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Synthesis of Benzamides Related to Anacardic Acid and Their Histone Acetyltransferase (HAT) Inhibitory Activities

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A group of benzamides related to anacardic acid amide CTPB with alkyl chains of defined length were prepared by a five-step sequence starting from 2,6-dihydroxybenzoic acid, and their activities were compared with those reported for the HAT inhibitor anacardic acid (AA). The subset of 4-cyano-3-trifluoromethylphenylbenzamides with shorter chains exhibited activities similar to that of AA, as they behaved as human p300 inhibitors, induced a decrease in histone acetylation levels in immortalized HEK cells, and counteracted the action of the HDAC inhibitor SAHA in MCF7 breast cancer cells. Moreover, an analogue with the shortest alkyl chain induced significant apoptosis at 50 μM in U937 leukemia cells.

Introduction

Epigenetic modulation, that is, the alteration of cellular phenotype without alteration of the genotype,[1] plays an increasingly important role in the development of new therapies for cancer.[2–5] Data accumulated over the last decade clearly links carcinogenesis and tumor progression to the deregulation of enzymes responsible for epigenetic modifications.[2,6–10] These epigenetic alterations occur at chromatin, a structure composed of nucleosomes, the 146-base-pair stretches of DNA wrapped around an octameric core which contains two molecules each of histones H2A, H2B, H3, and H4,[11] organized like beads on a string. The histone core contains flexible and highly conserved basic tail regions at the surface of the nucleosome, where various modifications such as acetylation, methylation, ADP-ribosylation, phosphorylation, sumoylation, and ubiquitylation can occur.[12] These often reversible covalent changes create docking sites for recognition and assembly of supramolecular structures.[13] Through specific readout mechanisms, including cross-regulation of these post-translational modifications,[14] fundamental cellular processes such as replication, chromatin assembly, repair and transcription can be tightly regulated.[3,15]

Acetylation at histone lysine residues has the net effect of neutralizing their positive charge, and consequently of weakening the interactions with phosphate groups of DNA. This in turn induces unwrapping and creates a more open chromatin state, ready for transcription.[16] The enzymes responsible for acetylation of histones are histone acetyl transferases (HATs). Nuclear HATs have been grouped into three families (Gcn/PCAF, MYST, and p300/CBP) with different specificities.[17] Although all HAT families require acetyl-CoA as cofactor, the precise mechanisms for acetyl transfer to the lysine residue within the HAT enzyme (via a ternary complex or a ping-pong mechanism)[17] remain to be determined. The acetylation of histones can be reversed by enzymes that hydrolyze the acetyl groups, the so termed histone deacetylases (HDACs). Inhibitors of HDAC enzymes (HDACis) have shown anticancer potential and already entered the clinic (SAHA, vorinostat, Zolinza have been approved for the treatment of advanced cutaneous T-cell lymphoma, CTCL).[9,18,19]

Figure 1 depicts selected HAT modulators of different structural classes including the bi-substrate competitors H3-CoA-20**1** and construct **2**,[20–22] simple molecules such as MC1626 **4**,[23] **5**[24] and MB-3 **3**[25] and also the natural products curcumin **6**,[26,27] garcinol **9**[28] (and its derivative LTK-14 **10**[29]) and anacardic acid (AA) **7**.[30] Whereas AA is a p300 and PCAF HAT inhibitor,[30] the *N*-(4-chloro-3-trifluoromethylphenyl)-2-ethoxy-6-pentadecylbenzamide (CTPB) **8** was reported as the most potent activator of the same enzymes, and moreover devoid of HDAC activity.

