In Vitro Transcription Assay**: Transcription assays were essentially carried out as described elsewhere (8), with minor modifications. The scheme of transcription is enumerated in Figure 5A. Briefly, 30 ng of DNA/equivalent amount of chromatin template was incubated with 30 ng of activator (Gal4-VP16) in a buffer containing 4 mM HEPES (pH 7.8), 20 mM KCl, 2 mM DTT, 0.2 mM PMSF, 10 mM sodium butyrate, 0.1 mg/ml BSA, 2% glycerol (8). The compound CTPB was added to the acetylation reaction along with p300 and acetyl-CoA, and incubated for 30 min. at 30°C. This was followed by addition of the p300 specific inhibitor Lysyl CoA (5 µM) to quench the acetylation reaction (see Results and Discussion). For AA, the HAT p300 was pre-incubated with indicated amounts of AA on ice for 20 min., following which it was added to the acetylation reaction in the transcription assay (see scheme, Figure 5A). For the DNA transcription assays and chromatin transcription inhibition assays, the Lysyl CoA step was omitted. After acetylation, HeLa nuclear extract (5 µl, which contains ~8 mg/ml protein) was added to initiate the pre-initiation complex formation. Transcription reaction was started by the addition of NTP-mix and α-[³²P]-UTP, after the pre-initiation complex formation. The incubation was continued for 40 min at 30°C. Transcription was terminated by the addition of 250 µl stop buffer (20 mM Tris-HCl pH 8.0, 1 mM EDTA, 100 mM NaCl, 1% SDS and 0.025 ng/µl tRNA). The ³²P-radiolabeled transcript was extracted with phenol-chloroform, ethanol precipitated, dried pellet dissolved in loading dye (8 M Urea, 0.005% bromophenol blue and xylene cyanol) and analyzed on 5% urea-polyacrylamide gel. Gels were then dried and subjected to autoradiography at -70°C. Quantification of transcription was done by Fuji BAS system. Quantitation of DNA and chromatin transcription data represents three independent experiments.
RESULTS AND DISCUSSION

There are several reports on the inhibitors of histone deacetylases (HDAC), while that regarding HAT inhibitors are scanty. Recently, two enzyme specific HAT inhibitors have been synthesized, which are peptide conjugates of acetyl-CoA, Lys-CoA for p300 and H3-CoA-20 for PCAF (10). However there is no report yet regarding naturally occurring inhibitors of any of the HATs. We have screened the plant extracts known to possess anti-cancer properties. The extracts (both polar and nonpolar) were tested for their HAT-inhibitory activity in filter binding assays (see Experimental Procedures), using highly purified HeLa core histones (Figure 1 A) as a substrate and baculo virus expressed PCAF or p300, as the histone acetyltransferases (Figure 1, C and D). This led us to find out that cashew nut shell liquid (CNSL) possessed inhibitory activity towards p300. The systematic bio-activity guided fractionation of CNSL yielded unsaturated anacardic acids mixture, namely, the 8’Z-monoene, the 8’Z, 11’Z-diene, and the 8’Z, 11’Z, 14’Z-triene, which are the chief constituents (~75%) of cashew nutshell liquid (18), having maximum HAT inhibitory activity (data not shown). The hydrogenation of unsaturated anacardic acids mixture yielded a single compound, Anacardic acid (2-hydroxy-6-pentadecylbenzoic acid) showing an equally potent inhibitory activity towards p300 (Figure 2A). This data indicated that absence of unsaturation in anacardic acid did not alter its HAT inhibitory property.

To further test the specificity and concentration dependence of inhibition, we compared the effect of a range of concentrations of anacardic acid on the HAT activities of p300 and PCAF. Since the compound was in DMSO, we added an appropriate control in the HAT assays. The solvent does not produce any appreciable change in the HAT activities of either p300 or PCAF (Figure 2A, bar 3 versus bar 2). Though at 5 µM concentration of anacardic acid p300 showed a minimal enhancement in HAT activity, that of PCAF showed an inhibition of 50% (Figure 2A bar 4 versus 3, see inset). Further increase in the concentration of anacardic acid produced a rapid inhibition of HAT activities of both p300 and PCAF; with 15 µM anacardic acid quenching HAT activity > 90% (Figure 2A bar 6 versus bar 3).

