Small-Molecule Inhibitors of Histone Acetyltransferase Activity: Identification and Biological Properties

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Starting from a yeast phenotypic screening performed on 21 compounds, we described the identification of two small molecules (**9** and **18**) able to significantly reduce the *S. cerevisiae* cell growth, thus miming the effect of GCN5 deletion mutant. Tested on a GCN5-dependent gene transcription assay, compounds **9** and **18** gave a high reduction of the reporter activity. In *S. cerevisiae* histone H3 terminal tails assay, the H3 acetylation levels were highly reduced by treatment with 0.6−1 mM **9**, while **18** was effective only at 1.5 mM. In human leukemia U937 cell line, at 1 mM **9** and **18** showed effects on cell cycle (arrest in G1 phase, **9**), apoptosis (**9**), and granulocytic differentiation (**18**). When tested on U937 cell nuclear extracts to evaluate their histone acetyltransferase (HAT) inhibitory action, both compounds were able to reduce the enzyme activity when used at 500 μM. Another quinoline, compound **22**, was synthesized with the aim to improve the activity observed with **9** and **18**. Tested in the HAT assay, **22** was able to reduce the HAT catalytic action at 50 and 25 μM, thereby being comparable to anacardic acid, curcumin, and MB-3 used as references. Finally, in U937 cells, compounds **9** and **18** used at 2.5 mM were able to reduce the extent of the acetylation levels of histone H3 (**9**) and α-tubulin (**9** and **18**). In the same assay, **22** at lower concentration (100 μM) showed the same hypoacetylating effects with both histone and non-histone substrates.

Introduction

The reversible process of histone acetylation occurring at the ϵ-amino group of lysine residues in the N-terminal tails of core histones mediates conformational changes in nucleosomes. Two superfamilies of enzymes are involved in such a process: histone acetyltransferases (HATs) and histone deacetylases (HDACs), which catalyze respectively the addition to and the removal of acetyl units to histones. These modifications affect the accessibility of transcription factors to DNA and regulate gene expression.[1−5] Indeed, hyperacetylation of histones has been associated with relaxed chromatin structure and active gene transcription, while hypoacetylated histones lead to transcriptional repression by condensing the structure of chromatin and restricting the access of transcription factors.[6−9]

The first nuclear HAT to be discovered, general control of nuclear-5 (GCN5), and its paralogue p300/CBP accessory factor (PCAF) are conserved in organisms from yeast to humans, and GCN5/PCAF HATs have served as paradigms for the biochemical analysis of HAT structure, mechanism, and function.[10−12] Similar to HDACs, GCN5/PCAF HATs are typically found in cells in large multiprotein complexes such as SAGA and ADA, yet unlike HDACs they exhibit robust catalytic activity as purified proteins, with high selectivity for Lys-14 in histone H3.[13−16] However, their substrate specificity in protein complexes appears to be broader, and in addition to histones, GCN5/PCAF HATs target non-histone protein substrates, such as transcription factors (i.e., p53)[17] and structural proteins (i.e., α-tubulin).[18]

The anticancer effects of HDAC inhibitors are well-known, and a number of them are in clinical trials,[19,20] while the chemotherapeutic potential of HAT targets has been less validated so far. Missense and deletion mutations in the p300 gene have been found in colorectal, gastric, and epithelial cancers, and the loss of heterozygosity of p300 gene has been related to glioblastoma.[21,22] Amplification and overexpression of AIB-1 (amplified in breast cancer-1), a steroid receptor coactivator with HAT activity, in breast, ovarian, and gastric cancers furnish an example of deregulated HATs in oncogenesis.[23,24] In acute myeloid leukemia (AML), the gene for CBP is translocated and fused to MYST family HATs such as MOZ and MORF.[25,26] p300 and PCAF have been reported to play an important role in MyoD dependent cell cycle arrest,[27] and deregulation of GCN5/PCAF in genetic diseases and cancer has led to the supposition that selective inhibitors of these HATs may exert therapeutic applications.[28,29]

To date, a small number of HAT inhibitors have been reported, the first being the bisubstrate analogues Lys-CoA and H3-CoA-20, respectively selective for p300 and PCAF.[30] As the second step, a few natural small molecules such as anacardic acid,[31] garcinol,[32] and curcumin[33] were described as potent p300

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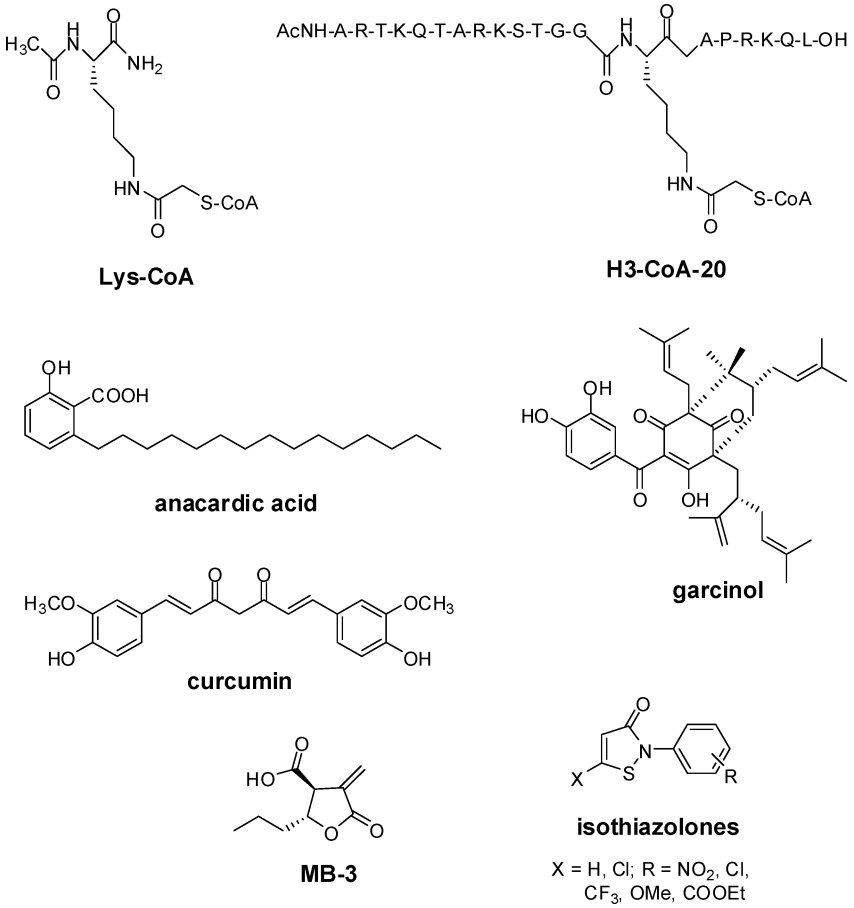


Figure 1. Known HAT inhibitors.

and PCAF inhibitors. Recently, the γ-butyrolactone MB-3 has been discovered as a small, cell-permeable GCN5 inhibitor,[34] and some isothiazolones were disclosed as inhibitors of p300 and PCAF HAT activity[35] (Figure 1).

Pursuing our searches on design, synthesis, and biological evaluation of small molecules as epigenetic tools for regulating gene expression and transcription,[36−46] we performed a yeast phenotypic screening on a set of both newly synthesized and commercially available molecules (**1**−**21**) (Figure 2) to find among them one or more modulators of GCN5 activity. Indeed, GCN5 is involved in maximal expression of certain amino acid biosynthesis genes,[47] and loss of *GCN5* leads to poor yeast growth under amino acid starvation. Small-molecule inhibitors of GCN5 activity in wild-type yeast, as well as *gcn5*Δ mutants, are expected to cause clear *gcn5*$^-$ phenotypes.

Among tested compounds, derivatives **1**−**4** were designed after a molecular pruning/isosterism approach on the transition-state analogue Lys-CoA. Anacardic acid (AA) **5**[31] was used as a template for the design and synthesis of analogues **6**−**10**, in which the AA benzene ring was replaced by a pyrimidine (**6**, **7**) or quinoline (**8**) moiety, including the two simpler quinolines **9** and **10**. *all-trans*-Retinoic acid (ATRA) **11**,[48] a well-known transcriptional modulator, and the corresponding hydroxamate **12** also show some structural analogies with AA and were thus included in the assay. Apicidin **13**[49] and HC-toxin **14**,[50] two HDAC inhibitors belonging to the cyclic tetrapeptide family, were also tested in the cell-based assay to investigate an eventual positive modulation of GCN5-mediated phenotypic effects. In fact, an amide analogue of AA, (*N*-(4-chloro-3-trifluoromethylphenyl)-2-ethoxy-6-pentadecylbenzamide, CTPB),[31] was reported as a p300 activator, and we consequently expected that HDACi and HAT activators could induce similar phenotypes in a cell-based assay.