CTPB **8** and its analogues have been prepared by alkylation of the phenol of the natural product (AA) as ethyl ether and condensation of the carboxylic acid (saponification of the ethyl ester formed in the previous reaction was required) with different anilines.[30] However, starting from the natural material, no modification of the alkyl chain could be performed.[30] In order to complete this study, we set out to prove the role of the hydrophobic alkyl chain of these salicylic acid amides on their HAT activity, while preserving the substituents that reportedly afforded the highest inhibitory potency on **8** (the ethoxy and the 4-chloro- or 4-cyano-3-trifluoromethyl groups on the benzamides).[30]

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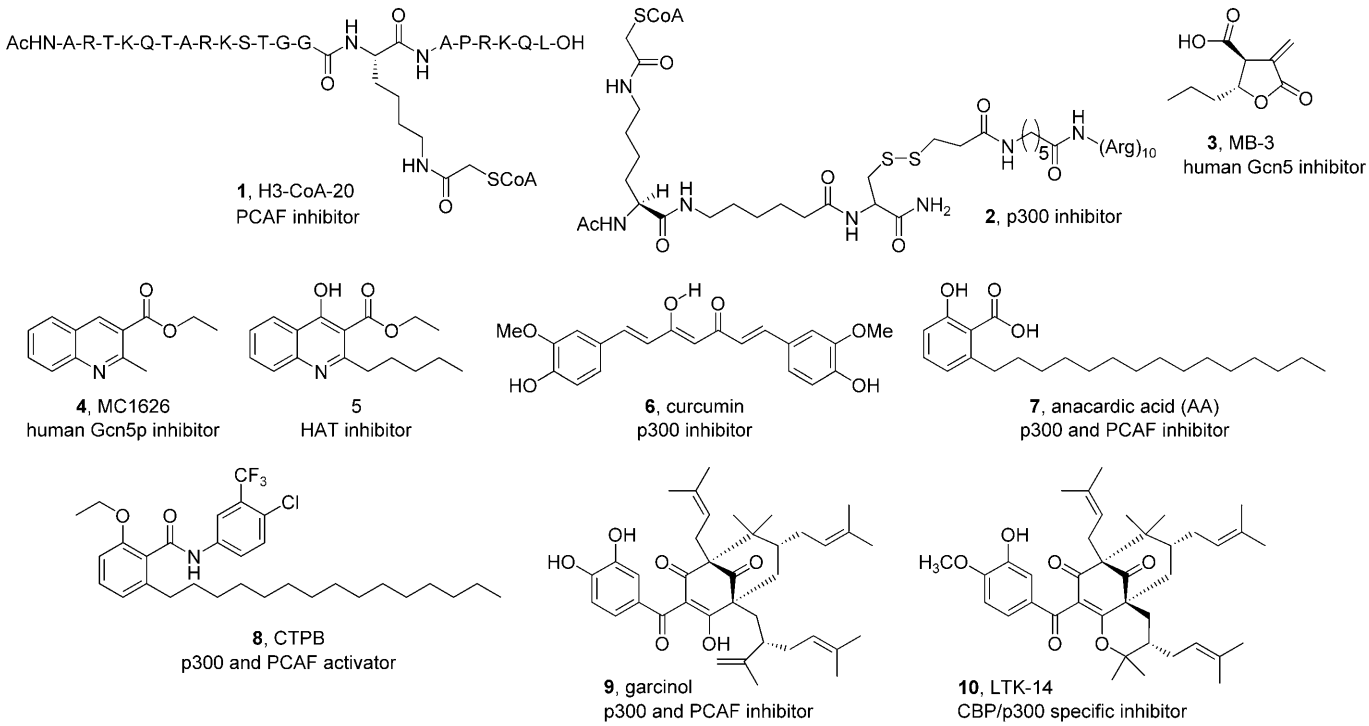


Figure 1. Histone acetyl transferase (HAT) modulators and their reported activities.