In order to visualize the anacardic acid mediated inhibition of p300 HAT activity on different core histones, we analyzed the radiolabelled histones on SDS-PAGE followed by fluorography. The results were similar to the observation made in the filter binding assays. In the presence of 5 µM anacardic acid, there is an increase in the acetylation of histone H4, (Figure 2B,
lane 4). However, the overall acetylation remains same as compared to acetylation in absence of the inhibitor. Further increase in the concentration of anacardic acid completely quenches the HAT activity (Figure 2B, lane 7). The gel assay for PCAF shows a correlation with the filter binding assay, (Figure 2C), with higher concentrations of anacardic acid completely shutting off HAT activity (Figure 2C, lane 7). The IC$_{50}$ values of anacardic acid for p300 and PCAF was found to be ~8.5 µM (data not shown) and ~5 µM (Figure 2A, bar 4) respectively. The filter binding assays were also repeated, using the human histone H3 N-terminal peptide as the substrate for p300 HAT to confirm the specificity of AA towards the HAT. Addition of AA to the reaction produced the characteristic inhibition of HAT activity (Figure 2D) and IC$_{50}$ value for H3 peptide was found to be ~500 nM (Figure 2D, bar 7 vs. bar 3). We went on to characterize the nature of inhibition of anacardic acid on the p300 HAT activity. The rate of the acetylation reaction at different concentrations of the inhibitors (and in its absence) was recorded with increasing concentrations of $[^3]$H-acetyl CoA and a constant amount of core histones. The double reciprocal plot for each inhibitor concentration and in its absence (1/c.p.m vs. 1/[Acetyl CoA]) was plotted as shown in Figure 2E. The results suggest that AA is a non-competitive type of p300-HAT inhibitor.

Due to the apparent lack of specificity towards HATs, we were interested to alter the various functional groups of anacardic acid, keeping the parent structure intact, to end up in a molecule with a better inhibitory effect or even selectivity. Since the side chain of the compound had already been negated from having any effect (no change in the HAT inhibition of unsaturated versus saturated anacardic acid), we modified the other functional groups on the phenolic ring in anacardic acid. The acidic group on the anacardic acid was modified to different amide derivatives using substituted anilides (Experimental Procedures). One of these compounds, with a 5-amino-2-chlorobenzotri fluoride moiety substituted on anacardic acid, CTPB, (Figure 3A and B) when tested in vitro HAT assay (filter binding), surprisingly showed an enhancement in the p300 HAT activity, while keeping the PCAF HAT activity mostly unperturbed (Figure 4A). The concentration dependent HAT activity profile revealed a maxima for p300 HAT activity at 275 µM of CTPB (Figure 4A, inset, bar 7c); an ~4 fold increase over the DMSO control (Figure 4A inset, lane 7c versus lane 3). Further increase in the concentration of CTPB to 300µM resulted in a drop in the activation levels. These results were confirmed using the H3 peptide as a substrate in the HAT assays with p300 (data not shown).
In order to analyze the effect of the compound on the acetylation of the individual core histones, the radiolabelled product of the HAT assays were separated on a 15% SDS-PAGE and visualized by fluorography. Addition of CTPB in increasing amounts in the presence of p300 resulted in the gradual increase in the acetylation of both histones H3 and H4, peaking at 200 µM (Figure 4B, lane 7). In contrast to the filter binding assays the enhancement in the gel assays is ~5 fold more than the DMSO control (Figure 4B, lane 7 versus lane 3). Further increase in the concentration of CTPB brings down the HAT activity. A drop in the enhancement of HAT activity at higher concentrations of the activator, CTPB, a non-polar compound, could be due to an alteration in the reaction condition affecting the efficiency of the HAT. In case of PCAF, there is a slight increase in the levels of H4 acetylation, while the H3 acetylation levels remain constant (Figure 4C). This reflects the congruence between the filter binding and gel assay data.

Despite using highly purified p300 and PCAF for our HAT assays, we went on to check the effect of CTPB on histone deacetylases, enzymes that catalyze the reverse reaction of HATs. This was done in order to ensure that CTPB does not affect other enzymes or the substrate (histones) in a non-specific manner. The HDAC assays protocol was modified (as elaborated in Experimental Procedures) to include acetylation of histones followed by the quenching of the HAT activity by the p300-selective HAT inhibitor, Lys-CoA, and the incubation with the purified recombinant human HDAC1, (Figure 1B), either in the presence or absence of CTPB.

Deacetylation of the core histones in the presence or absence of the compound, CTPB at 100 µM or 500 µM, shows no difference whatsoever (Figure 4D lanes 5 and 6 versus lane 3). Pre-incubation of HDAC1 with CTPB does not affect its deacetylation activity (data not shown). Interestingly, addition of CTPB, after quenching the p300 HAT activity by Lys-CoA does not show the characteristic enhancement (Figure 4D lane 7 versus lane 2). Addition of the solvent, DMSO, has a slight inhibitory effect on the deacetylase activity of HDAC1 (Figure 4D lane 4 versus lane 3). These results indicate the specific nature of CTPB towards p300. In order to confirm that the target of CTPB is p300, we have used a non-histone substrate, human transcription coactivator PC4, for p300 acetylation. PC4 is acetylated specifically by p300 (16). The addition of CTPB (100µM) enhances PC4 acetylation by p300 (Figure 4E, lane 5 versus lane 3) substantially. A further increase in the concentration of CTPB produces a drop in the
enhancement of PC4 acetylation (data not shown), as observed in the case of the histones. Taken together these results indicate that the probable target of CTPB is the enzyme p300.