Among the first tested compounds, **1**−**14**, only the 3-carbethoxy-2-methylquinoline **9**[52] was found to be active in inhibiting yeast cell growth by resembling a *GCN5* loss-of-function mutation. Thus, a series of analogues of compound **9** (compounds **15**−**21**) were prepared and tested in *Saccharomyces cerevisiae* as GCN5 inhibitors. Among them, only compound **18** was able to inhibit the yeast growth as well as compound **9**. Since compounds **9** and **18**, which have been characterized with a wide range of cellular activities, were active at (sub)millimolar concentrations, other functional targets may be involved and may explain the observed effects and phenotype. Thus, we tried to combine the structural features shown by compounds **9** and **18** (i.e., the C3-substituted quinoline moiety) with those typical of AA[31] and MB-3[34] (i.e., the salicylic acid or the 4-carboxy-γ-butyrolactone function together with the presence of a long alkyl chain), obtaining a new series of quinolines of which the 4-hydroxy-2-pentylquinoline-3-carboxylic acid **22** (Figure 3) can be considered a prototype.

Chemistry

The LysCoA-based compounds **1** and **2** were prepared by acylation of *N*α-acetyl-L-lysine methyl ester hydrochloride with 2-methylthioacetic acid or thiophene-2-carboxylic acid in the presence of *N*,*N*′-carbonyldiimidazole (Scheme 1). For the synthesis of **3** and **4**, the same acylation reactions were performed on the 2-[4-(4,5-dihydrooxazol-2-yl)phenoxy]ethylamine **23**, prepared by Mitsunobu reaction between *tert*-butoxycarbonyl- (BOC-) protected 2-aminoethanol and the 4-(4,5-dihydrooxazol-2-yl)phenol **24**[51] in the presence of diiso-

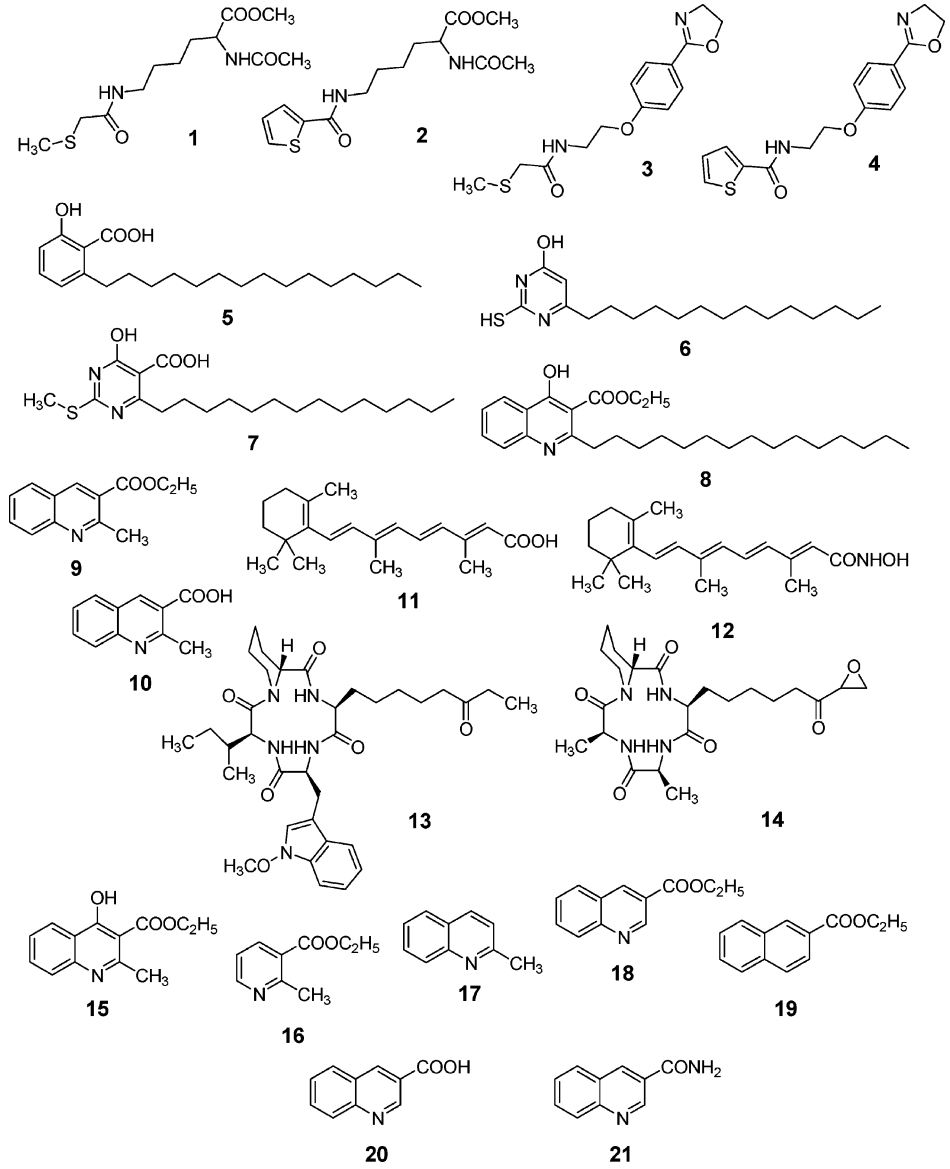


Figure 2. Compounds **1**−**21** subjected to the cell-based screening.

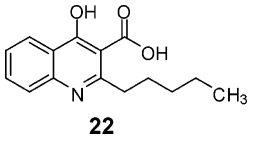


Figure 3. 4-Hydroxy-2-pentylquinoline-3-carboxylic acid **22.**

propyl azodicarboxylate (DIAD)/triphenylphosphine, followed by acidic removal of the protecting *tert*-butoxycarbonyl group (Scheme 1).

Acylation of potassium ethyl malonate with pentadecyl imidazolide in the presence of magnesium dichloride and triethylamine furnished the corresponding *β*-oxoester **25**, which was in turn condensed with thiourea and sodium ethoxide in ethanol to give the 2-thiouracil **6**. Following treatment of **6** with methyl iodide afforded the 2-methylthiopyrimidin-4(3*H*)-one **26**, which was converted into the corresponding 5-bromoderivative **27** with *N*-bromosuccinimide (NBS). Further reaction of **27** with *n*-butyllithium and carbon dioxide at −78 °C furnished the 4-hydroxy-2-methylthio-6-tetradecylpyrimidine-5-carboxylic acid **7** (Scheme 2).

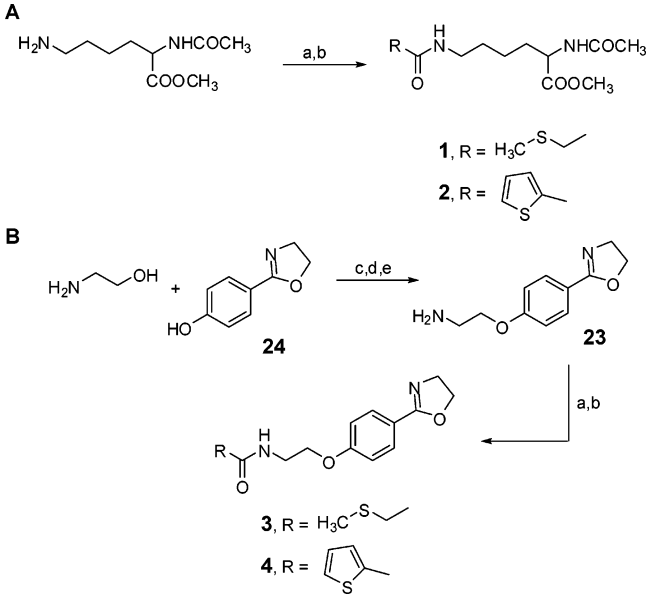
The 3-carbethoxy-4-hydroxyquinolines **8**, **15**, and **28** were obtained by reaction of ethyl 3-oxooctadecanoate (for **8**) or ethyl acetoacetate (for **15**) or ethyl 3-oxooctanoate (for **28**) with isatoic anhydride in the presence of sodium hydroxide as catalyst. Alkaline hydrolysis of **28** with 4 N KOH furnished the hydroxy acid **22** (Scheme 3). The synthesis of **9** was accomplished by a single-step variant of the Friedländer synthesis involving *o*-nitrobenzaldehyde and ethyl acetoacetate in the presence of $SnCl_2$ and $ZnCl_2$,[52] and the corresponding carboxylic acid **10**[53] was obtained from **9** by standard procedure (Scheme 3).