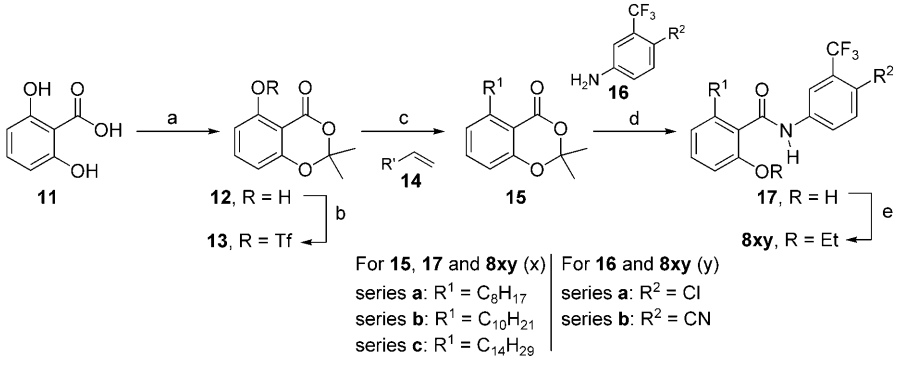
Results and Discussion

Synthesis

For attaching the alkyl chain to the aryl ring we selected the Suzuki cross-coupling reaction[31] between an aryl triflate and different trialkylboranes (Scheme 1). Starting from symmetrical 2,6-dihydroxybenzoic acid **11**, formation of the 1,3-benzodioxinone **12** was achieved with acetone and TFAA/TFA.[32] Although the yield is moderate (30%), the shortcut to the synthesis of these salicylic acid derivatives[33] by way of the desymmetrization of **11** makes this a convenient route relative to alternative preparations of anacardic acid derivatives.[34] Triflate **13**[35] was derived from the treatment of the 1,3-benzodioxinone **12** with TFAA and pyridine (77%), and then coupled to the trialkylboranes (obtained by hydroboration of the terminal alkenes **14** with 9-BBN) in the presence of [$PdCl_2$(dppf)], NaOMe, and KBr.[36] The coupling yields for the three representative alkenes surveyed (1-octene **14a**, 1-decene **14b** and 1-tetradecene **14c**) ranged from 50 to 80%. The acyl transfer/deprotection of the 1,3-benzodioxinone **15** required deprotonation of aniline **16** with *n*BuLi in DMPU[37] and the derived lithium amide was heated with **15** at 80 °C. Formation of the ethyl ether from salicylamides **17** proceeded uneventfully and provided the final benzamides **8**. See Table 1 for percent yields.

Biological evaluation

We first tested the effects of series **8xy** and AA (at 50 μM) on cell cycle analysis, differentiation and apoptosis of the human U937 leukemia cell line and compared them with known HDAC inhibitors (HDACis) such as SAHA[38] and MS275[39] (at 5 μM), used as positive controls. Figure 2A shows a cell cycle FACS analysis at the time course of 30 h. The compounds did not show major effects on the regulation of cell cycle phases, in contrast to both known HDAC inhibitors. Compound **8bb** was able to fully block the G2/M phase, but did not result in a G1 block. As for **8aa** and **8ab** a slight S phase increase was noticed. Analysis of differentiation, measured as CD11c positive, PI



Scheme 1. Reagents and conditions: a) reference [32]; b) trifluoromethanesulfonic anhydride, pyridine, 2 h (77%); c) terminal alkene **14**, 9-BBN, NaOMe, [$PdCl_2$(dppf)], KBr, 70 °C, THF (Table 1); d) aniline **16a**/**16b**, *n*BuLi, DMPU, 80 °C, 2 h (Table 1); e) Et_2SO_4, K_2CO_3, acetone (Table 1).

Table 1. Yields for steps c–e of the synthesis shown in Scheme 1.