p300, along with its homologue CBP, is known to be a prominent transcription coactivator, capable of interacting with a large number of transcriptional activators possessing HAT activity. Acetylation of histones is a distinctive feature of active genes (19). It has been conclusively proved that the acetylation of the promoter proximal histones by p300 is necessary and sufficient for the initiation of transcription. Thus, modulators of the HAT activity of p300 can be applied in the study of transcriptional regulation. In order to address the effect of CTPB on transcription from a chromatin template, we used the in vitro chromatin based transcription system (8). This system requires the HAT activity of p300 for the initiation of transcription (Figure 5A). Such a system would be ideal for testing CTPB. In order to establish the HAT specific nature of our compound, we first tested its effect on transcription from a histone free DNA template. This system does not require p300 HAT activity for transcriptional initiation (Figure 5B). Addition of the solvent, DMSO, to this assay, produces a drop in transcript levels (Figure 5B, lane 3 versus lane 2). But the addition of increasing concentration of either AA or CTPB does not produce any variation in the transcript levels as compared to the DMSO control (Figure 5B lanes 4-7 for AA and lanes 8-10 for CTPB versus lane 3 and also see Figure Legends). This suggests that the compounds do not affect any component of the basal transcription machinery. The drop in transcript levels upon addition of DMSO may be due to the disruption of certain key protein-protein interactions. We went on to test the effect of the compound CTPB on HAT-dependent transcription from a chromatin template. The template pG5ML-array (8) was assembled into chromatin using the NAP1 mediated assembly method (Experimental Procedures). Addition of CTPB to the HAT-dependent transcription reaction along with the p300 and acetyl CoA (without addition of Lys-CoA) did not produce a significant variation in the transcript levels with or without the compound (data not shown). A close scrutiny of the transcription assay scheme revealed that the HAT activity of p300 remains active throughout the assay period; within which time the promoter proximal histones could be completely acetylated. In order to characterize a HAT activator, it would be necessary to limit the period of acetylation to a small window. We achieved this by adding the p300 specific inhibitor Lys-CoA (10) after allowing for 30 min of acetylation either in the presence or absence of the compound. Under these conditions we found the addition of DMSO produced a slight drop in the transcript levels (Figure 5C, lane 5 versus lane 4), while the addition of CTPB enhanced the
levels of transcription 1.6 fold over the DMSO control (Figure 5C, lane 6 versus lane 5). Thus this result indicates that CTPB specifically enhances the HAT activity of p300, a function that is reflected even at the transcriptional level. In order to explain the 1.6-fold increase in transcription levels, in contrast to the ~5-fold increase in the histone acetylation levels; we carried out a time course experiment to analyze the effect of CTPB on histone acetylation over a 30 min. time period. We used DMSO treated p300 acetylation reactions as the control on which the activation levels were calculated (Figure 5D). Since the histone concentration remains constant, the difference in the levels of histone acetylation drops over time. After a 30-minute incubation, the difference stands at 1.6-fold (Figure 5D, bar 4), same as what we observe in the transcription assay. Anacardic acid did not affect the transcription from the DNA template, but the HAT-dependent transcription from chromatin template was inhibited by anacardic acid even at 10µM concentration (Figure 5C, lane 10 versus lane 9).

We have identified a natural compound, which can broadly inhibit the HAT activity but not any other enzymatic activity as revealed by the DNA transcription. Though anacardic acid is not specific for any particular group of HATs, it may serve as a lead compound to synthesize other non-peptide based specific HAT activity modulators. The most significant finding of this study is the synthesis of a specific activator of p300 HAT activity, CTPB, using anacardic acid as a synthon. The enhancement of p300 HAT activity by CTPB is also reflected at the transcriptional level, where acetylation of histones in the promoter proximal region dictates transcription initiation. Further investigation of the effect of CTPB in in vivo histone acetylation and the functions thereof should be studied in order to understand the mechanism of action of the compound. This information would be very useful in order to design a novel group of antineoplastic drugs targeted towards histone acetyltransferases.
FIGURE LEGENDS

Figure 1:
Purified proteins used in different experiments:
(A) Highly purified HeLa core histones (2 µg) isolated from HeLa nuclear pellet used in the experiment were analyzed by 15% SDS-PAGE. Recombinant proteins were purified from respective baculovirus infected Sf21 cells and analysed by different percentages (indicated in the parentheses) of SDS-PAGE, (B) 150 ng of FLAG epitope tagged full length human HDAC1 (12%), (C) 500 ng FLAG epitope tagged full length human PCAF (8%), (D) 800 ng His₆-tagged full length human p300 (8%), (E) Purified PC4 (500 ng) resolved on a 15% gel. All proteins were visualized by coomassie blue staining.