The *N*-hydroxyretinamide **12**[54] was prepared by reaction of the commercial *all-trans*-retinoic acid **11** with ethyl chloroformate, followed by addition of *O*-(2-methoxy-2-propyl)hydroxylamine[55] and subsequent acidic treatment in the presence of Amberlyst 15 ion-exchange resin (Scheme 4).

The commercial 3-quinolinecarboxylic acid **20** was the starting material for the preparation of both the corresponding ethyl ester **18**[56] (by standard method) and the carboxyamide **21**[57] (by a two-step, one-pot reaction with ethyl chloroformate/0.5 M ammonia solution in dioxane). Finally, the ethyl 2-naphthoate **19** was prepared from 2-naphthoyl chloride by standard reaction.[58]

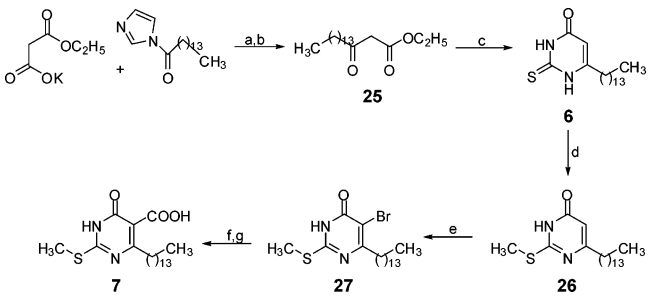
Chemical and physical data for compounds **1**−**4**, **6**−**8**, **12**, **15**, **21**−**23**, and **25**−**28** are reported as Supporting Information.

Scheme 1[a]



[a] Reagents and conditions: (a) 2-methylthioacetic acid or 2-thiophenecarboxylic acid; (b) *N*,*N*′-carbonyldiimidazole, triethylamine, dichloromethane, room temp; (c) di-*tert*-butyl dicarbonate, dioxane, room temp; (d) DIAD/PPh_3, THF, room temp; (e) CF_3COOH, dichloromethane, room temp.

Scheme 2[a]



[a] Reagents and conditions: (a) $MgCl_2$, Et_3N, acetonitrile, room temp; (b) 13% HCl, room temp; (c) thiourea, EtONa, EtOH, reflux; (d) MeI, DMF, room temp; (e) NBS, benzoyl peroxide, carbon tetrachloride, reflux; (f) 2.5 M *n*-butyllithium, diethyl ether, −70 °C; (g) CO_2, room temp.

Synthetic procedures for known compounds have been reported only if different from those already published.

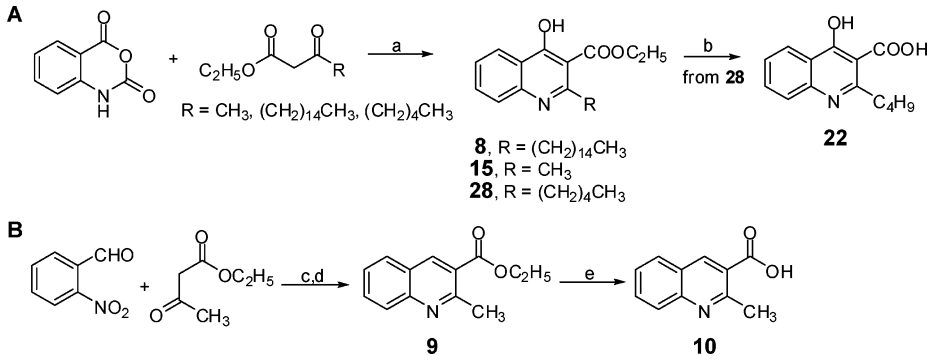
Results and Discussion

Yeast Phenotypic Screening. Compounds **1**−**21** at doses ranging from 0.2 to 1 mM were tested on *S. cerevisiae* to evaluate the effects on cell growth inhibition (Figure 4; only the effects of compounds at 0.6 mM is reported). Because yeast strains lacking *GCN5* (*gcn5*Δ) showed poor growth under amino acid starvation and alteration in cell cycle phases,[59] we expected that compounds able to reduce yeast cell growth could mimic the effects produced by the deletion of *GCN5*. Among the first tested compounds, **1**−**14**, only **9** gave a significant reduction of cell growth.[52] Tested on *gcn5*Δ yeast strain, compound **9** had little effect on cell growth.[52] Prompted by these results, we prepared and tested in our cell-based phenotypic screen seven novel analogues of compound **9** (compounds **15**−**21**) to acquire SAR information about the inhibiting activity. Among compounds **15**−**21**, only the 3-carbethoxyquinoline **18** was able to reduce the growth of wild-type *S. cerevisiae*, being less effective in inhibiting the *gcn5*Δ strain growth (Figure 5). The insertion of a hydroxyl group at the C4 position of **9** (compound **15**) as well as the replacement of the quinoline ring of **9** with a pyridine (compound **16**) or the deletion of the C3-carbethoxy function (compound **17**) abolished the GCN5 inhibiting activity. In contrast, compound **18**, lacking the C2-methyl substituent, retained the GCN5 inhibitory activity, but again the replacement of its quinoline moiety (with naphthalene, **19**) or the modification of the 3-carbethoxy group at the quinoline C3 position into a carboxyl (**20**) or carboxamide (**21**) function yielded inactive products.

To ascertain the existence of a direct correlation between the activities of compounds **9** and **18** and the HAT catalytic activity of GCN5, we tested the effects of **9** and **18** (0.6 mM) on cell growth in the mutant F221A and E173H yeast strains, and these effects were compared with those obtained with the respective isogenic wild-type MK839 strain. As negative control, the ineffective compound **16** (0.6 mM) was also tested. F221A mutant strain contains the F221A point mutation in the HAT minimal catalytic domain of GCN5, this causing a major defect in cell growth.[60] In the E173H mutant strain the glutamic acid 173 playing an essential role in the HAT catalytic mechanism and function of GCN5 is replaced by a histidine residue, unable to give the deprotonation of the histone substrate essential for the HAT catalytic activity.[61] In comparison with the effects exerted by compounds **9** and **18** on wild-type MK839, the reduction of the cell growth on F221A and E173H mutant strains was lower, thus providing the evidence that the inhibition observed with **9** and **18** is exerted on the catalytic activity of GCN5 but not on the whole protein (Figure 6). Finally, we tested compounds **9** and **18** on spt20Δ, a *S. cerevisiae* mutant strain containing null mutation in the *SPT20* gene that selectively disrupts the SAGA but not the ADA complex among the two high-molecular-mass native HAT complexes recruiting GCN5 as catalytic subunit for acetylation of chromosomal histones.[15,62] In *S. cerevisiae*, the mutation of *SPT20* causes poor cell growth and a set of severe transcriptional defects.[63] Interestingly, the yeast spt20Δ mutant strain was more susceptible than the wild-type MK839 strain toward compounds **9** and **18** inhibition (Figure 6). Compound **16** did not exert any effect on WT MK839, F221A, E173H, and spt20Δ yeast strains.

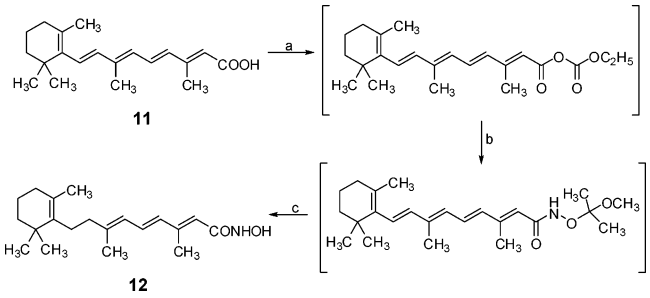
Effect on Transcriptional Activation. The HAT activity of GCN5 is required for transcriptional activation of specific target genes in vivo, through nucleosomal acetylation in the gene promoter regions.[59] In yeast, GCN5 was first described as a transcriptional coactivator of amino acid biosynthetic genes, *HIS3* being one of the most affected.[47] The 3-carbethoxyquinoline **18** and the 3-carbethoxy-2-methylpyridine **16** (as negative control) (0.6 mM) were tested in a transcription assay using the reporter *HIS3-LacZ* in comparison with compound **9** (0.6 mM), to evaluate the functional inhibition of GCN5-dependent gene transcription. The test was performed in basal and in activated conditions, in which yeast strain was starved for amino acids including histidine to induce *HIS3* expression, by applying 3-aminotriazole (3-AT), an amino acid analogue, to the medium. The levels of β-galactosidase (β-Gal) activity produced were used to measure the transcriptional activity. In this test, the reporter activity was highly reduced in the presence of **9** and **18** both in basal (minimal SD medium; see Experimental Section) and, more drastically, in activated (SD + 3AT) conditions, while the treatment with **16** was ineffective (Figure 7A). To check the effects of our compounds toward global transcription, a promoter (*GAL10-CYC1*) not responding to GCN5 fused to a *Lac-Z* reporter was used. In this case activated transcription was induced by addition of galactose to the medium, and the same reporter assay was repeated for com-

Scheme 3[a]



[a] Reagents and conditions: (a) NaOH, dioxane, reflux; (b) 4 N KOH, EtOH, reflux; (c) $SnCl_2$, $ZnCl_2$, EtOH, 80 °C; (d) 10% $NaHCO_3$, room temp; (e) KOH, EtOH, 80 °C.