R^1 in **15**	Step c [%]	R^2 in **16**	Step d [%]	Step e [%]
C_8H_{17}	**15a**, 61	Cl	**17aa**, 86	**8aa**, 94
C_8H_{17}		CN	**17ab**, 87	**8ab**, 62
$C_{10}H_{21}$	**15b**, 50	Cl	**17ba**, 94	**8ba**, 91
$C_{10}H_{21}$		CN	**17bb**, 97	**8bb**, 72
$C_{14}H_{29}$	**15c**, 80	Cl	**17ca**, 90	**8ca**, 99
$C_{14}H_{29}$		CN	**17cb**, 90	**8cb**, 42

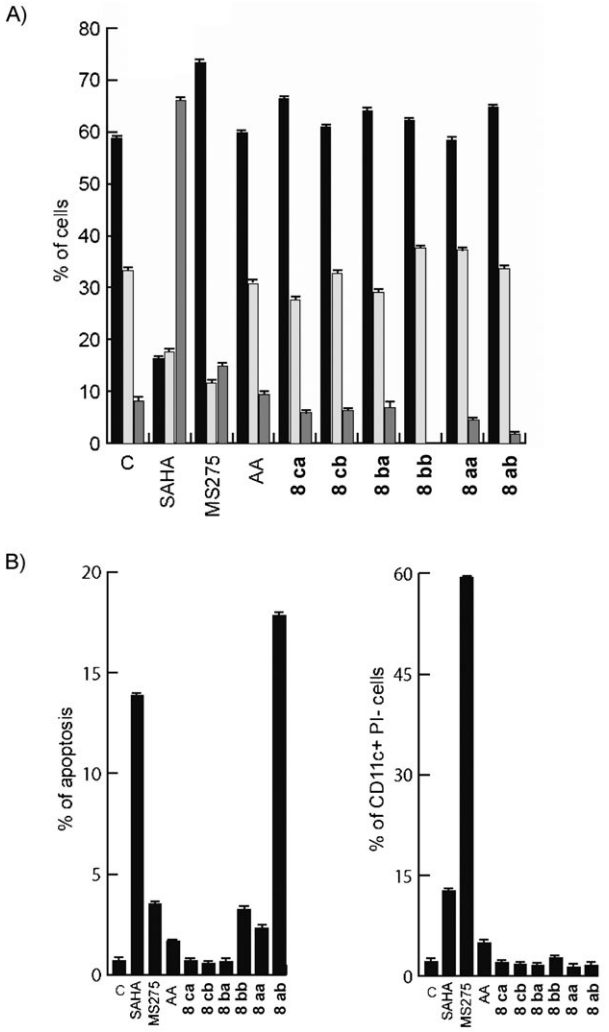


Figure 2. A) Cell cycle analysis of U937 leukemia cells upon treatment with the indicated compounds for 30 h; bars represent the percentage of cells in the cell cycle phases indicated (■: G1, □: S, ■: G2/M). B) Left side: evaluation of apoptosis measured as caspase 3 activation; bars represent the number of cells containing active caspase 3. Right side: evaluation of cell differentiation measured as CD11c-positive cells in PI-negative cells. Exclusion of the PI-positive cells prevents the analysis in dead cells.

negative cells, indicates low activities for compounds **8xy** as shown in Figure 2B. In contrast, we did observe significant apoptosis when U937 cells were treated with **8ab** at the dose of 50 μM for 30 h (Figure 2B), thus indicating a selective profile for this derivative.

These preliminary biological data prompted us to check the effects of selected inhibitors on the histone H4 acetylation levels in immortalized non-tumorigenic HEK cells (HEK-TE), which present higher basal histone acetylation levels. As shown by WB analysis in Figure 3, compounds **8bb** and **8ab**

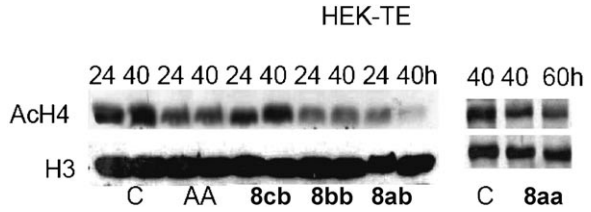


Figure 3. Histone H4 acetylation levels upon treatment of HEK-TE cells with selected benzamides **8xy** (50 μM). The levels of total histone H3 are used to normalize for equal loading.

were able to down-regulate acetylation levels to a similar extent as the reference compound (AA). Analogue **8cb** did not show inhibition on this assay.