Figure 2:
Anacardic acid is a HAT inhibitor:
Histone acetyltransferase assays were performed in the presence and absence of AA using highly purified HeLa core histones (800 ng) and either with p300 (5 ng) or PCAF (15 ng) and processed for filter binding (A) or flurography (B and C). (A) Filter binding assay using p300 and PCAF, in the absence or presence of different concentrations of AA (3 µM – 25 µM) as depicted. The results represent the average values with error bars (+/- standard deviation) of three independent experiments. Fluorographic analysis of acetylated histones by p300 (B) and PCAF (C) in the presence of AA; Lane 1, core histones without any HAT; lane 2, histones with HAT; lane 3, with DMSO as a control, lanes 4-7, with 5, 10, 15, 20 µM concentrations of AA. (D) Filter binding assay was performed using p300 and H3 N-terminal peptide (45 ng) in the absence or presence of different concentrations of AA (50 nM – 10 µM). The results represent the average values with error bars (+/- standard deviation) of three independent experiments. (E) Inhibition kinetics of anacardic acid; filter binding HAT assay was done with a fixed concentration of histones (800 ng) and increasing concentrations of [³H]-acetyl CoA in the absence or presence (7 µM and 7.5 µM) of AA. The results were plotted using the Graph Pad Prism software.
Figure 3:
Synthesis and structural elucidation of N- (4-Chloro-3- trifluoromethyl-phenyl)-2-ethoxy-6- pentadecyl-benzamide (CTPB) from anacardic acid:
(A) General scheme for synthesis of substituted anilide derivatives of anacardic acid. (B) X-ray crystal structure CTPB showing the ORTEP view of the compound.

Figure 4:
CTPB activates histone acetyltransferase activity of p300.
Histone acetyltransferase assays were performed in the presence and absence of CTPB using highly purified HeLa core histones (800 ng) and either with p300 (5 ng) or PCAF (15 ng) and processed for filter binding (A) or fluorography (B and C). (A) Filter binding assay using p300 and PCAF, in the absence or presence of different concentrations of CTPB (25 µM – 300 µM) as depicted. The results represent the average values with error bars (+/- standard deviation) of three independent experiments. Fluorographic analysis of acetylated histones by p300 (B) and PCAF (C) in the presence of CTPB; Lane 1, core histones without any HAT; lane 2, histones with HAT; lane 3, with DMSO as a control, lanes 4-7, with 50, 100, 200, 300 µM concentrations of CTPB. (D) CTPB does not affect the histone deacetylase activity of HDAC1. 2.5 µg of [3H]-labeled acetylated histones (by p300) (lane 2) was subjected to deacetylation with 60 ng of recombinant HDAC1 in the absence (lane 3) or presence of 100 µM (lane 5) or 500µM (lane 6) of CTPB. (E) CTPB activates p300-mediated acetylation of nonhistone protein PC4, human positive coactivator (500ng). Lane 1, core histones with DMSO; lane 2, histones with p300 with 200 µM of CTPB; lane 3, PC4 with p300; lane 4, PC4 with p300 in the presence of DMSO; lane 5, PC4 with p300 in the presence of 100µM of CTPB.

Figure 5:
Effect of CTPB and AA on the in vitro transcription from DNA and chromatin template:
(A) Schematic representation of the in vitro transcription protocol.
Transcription from DNA (B) and chromatin template (C). Freshly assembled chromatin templates (50 ng) or histone free DNA were subjected to the protocol in (A) with or without activator/inhibitor, 30 ng Gal4-VP16, 25 ng of baculo-expressed, purified His6-tagged p300 (full-length), 1.5 µM of acetyl-CoA in the presence/absence of 100 µM CTPB (lane 6), 10 µM and 15 µM (lanes 10 and 11) anacardic acid as depicted. The reaction mixtures were analyzed on 5%
urea-polyacrylamide gel and processed by autoradiography. (D) Histone acetyltransferase assays were performed in the presence of 100 µM CTPB or DMSO as a control using 800 ng of highly purified HeLa core histones and 5 ng p300 for the different time periods indicated and processed for filter binding. The fold activation (C.P.M_{CTPB}/C.P.M_{DMSO}) is shown for each time interval.
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Figure 1
Figure 2
**Figure 3**

A

![Chemical structure diagram](image)

B

![Chemical structure diagram](image)
Figure 4
Figure 5
Small molecule modulators of histone acetyltransferase p300
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