Scheme 4[a]



[a] Reagents and conditions: (a) ClCOOEt, Et_3N, THF, room temp; (b) $H_2NOC(CH_3)_2OCH_3$, room temp; (c) Amberlyst 15, MeOH, room temp.

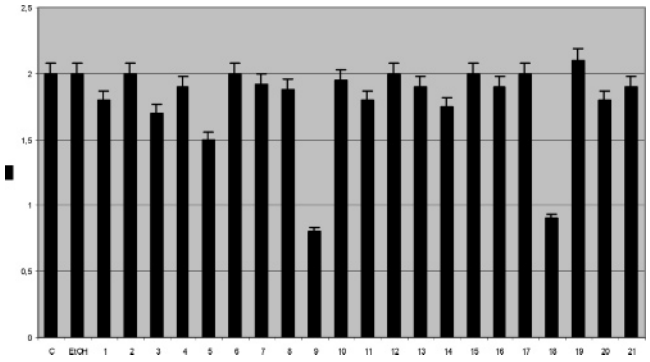


Figure 4. Inhibition of yeast cell growth observed with compounds **1**−**21** (0.6 mM). The numbers of yeast cells were evaluated as arbitrary units by optical density.

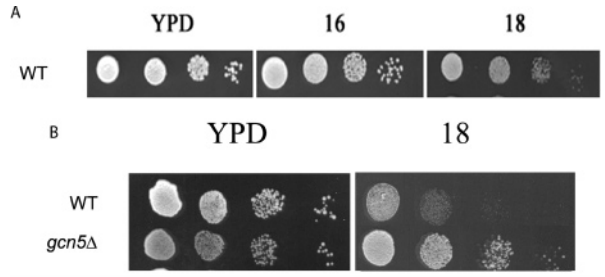


Figure 5. (A) Effects on yeast (WT) cell growth exerted by **18** and by the ineffective compound **16** (both at 0.6 mM). (B) Inhibitory effects of **18** (0.6 mM) on WT and *gcn5*Δ yeast strains. Ten-fold serial dilutions of the liquid yeast cell culture were spotted on solid medium.

parison. Figure 7B clearly shows that **9** and **16** were completely ineffective in inhibiting the GCN5-independent *GAL10-CYC1-LacZ* transcription in both basal and activated conditions, while **18** displayed little inhibitory effect in activated conditions, thus showing that the inhibitory effect of **18** on transcription is not fully GCN5-specific such as that observed with **9**.

Yeast Acetylation Level of Histone H3. To confirm that the acetylation reaction is the primary target for the inhibiting activity of compounds **9** and **18**, an in vivo assay was performed by measuring the global acetylation level of histone H3 N-terminal tails in protein extracts from wild-type *S. cerevisiae* cells, grown for 16 h in YPD medium (see Experimental Section) alone and in the presence of compounds **9** and **18** used at 0.6, 1, and 1.5 mM (Figure 8). In parallel, the assay was performed on the *gcn5*Δ strain for comparison purposes. To determine the acetylation levels, histone-specific antibodies anti-acetyl H3 were used. In these conditions, **9** already inhibited H3 histone tail acetylation at 0.6−1 mM, while **18** showed a low inhibitory activity only at 1.5 mM.

Cell Cycle, Apoptosis, and Cytodifferentiating Effect on Human Leukemia U937 Cells. Compounds **9** and **18** were also tested for their effects on cell cycle, apoptosis induction, and granulocytic differentiation in human leukaemia U937 cell line. After 24 h of treatment at 1 mM, compound **9** prompted arrest of the cell cycle in the G1 phase and was able to induce 27% of apoptosis, whereas compound **18** had very weak effects on cell cycle and apoptosis induction at the same conditions (Figure 9A). To test differentiation, the granulocytic maturation of U937 cells was determined by CD11c expression levels upon 30 h of stimulation with compounds **9** and **18** (both used at 1 mM). As indicated in Figure 9B, whereas compound **9** was similar to the control (8.8% vs 8.2%), **18** was able to induce a weak increase in the CD11c expression levels (14% of CD11c positive cells). Note that propidium iodide (PI) positive cells (dead cells) have been excluded from the analysis.

HAT Inhibitory Assay. To ascertain that compounds **9** and **18** were biologically active as HAT inhibitors, we performed a HAT assay using AA,[31] curcumin,[33,64,65] and MB-3[34] (all at 50 μM) as references. As shown in Figure 10, both **9** and **18** at 500 μM were able to reduce the HAT enzymatic activity in U937 cell nuclear extracts (32% (for **9**) and 25% (for **18**) of inhibition of HAT activity). Given that **9** and **18**, active at the submillimolar level, were 1 order of magnitude less potent than the references, other functional targets may be involved and may explain the observed cellular effects and phenotypes. Thus, by combining some structural features shown by **9** and **18** (i.e., the C3-substituted quinoline moiety) with those typical of AA[31] and MB-3[34] (i.e., the salicylic acid or the 4-carboxy-γ-lactone function together with the presence of a long alkyl chain), we designed a new series of quinolines of which the 4-hydroxy-2-pentylquinoline-3-carboxylic acid **22** can be considered a prototype. Tested in the same assay, compound **22** showed HAT

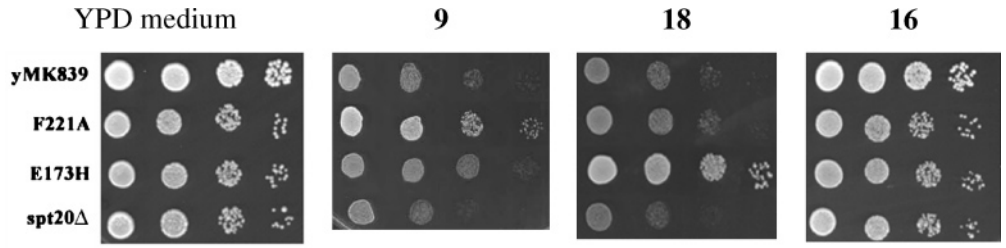


Figure 6. Cell growth inhibitory effects of **9**, **18**, and **16** on a panel of WT and mutant yeast strains. Ten-fold serial dilutions of the liquid yeast cell culture were spotted on solid medium.

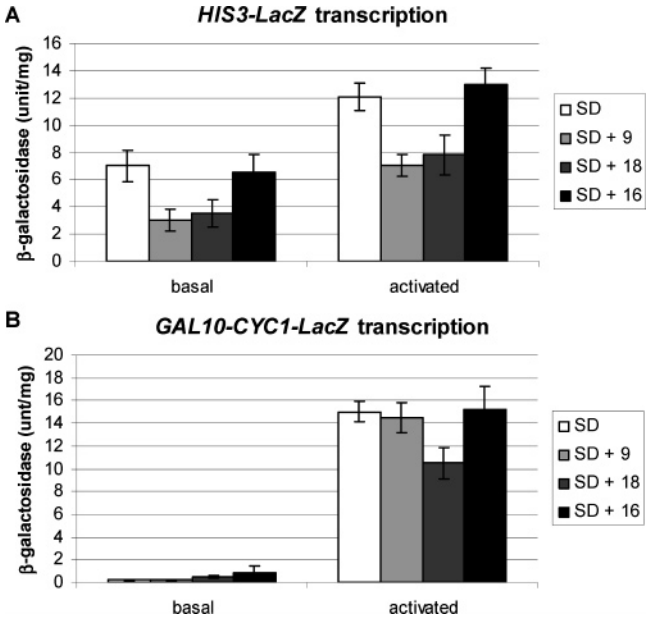


Figure 7. (A) Effects of **9**, **18**, and **16** (0.6 mM) on GCN5-dependent (*HIS3-LacZ*) transcription. (B) Effects of the same compounds on GCN5-independent (*GAL10-CYC1-LacZ*) transcription.