We also investigated whether the AA-related benzamides were able to counteract the action of known HDACis such as SAHA. WB analysis of H3 acetylation levels in MCF7 breast cancer cells treated with 5 μM SAHA and co-treated with the HAT modulators (100 μM) for 3 h revealed that compounds **8bb**, **8aa** and **8ab** showed deacetylation activities similar if not higher than those of anacardic acid (Figure 4). This finding

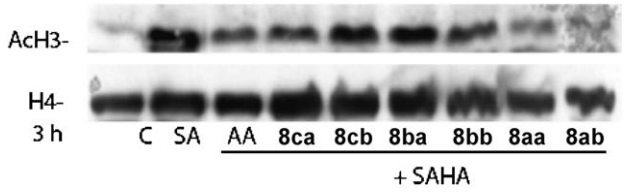


Figure 4. Histone H3 acetylation levels upon incubation of SAHA-treated MCF7 cells with benzamides **8xx**. The levels of total histone H4 are used to normalize for equal loading.

confirms that these molecules display HAT inhibitory activities and oppose the known hyper-acetylating effects of SAHA. Of note compounds **8cb** and **8ba** did not display detectable histone acetylation inhibitory actions (as also shown in the HEK-TE cells in Figure 3 for **8cb**).

Finally, we checked the activities of the 4-cyanobenzamides **8ab** and **8bb** (the 4-chlorobenzamides were less active, not shown) on the human recombinant p300 enzyme, in comparison with the known inhibitor anacardic acid. The in vitro p300 activity assay (Figure 5) clearly shows that compounds **8bb** and **8ab** used at 100 μM concentrations inhibit the human p300 recombinant enzyme at levels similar to AA (the potency of AA reported in reference [30] is, however, about 10-fold lower than our measurement).

The above studies clearly show that the benzamides related to the previously described CTPB **8**[30] behave as inhibitors of p300, and exhibit a profile that is similar to the natural product anacardic acid, with some of the amides surpassing the latter

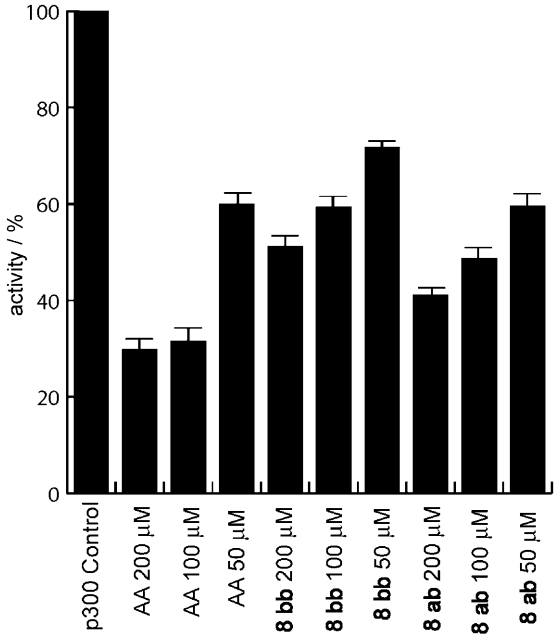


Figure 5. p300 HAT activity assay measured in the presence and absence of the indicated compounds. Data represent the percentage of activity of p300 relative to control, taken as 100%.

in its ability to decrease the H4 acetylation levels. Moreover, the potency of these compounds to counteract the activity of the known HDAC inhibitor SAHA is inversely correlated with the chain length for the same benzamide group. The highest potency was measured for the *n*-octyl analogue **8ab**, which moreover showed promising induction of apoptosis in U937 cells.