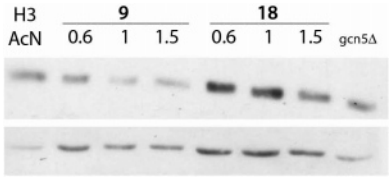


Figure 8. Effect of **9** and **18** on acetylation levels of yeast H3 histones.

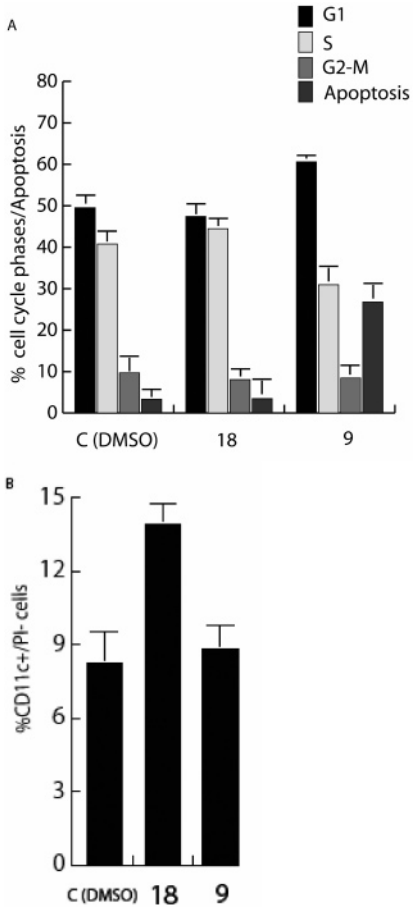


Figure 9. (A) Cell cycle analysis and apoptosis induction for compounds **9** and **18** in U937 cells after 24 h of stimulation. (B) Cytodifferentiating effect of compounds **9** and **18** in U937 cells after 30 h of treatment.

inhibitory activity at 50 μM (30%) and 25 μM (24%) concentrations, thereby being more potent than AA (15% of HAT inhibiting activity at 50 μM) and curcumin (17.5% of inhibition at 50 μM) and just a bit weaker than MB-3 (44% of inhibition at 50 μM) in this assay (Figure 10).

Effects on Histone H3 and α-Tubulin Acetylation. The capability of compounds **9** and **18** to reduce the acetylation levels of H3 terminal tails as well as the non-histone substrate α-tubulin was assessed in the human U937 leukemia cell line. To this aim, the U937 cell line was initially treated for 2 h with 5 μM suberoylanilide hydroxamic acid (SAHA),[66] a well-known HDAC inhibitor, to increase H3 as well as α-tubulin acetylation levels. As second step, cells were washed and incubated with vehicle or compounds **9** and **18** (at 2.5 mM) for an additional 4 h. As shown in Figure 11A by Western blot analysis, compound **9** was highly efficient in reduction of the H3 acetylation level whereas **18** was much less effective in the same conditions. Tested on α-tubulin, both compounds **9** and **18** were able to decrease the acetylation level of the non-histone substrate, **18** being slightly more potent than **9** (Figure 11B). These results suggest that, mainly for compound **18**, the potential activity of HAT inhibition may have non-histone molecular targets.

The same assay was repeated with compound **22** at 100 μM, using AA, curcumin, and MB-3 (all at 100 μM) as reference drugs. As shown in Figure 12, the acetylation levels of α-tubulin (lane 1) as well as histone H3 (lane 2) increased with the 2 h pretreatment with SAHA (5 μM). After washing out and incubation for a further 4 h with several compounds, the addition of SAHA (5 μM) to the medium produced higher acetylation levels of both tubulin and histone H3, whereas the incubation with curcumin, AA, MB-3 as well as with **22** (all at 100 μM) clearly reduced the acetylation extents of the histone (lane 2) and the non-histone (lane 1) substrates, **22** being more efficient than AA and slightly less active than curcumin and MB-3 with the histone H3 as substrate. The same assay repeated without the 2 h pretreatment with SAHA (lane 4 for histone H3 and

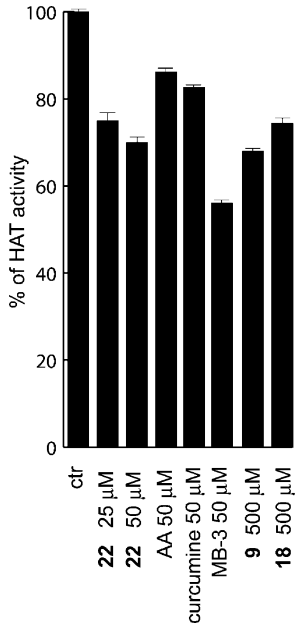


Figure 10. HAT assay performed with **9**, **18**, and **22** in U937 cell nuclear extracts. Anacardic acid (AA), curcumin, and MB-3 were used as reference drugs. The concentrations used are indicated.

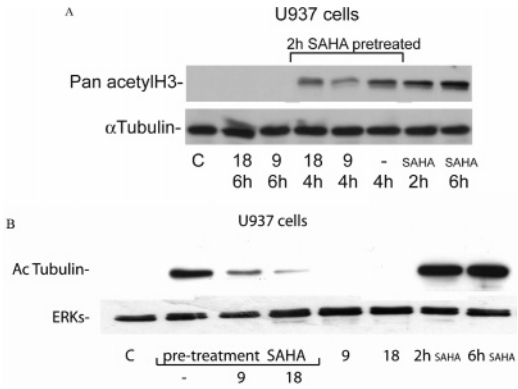


Figure 11. (A) Effects exerted by **9** and **18** (2.5 mM) on H3 acetylation. (B) Effects exerted by **9** and **18** (2.5 mM) on α-tubulin acetylation.

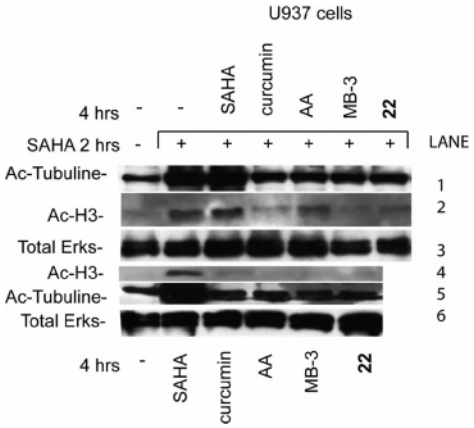


Figure 12. Histone H3 and α-tubulin hypoacetylating effects exerted by AA, curcumin, MB-3, and **22** (all at 100 μM).

lane 5 for tubulin) produced no observed effects (with the exception of the SAHA-dependent hyperacetylation).

Conclusion

Starting from a yeast phenotypic screening, we described the discovery of two small molecules as GCN5 inhibitors. As the first approach, we performed a screening of various molecules (**1**−**14**) ranging from transition-state mimics, anacardic acid analogues, to HDAC inhibitors to evaluate the modulating effect on the cell growth of the wild-type *S. cerevisiae* strain in comparison with that of the *gcn5*Δ mutant. Among compounds **1**−**14**, only compound **9** was able to significantly reduce the yeast cell growth, thus miming the effect of GCN5 deletion mutant. On the basis of these findings, we synthesized and tested in a cell-based assay some analogues of **9** (derivatives **15**−**21**) to acquire SAR information. Among compounds **15**−**21**, only the **9**-desmethyl derivative **18** was active in inhibiting the yeast cell growth. Tested on the *HIS3-LacZ* transcription assay, compounds **9** and **18** gave a high reduction of the GCN5-dependent gene transcription both in basal and in activated (amino acid starvation) conditions while the 3-carbethoxy-2-methylpyridine **16** was ineffective. Nevertheless, when tested on the GCN5-independent *GAL-10-CYC1-LacZ* reporter, **9** was completely inactive to inhibit transcription, whereas **18** showed a little inhibiting activity, thus indicating that activity profiles of the two derivatives are not fully superimposable. To determine the in vivo effects of **9** and **18** on the GCN5 acetylation activity, the acetylation of histone H3 N-terminal tails in the presence of the two compounds was determined in *S. cerevisiae*. Under steady-state conditions, the H3 acetylation levels were highly reduced by treatment with 0.6 mM **9**, while **18** was effective only at 1.5 mM.

In the human leukemia U937 cell line, at 1 mM, compound **9** showed effects on cell cycle (arrest in G1 phase) and apoptosis induction while **18** did not alter cell cycle and apoptosis. Moreover, both compounds at higher concentrations were able to induce apoptosis, as determined by Annexin V/PI double staining and by caspase3 cleavage, with compound **9** being more powerful than **18** in both cases (data not shown). When the granulocytic cytodifferentiating effect in U937 cells was tested, compound **18** displayed a higher number of CD11c positive PI negative (14%) cells while **9** was ineffective. When the HAT inhibitory activity was tested in U937 cell nuclear extracts, both **9** and **18** used at 500 μM were able to reduce the enzymatic activity (32% (for **9**) and 25% (for **18**) of HAT activity inhibition). With the aim of looking for small molecules active at lower concentrations such as the compounds used as reference drugs (AA, curcumin, and MB-3), we designed a new series of quinolines with the 4-hydroxy-2-pentylquinoline-3-carboxylic acid **22** as a prototype. In the HAT assay, compound **22** was able to reduce the HAT activity at 50 μM (30%) and 25 μM (24%). Finally, in human U937 cells, compound **9** (2.5 mM) was able to reduce the acetylation level of H3 terminal tails whereas **18** (2.5 mM) showed just a weak, if any, effect. On the other hand, both compounds showed high hypoacetylating properties on the non-histone substrate α-tubulin, **18** being slightly more potent than **9**. In the same assay, at the lower concentration of 100 μM, compound **22** showed a clear reduction of the acetylation extents of both histone H3 and α-tubulin comparable to that obtained with curcumin, AA, and MB-3 (all at 100 μM).

Further studies on compound **22** and analogues will be performed to further increase their HAT inhibiting properties, substrate specificities, and modulating effects on HAT-related phenomena.

Experimental Section

Chemistry. Melting points were determined on a Buchi 530 melting point apparatus and are uncorrected. Infrared (IR) spectra (KBr) were recorded on a Perkin-Elmer Spectrum One instrument. ^{1}H NMR spectra were recorded at 400 MHz on a Bruker AC 400

spectrometer; chemical shifts are reported in δ (ppm) units relative to the internal reference tetramethylsilane (Me_4Si). Electronic impact mass spectrometry (EI-MS) was performed on a Finnigan LCQ DECA TermoQuest (San Jose, CA) mass spectrometer. All compounds were routinely checked by TLC and ^{1}H NMR. TLC was performed on aluminum-backed silica gel plates (Merck DC, Alufolien Kieselgel 60 F_{254}) with spots visualized by UV light or using a $KMnO_4$ alkaline solution. All solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of solutions after reactions and extractions involved the use of a rotary evaporator operating at a reduced pressure of ~20 Torr. Organic solutions were dried over anhydrous sodium sulfate. Analytical results are within 0.40% of the theoretical values. A SAHA sample for biological assays was prepared as previously reported by us.[67] All chemicals were purchased from Aldrich Chimica (Milan, Italy) or from Lancaster Synthesis GmbH (Milan, Italy) and were of the highest purity.

As a rule, samples prepared for physical and biological studies were dried in high vacuum over P_2O_5 for 20 h at temperatures ranging from 25 to 110 °C, depending on the sample melting point.

Synthetic procedures for known compounds have been reported only if different from those already published.

2-[4-(4,5-Dihydrooxazol-2-yl)phenoxy]ethylamine (23). To a stirred solution of 2-ethanolamine (8.19 mmol, 0.50 g) in anhydrous dioxane (30 mL) at 0 °C a solution of di-*tert*-butyl dicarbonate (6.96 mmol, 0.15 g) in dioxane (10 mL) was added dropwise. The resulting mixture was stirred at room temperature for an additional 4 h. After completion (TLC monitoring, $CHCl_3$ on silica gel plate), the solvent was distilled in vacuo at 40−50 °C until dry and the residue was dissolved in water (30 mL) and extracted with dichloromethane (3 × 20 mL). The organic layers were collected, washed with brine (3 × 20 mL), dried overnight, then evaporated to furnish a TLC pure colorless oily residue (4.17 mmol, 0.76 g, 60%), which was redissolved in anhydrous tetrahydrofuran (20 mL) and mixed with 4-(4,5-dihydrooxazol-2-yl)phenol **24**[51] (4.17 mmol, 0.68 g) and anhydrous triphenylphosphine (4.17 mmol, 1.09 g). To the magnetically stirred resulting mixture, a solution of diisopropyl azodicarboxylate (4.17 mmol, 0.84 g) in anhydrous tetrahydrofuran (10 mL) was added dropwise. The resulting mixture was stirred at room temperature for 12 h and then filtered, and the solvent was removed under reduced pressure. The residue was taken up with water (50 mL) and extracted with dichloromethane (3 × 30 mL). The organic layer was washed with 2 N KOH (2 × 20 mL) and then with brine (3 × 30 mL), dried, and evaporated. Column chromatography on silica gel (ethyl acetate as eluent) provided a pure colorless oil (0.70 g, 55%), which was dissolved in dichloromethane (10 mL) and treated with trifluoroacetic acid (3 mL). The resulting mixture was stirred at room temperature overnight. Afterward, the solvent was removed under reduced pressure to give TLC pure **23** as a white solid, which was further purified by crystallization. MS (EI, 70 eV) *m*/*z*: 206. ^{1}H NMR ($CDCl_3$) δ 1.62 (s, 2H, NH_2), 3.62−3.66 (t, 2H, CH_2NH$_2$), 4.01−4.15 (t, 2H, CH_2N), 4.40−4.46 (m, 4H, OCH_2CH$_2$NH$_2$ and OCH$_2$ oxazoline ring, overlapped signals), 6.89−6.92 (d, 2H, $H_{2,6}$ benzene ring), 7.87−7.90 (d, 2H, $H_{3,5}$ benzene ring).

General Procedure for the Preparation of Derivatives 1−4. Example: Synthesis of N_2-Acetyl-N_6-methylthioacetyl-L-lysine Methyl Ester (1). A mixture of *N*α-acetyl-L-lysine methyl ester hydrochloride (0.42 mmol, 100.0 mg) and triethylamine (1.26 mmol, 0.18 mL) in anhydrous dichloromethane (5 mL) was treated with a previously prepared solution of methylthioacetic acid (0.42 mmol, 36.5 μL) and *N*,*N*′-carbonyldiimidazole (0.46 mmol, 74.9 mg) in anhydrous dichloromethane (5 mL). The resulting mixture was magnetically stirred at room temperature until completion (TLC monitoring, 9:1 $CHCl_3$/MeOH on silica gel plate), then diluted to 30 mL with dichloromethane and washed with water (3 × 10 mL) and brine (3 × 10 mL). The organic layer was dried and evaporated under reduced pressure to give compound **1** as a pure solid, which was further purified by crystallization. MS (EI, 70 eV) *m*/*z*: 290. ^{1}H NMR ($CDCl_3$) δ 1.59−1.62 (m, 2H, χ-CH_2), 1.63−1.65 (m, 2H, δ-CH_2), 1.78−1.81 (m, 2H, β-CH_2), 2.29 (s, 3H, CH_3−C═O), 2.37 (s, 3H, CH_3−S), 3.45 (s, 2H, CH_2−S), 3.51−3.55 (t, 2H, CH_2−NH), 3.98 (s, 3H, CH_3−O), 4.80−4.83 (t, 1H, CH), 6.58 (s, broad, NH), 7.50 (s, broad, NH).

Ethyl 3-Oxoheptadecanoate (25). Triethylamine (1.83 mL, 13.20 mmol) and magnesium chloride (0.98 g, 10.31 mmol) were added to a stirred suspension of potassium ethyl 2-methylmalonate (1.47 g, 8.66 mmol) in dry acetonitrile (28 mL), and stirring was continued at room temperature for 2 h. Then a previously prepared mixture of pentadecanoic acid (1.00 g, 4.12 mmol) and *N*,*N*′-carbonyldiimidazole (0.73 g, 4.53 mmol) in dry acetonitrile (15 mL) was added, and the resulting slurry was stirred overnight. After completion (TLC monitoring, SiO_2/$CHCl_3$), the mixture was cautiously acidified with 13% HCl while keeping the temperature below 25 °C, and the resulting mixture was stirred for a further 15 min. The organic layer was separated and evaporated, and the residue was treated with ethyl acetate (20 mL). The aqueous layer was extracted with ethyl acetate (2 × 20 mL), and the organic phases were combined, washed with saturated sodium bicarbonate solution (2 × 30 mL) and brine (3 × 30 mL), dried, and concentrated to give **25** (96%) as a white solid purified by column chromatography (SiO_2/$CHCl_3$). MS (EI, 70 eV) *m*/*z*: 312. ^{1}H NMR ($CDCl_3$) δ 1.07−1.11 (t, 3H, CH_3-aliphatic chain), 1.39−1.42 (m, 22H, aliphatic chain), 1.63−1.65 (m, 2H, CH_2CH$_2$C═O), 2.54−2.58 (t, 2H, CH$_2$CH_2C═O), 3.45 (s, 2H, CH_2), 4.13−4.15 (m, 2H, CH_2O). IR: 1725, 1700 cm^{-1}.