Conclusions

Several 2-ethoxy salicylamides with linear alkyl chains of defined lengths have been prepared by a 5-step sequence starting from 2,6-dihydroxybenzoic acid. The sequence is highly versatile and amenable to diversity-oriented synthesis, because the three last steps each incorporate one of the components of the series. The ethyl ether on the phenol group and two different salicylamides, the 4-cyano-3-trifluoromethylbenzamide (series **b**) and 4-chloro-3-trifluoromethylbenzamide (series **a**) reported as the most potent[30] were selected, and we therefore focused on the role of the alkyl chain in these derivatives. The biological activities of the ethyl salicylbenzamides with C8, C10 and C14 chains were compared with those reported for the HAT inhibitor anacardic acid, which has a linear C15-alkyl chain.

Whereas none of these synthetic amides exhibited significant effects on cell cycle and differentiation, the shortest analogue with a *p*-cyanophenyl group **8ab** induced apoptosis at 50 μM, as did analogues **8aa** and **8bb** to a lesser extent. Whether the induction of apoptosis is related to the epigenetic activities remains to be demonstrated. Measurement of histone acetylation levels on immortalized HEK cells upon treatment with the subset of *p*-cyanophenylbenzamides indicates that those with shorter chains (**8ab**, **8bb**) have activities similar to AA. Moreover, on a competition experiment in MCF7 breast cancer cells treated with SAHA, compounds **8aa**, **8bb** and **8ab** showed an inhibitory potency similar to or higher than AA, and this was consistent with their effects on the histone acetylation levels. The two most effective benzamides, **8bb** and **8ab**, were confirmed as human p300 inhibitors with a potency similar to AA.

Work is underway to extend the study to a more complete series in order to clarify the role of the different modifications (ether, benzamides, chain length) on their epigenetic modulatory activities, discover novel apoptosis-inducing agents and deepen our understanding of the beneficial effects of HAT modulators in the treatment of human diseases with alterations of the epigenetic circuitry.

Experimental Section

General procedures: Solvents were dried according to published methods and distilled before use. All other reagents were commercial compounds of the highest purity available. All reactions were carried out under argon atmosphere, and those not involving aqueous reagents were carried out in oven-dried glassware. Analytical thin layer chromatography (TLC) was performed on aluminum plates with Merck Kieselgel 60F254 and visualized by UV irradiation (254 nm) or by staining with an ethanolic solution of phosphomolibdic acid. Flash column chromatography was carried out using Merck Kieselgel 60 (230–400 mesh) under pressure. UV/Vis spectra were recorded on a Cary 100 Bio spectrophotometer. IR spectra were obtained on JASCO FTIR 4200 spectrophotometer, from a thin film deposited onto a NaCl glass plate. Mass spectra were obtained on a Hewlett–Packard HP59970 instrument operating at 70 eV by electron ionization. High Resolution mass spectra were taken on a VG Autospec instrument. ^{1}H NMR spectra were recorded in $CDCl_3$ and $(CD_3)_2CO$ at ambient temperature on a Bruker AMX-400 spectrometer at 400 MHz with residual protic solvent as the internal reference [$CDCl_3$, $\delta_H = 7.26$ ppm; $(CD_3)_2CO$, $\delta_H = 2.05$ ppm]; chemical shifts (δ) are given in parts per million (ppm), and coupling constants (J) are given in Hertz (Hz). The proton spectra are reported as follows: δ (multiplicity, coupling constant J, number of protons) ^{13}C NMR spectra were recorded in $CDCl_3$ and $(CD_3)_2CO$ at ambient temperature on the same spectrometer at 100 MHz, with the central peak of $CDCl_3$ ($\delta_C = 77.0$ ppm) or $(CD_3)_2CO$ ($\delta_C = 30.8$ ppm) as internal reference. DEPT 135 was used to aid the assignment of signals in the ^{13}C NMR spectra.

2,2-Dimethyl-4-oxo-4*H*-benzo[*d*-1,3]dioxin-5-yl Trifluoromethanesulfonate (13): To a solution of 5-hydroxy-2,2-dimethyl-4*H*-benzo[*d*-1,3]dioxin-4-one **12** (0.71 g, 3.86 mmol) in pyridine (8 mL) was added, at 0 °C, trifluoromethanesulfonyl anhydride (0.73 mL, 4.42 mmol) and the reaction was stirred at 0 °C for 2 h. An aqueous saturated solution of $NaHCO_3$ (10 mL) was added and the aqueous mixture was extracted with EtOAc (3×), dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by chromatography (silica gel, 90:10 hexane/EtOAc) to afford 948 mg (30%) of a white solid identified as 2,2-dimethyl-4-oxo-4*H*-benzo[*d*-1,3]dioxin-5-yl trifluoromethanesulfonate (**13**); mp: 114 °C (hexane/acetone); ^{1}H NMR (400.13 MHz, $CDCl_3$): $\delta = 7.60$ (t, $J = 8.3$ Hz, 1H), 7.05 (d, $J = 8.4$ Hz, 1H), 6.99 (d, $J = 8.2$ Hz, 1H), 1.76 ppm (s, 6H); ^{13}C NMR (100.62 MHz, $CDCl_3$): $\delta = 157.3$ (s), 157.1 (s), 148.5 (s), 136.4 (d), 118.6 (q, $J = 321$ Hz, CF_3), 118.0 (d), 116.5 (d), 108.2 (s), 106.8 (s), 25.6 ppm (q, 2×); MS (FAB$^+$): 328 (14, [M^++2]), 327 (100, [M^++1]), 269 (85), 268 (13), 154 (17); HRMS (FAB$^+$): calcd for $C_{11}H_9F_3O_6S$,

327.0150, found: 327.0151; IR (NaCl): $\tilde{\nu}$ = 3000–2800 (w), 1784 (s), 1622 (s) cm^{-1}; Elemental analysis: calcd for $C_{11}H_9F_3O_6S$: C 40.50, H 2.78, found: C 40.53, H 2.75.

2,2-Dimethyl-5-tetradecyl-4*H*-benzo[*d*-1,3]dioxin-4-one (15 c). General procedure for Suzuki coupling: To a solution of tetradec-1-ene **14 c** (0.15 mL, 0.576 mmol) in THF (0.61 mL) was added 9-BBN (1.15 mL, 0.5 M in THF, 0.575 mmol) and the reaction was stirred at 25 °C for 24 h. The mixture was transferred to a flask containing NaOMe (0.031 g, 0.576 mmol) and the solution was stirred for 2 h. A mixture of dichloro-1,1′-bis-(diphenylphosphino)ferrocene palladium (0.012 g, 0.015 mmol), KBr (0.076 g, 0.638 mmol) and 2,2-dimethyl-4-oxo-4*H*-benzo[*d*-1,3]dioxin-5-yl trifluoromethanesulfonate **13** (0.2 g, 0.614 mmol) in THF (4 mL) were added and the reaction was stirred at 70 °C. After 4 h, hexane (1 mL), a 2 M aqueous NaOH solution (1 mL) and H_2O_2 (1 mL, 30% *w/w*) were added and the mixture was stirred at 25 °C. The aqueous mixture was extracted with Et_2O (3×) and the combined organic layers were washed with an aqueous saturated solution of $NaHCO_3$ (3×), dried (Na_2SO_4) and the solvent was evaporated. The residue was purified by chromatography (silica gel, 90:10 hexane/EtOAc) to afford 174 mg (80%) of a yellow oil identified as 2,2-dimethyl-5-tetradecyl-4*H*-benzo[*d*-1,3]dioxin-4-one (**15 c**). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 7.38 (t, J = 7.5 Hz, 1H), 6.92 (d, J = 7.6 Hz, 1H), 6.99 (d, J = 8.0 Hz, 1H,), 3.08 (t, J = 7.7 Hz, 2H), 1.69 (s, 6H), 1.6–1.5 (m, 2H), 1.4–1.2 (m, 22H), 0.87 ppm (t, J = 6.5 Hz, 3H); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 160.2 (s), 157.1(s), 148.5 (s), 135.0 (d), 125.1 (d), 115.0 (d), 112.1 (s), 104.9 (s), 34.3 (t), 31.9 (t), 31.2 (t), 29.8 (t, 3×), 29.7 (t, 3×), 29.6 (t), 29.5 (t), 29.3 (t), 25.6 (q, 2×), 22.7 (t), 14.1 ppm (q); MS (FAB$^+$): 376 (10, [M^++2]), 375 (36, [M^++1]), 373 (11), 318 (23), 317 (100), 316 (14), 161 (11); HRMS (FAB$^+$): calcd for $C_{24}H_{39}O_3$, 375.2899, found: 375.2903; IR (NaCl): $\tilde{\nu}$ = 3000–2800 (br), 1741 (s), 1580 (m), 1540 (m) cm^{-1}.