3,4-Dihydro-2-thioxo-6-tetradecylpyrimidin-4(3*H*)-one (6). Sodium metal (88.3 mg, 3.84 mmol) was dissolved in 20 mL of absolute ethanol, and thiourea (0.15 g, 1.92 mmol) and **25** (0.50 g, 1.60 mmol) were added to the clear solution. The mixture was heated at reflux for 5 h. After the mixture was cooled, the solvent was distilled in vacuo at 40−50 °C until dry and the residue was dissolved in a little water (20 mL) and made acidic with 0.5 N acetic acid. The resulting precipitate was filtered under reduced pressure, washed with water and then with diethyl ether, and vacuum-dried at 80 °C for 12 h to give title compound as a pure solid, which was further purified by crystallization. MS (EI, 70 eV) *m*/*z*: 324. ^{1}H NMR (DMSO-d_6): δ 1.06−1.08 (t, 3H, CH_3), 1.45−148 (m, 24H, CH_2), 1.73−1.74 (d, 2H, CH_2-Ar), 5.90 (s, 1H, CH), 12.51 (d broad, 2H, NH, exchangeable with D_2O).

3,4-Dihydro-2-methylthio-6-tetradecylpyrimidin-4(3*H*)-one (26). A mixture of 3,4-dihydro-2-thioxo-6-tridecylpyrimidin-4(3*H*)-one **6** (0.40 g, 1.23 mmol), methyl iodide (0.15 mL, 2.46 mmol), and 2 mL of anhydrous *N*,*N*-dimethylformamide was stirred at room temperature for 2 h. The reaction content was poured on cold water (80 mL) and extracted with chloroform (3 × 30 mL). The organic layers were collected, washed with brine (3 × 50 mL), dried overnight, then evaporated to furnish crude **26** as a white solid, which was purified by crystallization. MS (EI, 70 eV) *m*/*z*: 338. ^{1}H NMR (DMSO-d_6) δ 1.07−1.12 (t, 3H, CH_3-aliphatic chain), 1.30−1.37 (m, 22H, aliphatic chain), 1.66−1.73 (m, 2H, CH_2-CH$_2$C═), 2.63−2.68 (t, 2H, CH$_2$CH_2C═), 2.72 (s, 3H, CH_3), 6.12 (s, 1H, C*H*), 11.02 (s, 1H, NH, exchangeable with D_2O). IR: 2900, 1640 cm^{-1}.

5-Bromo-3,4-dihydro-2-methylthio-6-tetradecylpyrimidin-4(3*H*)-one (27). To a stirred supension of **26** (0.59 mmol, 0.20 g) and anhydrous carbon tetrachloride (50 mL) were added *N*-bromosuccinimide (0.59 mmol, 0.11 g) and catalytic benzoyl peroxide (10 mg), and the resulting mixture was refluxed for 2.5 h. After completion, the mixture was diluted with water (30 mL), the two phases were separated, and the aqueous layer was extracted with $CHCl_3$ (3 × 20 mL). The organic phases were combined, washed with saturated sodium bicarbonate solution (2 × 30 mL) and brine (3 × 30 mL), dried, and concentrated to give TLC pure **27** as a white solid, which was further purified by crystallization. MS (EI, 70 eV) *m*/*z*: 417. ^{1}H NMR (DMSO-d_6) δ 1.05−1.08 (t, 3H, CH_3-aliphatic chain), 1.28−1.35 (m, 22H, aliphatic chain), 1.66−1.73 (m, 2H, CH_2CH$_2$C═), 2.66 (s, 3H, CH_3), 2.73−2.78 (t, 2H, CH$_2$CH_2C═).

3,4-Dihydro-2-methylthio-4-oxo-6-tetradecylpyrimidin-5-carboxylic Acid (7). To a solution of **27** (0.48 mmol, 0.20 g) in anhydrous diethyl ether (20 mL), magnetically stirred at −70 °C,

a 2.5 M solution of *n*-BuLi in hexanes (1.2 mmol, 0.48 mL) was added dropwise. The resulting mixture was stirred for an additional 30 min, then poured on anhydrous diethyl ether (20 mL) saturated with dry ice, and kept stirring until warmed to room temperature. The mixture was then poured on cold water (80 mL). The aqueous layer was acidified with 10% HCl and extracted with ethyl acetate (3 × 25 mL). The combined organic extracts were washed with brine (3 × 20 mL), dried, and concentrated to give TLC pure **7**, which was further purified by crystallization. MS (EI, 70 eV) *m*/*z*: 382. ^{1}H NMR (DMSO-d_6) δ 1.06−1.10 (t, 3H, CH_3-aliphatic chain), 1.39−1.49 (m, 22H, aliphatic chain), 1.83−1.87 (m, 2H, CH_2-CH_2C═), 2.72 (s, 3H, CH_3), 2.80−2.86 (t, 2H, CH_2CH_2C═), 11.2 (s broad, 1H, NH), 11.3 (s, 1H, COOH, exchangeable with D_2O).

General Procedure for the Synthesis of Derivatives 8, 15, 28. Example: 3-Carbethoxy-4-hydroxy-2-pentadecilquinoline (8). To a stirred solution of isatoic anhydride (1.50 g, 9.2 mmol) and ethyl 2-oxostearate (4.50 g, 13.8 mmol) in dry dioxane (12 mL) was added NaOH pearl (55 mg, 1.4 mmol). The mixture was heated at reflux for 36 h. After the completion of reaction, the cooled mixture was poured on water (36 mL) and extracted with ethyl acetate (3 × 40 mL). The organic layers were collected, washed with brine (3 × 40 mL), dried, and evaporated to afford a residue, which was purified by chromatography on silica gel column (eluent, 3:1 ethyl acetate/hexane) to furnish **8** as a white solid. ^{1}H NMR (DMSO-d_6) δ 0.81 (t, 3H, $C_{15}H_3$ alkyl chain), 1.22 (m, 27H, OCH_2CH_3 and $C_{(3-14)}H_2$ alkyl chain), 1.63 (m, 2H, C_2H_2), 2.61 (m, 2H, C_1H_2 alkyl chain), 4.21 (q, 2H, OCH_2CH_3), 7.32 (m, 1H, *H* C_6 quinoline ring), 7.55 (m, 1H, *H* C_7 quinoline ring), 7.65 (m, 1H, *H* C_8 quinoline ring), 8.04 (m, 1H, *H* C_5 quinoline ring), 11.78 (s, 1H, O*H*).

4-Hydroxy-2-pentil-3-quinolinecarboxylic Acid (22). To a stirred solution of 3-carbethoxy-4-hydroxy-2-pentilquinoline **28** (0.44 g, 1.5 mmol) in ethanol (8 mL) was added 4 N KOH solution (15 mL). The mixture was then heated at 70 °C for 1 week. After the completion of reaction, the cooled mixture was poured on water (36 mL) and extracted with ethyl acetate (3 × 20 mL). Then 2 N HCl was added to the aqueous layer until the pH was 2. The precipitate was filtered and recrystallized from MeOH to yield the title compound as a TLC pure white solid. ^{1}H NMR (DMSO-d_6) δ 0.85 (t, 3H, CH_3), 1.33 (m, 4H, $CH_2CH_2CH_3$), 1.63 (m, 2H, CH_2-$CH_2CH_2CH_3$), 3.27 (m, 2H, $CH_2CH_2CH_2CH_2CH_3$), 7.51 (m, 1H, *H* C_6 quinoline ring), 7.73 (m, 1H, *H* C_7 quinoline ring), 7.83 (m, 1H, *H* C_8 quinoline ring), 8.20 (m, 1H, *H* C_5 quinoline ring), 12.42 (s, 1H, O*H*).

***N*-Hydroxyretinamide (12).** To a 0 °C cooled solution of **11** (0.50 g, 1.67 mmol) in dry tetrahydrofuran (10 mL) were added ethyl chloroformate (0.19 mL, 2.0 mmol) and triethylamine (0.30 mL, 2.17 mmol), and the mixture was stirred for 10 min. The solid was filtered off, and to the filtrate was added *O*-(2-methoxy-2-propyl)hydroxylamine[55] (0.37 mL, 5.0 mmol). The resulting mixture was stirred at room temperature for 1 h. Then the solvent was evaporated under reduced pressure and the residue was diluted in MeOH (6 mL). Amberlyst 15 ion-exchange resin (0.17 g) was added to the solution of the O-protected hydroxamate, and the mixture was stirred at room temperature for 1 h. Afterward, the mixture was filtered and the filtrate was concentrated in vacuum to give a residue, which was purified by crystallization. ^{1}H NMR (DMSO-d_6) δ 0.99 (s, 6H, two CH_3 at C_6 cyclohexene ring), 1.42 (m, 2H, CH_2 position 5 cyclohexene ring), 1.54 (m, 2H, CH_2 position 4 cyclohexene ring), 1.66 (s, 3H, CH_3 at C_7 position alkenylic chain), 1.96 (s, 3H, CH_3 at C_2 cyclohexene ring), 1.98 (m, 2H, CH_2 position 3 cyclohexene ring), 2.25 (s, 3H, CH_3 at C_3 position alkenylic chain), 5.75 (s, 1H, CH position 2 alkenylic chain), 6.19 (m, 3H, CH positions 4, 6, 8 alkenylic chain), 6.37 (m, 1H, CH position 9 alkenylic chain), 6.99 (m, 1H, CH position 5 alkenylic chain), 10.55 (s, 1H, N*H*OH), 11.06 (s, 1H, NHO*H*).

3-Carboxamidoquinoline (21). To a 0 °C cooled solution of **20** (0.50 g, 2.9 mmol) in dry tetrahydrofuran (25 mL) were added ethyl chloroformate (0.66 mL, 6.9 mmol) and triethylamine (1.05 mL, 7.5 mmol), and the mixture was stirred for 10 min. The solid was filtered off, and to the filtrate was added 0.5 M ammonia solution in dioxane (30 mL). The resulting mixture was then stirred at room temperature for 1 h. After the completion of the reaction, the solvents were removed in vacuum and the residue was poured into water (30 mL) and extracted with ethyl acetate (3 × 30 mL). Organic phases were combined, washed with 0.5 N HCl (3 × 30 mL) and brine (3 × 30 mL), then dried and evaporated to afford **21**, which was purified by crystallization. ^{1}H NMR (DMSO-d_6) δ 7.67 (m, 1H, *H* C_6), 7.84 (m, 1H, *H* C_7), 8.06 (m, 2H, *H* C_5 and *H* C_8), 8.32 (s, 2H, NH_2), 8.84 (s, 1H, *H* C_4), 9.31 (s, 1H, *H* C_2).

Cell-Based Screening. Yeast Strains and Growth. All yeast strains used in this study are listed in Table C (Supporting Information). The yPO4 and yPO13 strains were produced by gene disruption using a polymerase chain reaction (PCR) cassette carrying kanMX4[68] or the HIS3 gene. Cells were grown at 28 °C in YPD-rich medium (1% yeast extract, 2% bactopeptone, 2% glucose), in SD minimal medium (0.67% yeast nitrogen base (YNB), 2% glucose), or in SG minimal medium (0.67% YNB, 2% galactose), the last two supplemented with 0.01% of requested amino acids. For amino acid deprivation experiments, the yPO14 strain was inoculated into 20 mL of SD and incubated at 28 °C for 16 h. Then 25 mM 3-aminotriazole (3-AT) was added for 4 h at 28 °C with shaking. Cells were harvested and used for β-galactosidase determinations. For galactose induction experiments, the yPO15[69] strain was inoculated into 20 mL of SD and incubated at 28 °C for 16 h. The cells were then washed and grown in SG for 4 h at 28 °C. Microscopic observations were performed using a fluorescence microscope (Axioskop Zeiss) equipped with oil immersion ocular objectives at 100× and 10×.

Determination of β-Gal Activity. Protein extracts were prepared from 0.8 OD_{600} harvested yeast cultures. Cells were washed twice with H_2O and once with extraction buffer (100 mM Tris-HCl, 1 mM DTT, 20% glycerol). Soluble extracts were prepared by resuspending whole cells in extraction buffer and grinding them with glass beads in a Vortex mixer. Protein concentration was determined by the Bradford method.[70] β-Gal activities were determined as previously described.[71] One unit of β-galactosidase corresponds to 1 μmol of *o*-nitrophenol produced per minute.

Protein Extraction and Western Blot Analysis. Protein extraction from yeast was performed using an alkaline protocol.[72] Extracted proteins were run on 15% SDS-PAGE gels and blotted onto Hybond membranes (Amersham). Histones H3 and H3-Ac were detected using primary histone antibodies (Upstate Biotechnology) and HRP-labeled IgG secondary antibody, diluted 1:10000. Detected proteins signals were visualized using an enhanced chemiluminescence (ECL) system.

Determination of Cell Cycle Effect, Cell Differentiation, and Apoptosis in U937 AML Cells. Cell Culture and Ligands. Human leukemia cell line U937 was propagated in RPMI medium supplemented with 10% FBS (fetal bovine serum, Hyclone) and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin, and 250 ng/mL amphotericin-B). Cells were kept at a constant concentration of 200 000 cells per milliliter of culture medium. SAHA[67] was used at 5 μM. All compounds described were resuspended in DMSO (Sigma-Aldrich).

Cell Cycle Analysis. The 2.5 × 10^5 cells were collected and resuspended in 500 μL of an hypotonic buffer (0.1% Triton X-100, 0.1% sodium citrate, 50 μg/mL propidium iodide, RNAse A). Cells were incubated in the dark for 30 min. Samples were acquired on a FACS-Calibur flow cytometer using the Cell Quest software (Becton Dickinson) and analyzed with standard procedures using the Cell Quest software (Becton Dickinson) and the ModFit LT version 3 software (Verity). All the experiments were performed three times.

FACS Analysis of Apoptosis. Apoptosis was measured with Annexin V/propidium iodide double-staining detection (Roche and Sigma-Aldrich, respectively), as recommended by the suppliers. Samples were analyzed by FACS with Cell Quest technology (Becton Dickinson). As second assays, the caspase 3 detection (B-Bridge) was performed and quantified by FACS (data not shown, Becton Dickinson).

Granulocytic Differentiation. Granulocytic differentiation was carried out according to Altucci . et al.[73] Briefly, U937 cells were harvested and resuspended in 10 μL of phycoerythrine-conjugated CD11c (CD11c-PE). Control samples were incubated with 10 μL of PE conjugated mouse IgG1, incubated for 30 min at 4 °C in the dark, washed in PBS, and resuspended in 500 μL of PBS containing propidium iodide (0.25 μg/mL). Samples were analyzed by FACS with Cell Quest technology (Becton Dickinson). Propidium iodide positive cells have been excluded from the analysis.

HAT Assay. HAT inhibition assay has been performed as recommended by the suppliers (Upstate). Briefly, an indirect ELISA assay has been performed for the detection of acetyl residues on histone H3 substrate using 10 μg of U937 cell nuclear extract (prepared according to Nebbioso et al.)[74] per assay as source of HAT enzymes. The incubations with DMSO alone (control) or with **9** (500 μM), **18** (500 μM), **22** (25 and 50 μM), AA (50 μM), curcumin (50 μM), and MB-3 (50 μM) have been carried out for 90 min. Acetylated histone H3 peptides (Upstate) have been included as positive controls and have been used to make standard curves for the assay quantitation. Data have been expressed as a percentage of HAT activity inhibition, taking the untreated control of U937 nuclear extract as 100%.

Determination of α-Tubulin and Histone H3 Specific Acetylation. For α-tubulin, an amount of 50 μg of total protein extracts was separated on 10% polyacrylamide gels and blotted.[74] Western blots were shown for acetylated α-tubulin (Sigma, dilution of 1:500), and total ERKs (Santa Cruz) were used to normalize for equal loading. For histone H3 specific acetylations, an amount of 100 μg of total protein extract was separated on 15% polyacrylamide gels and blotted.[74] Western blots were shown for pan-acetylated histone H3 (Upstate), and total tubulin (Sigma) was used to normalize for equal loading.

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Supporting Information Available: Characterization and elemental analysis data for compounds **1**−**4**, **6**−**8**, **12**, **15**, **21**, **22**, **24**−**26** and table of yeast strains used in this study. This material is available free of charge via the Internet at http://pubs.acs.org.

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