5-Decyl-2,2-dimethyl-4*H*-benzo[*d*-1,3]dioxin-4-one (15 b): Following the general procedure for Suzuki coupling, the reaction of 2,2-dimethyl-4-oxo-4*H*-benzo[*d*-1,3]dioxin-5-yl trifluoromethanesulfonate **13** (0.2 g, 0.614 mmol) with dec-1-ene **14 b** (0.08 g, 0.576 mmol), 9-BBN (1.15 mL, 0.576), NaOMe (0.031 g, 0.576 mmol), [$PdCl_2$(dppf)] (0.012 g, 0.015 mmol), KBr (0.076 g, 0.638 mmol) in THF (4.61 mL) afforded, after chromatography (silica gel, 90:10 hexane/EtOAc), 90 mg (50%) of a yellow oil identified as 5-decyl-2,2-dimethyl-4*H*-benzo[*d*-1,3]dioxin-4-one (**15 b**). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 7.38 (t, J = 7.9 Hz, 1H), 6.92 (d, J = 7.6 Hz, 1H), 6.79 (d, J = 8.1 Hz, 1H), 3.10 (t, J = 7.5 Hz, 2H), 1.69 (s, 3H), 1.71 (s, 3H), 1.6–1.5 (m, 2H), 1.4–1.2 (m, 14H), 0.87 ppm (t, J = 6.5 Hz, 3H); ^{13}C NMR (100.62 MHz, $CDCl_3$): δ = 160.2 (s), 157.1 (s), 148.5 (s), 135.0 (d), 125.0 (d), 115.0 (d), 112.1 (s), 104.9 (s), 33.3 (t), 31.9 (t), 31.2 (t), 29.7 (t), 29.6 (t, 2×), 29.5 (t), 29.3 (t), 25.6 (q, 2×), 22.6 (t), 14.1 ppm (q); MS (FAB$^+$): 320 (14, [M^++2]), 319 (62, [M^++1]), 318 (11, [M^+]), 317 (23, [M^+−1]), 262 (19), 261 (100), 260 (24); HRMS (FAB$^+$): calcd for $C_{20}H_{30}O_3$, 319.2273, found: 319.2277; IR (NaCl): $\tilde{\nu}$ = 3000–2800 (br), 1740 (s), 1605 (m), 1582 (m) cm^{-1}.

2,2-Dimethyl-5-octyl-4*H*-benzo[*d*-1,3]dioxin-4-one (15 a): Following the general procedure for Suzuki coupling, the reaction of 2,2-dimethyl-4-oxo-4*H*-benzo[*d*-1,3]dioxin-5-yl trifluoromethanesulfonate **13** (0.2 g, 0.614 mmol) with hept-1-ene **14 a** (0.064 g, 0.576 mmol), 9-BBN (1.15 mL, 0.576), NaOMe (0.031 g, 0.576 mmol), [$PdCl_2$(dppf)] (0.012 g, 0.015 mmol), KBr (0.076 g, 0.638 mmol) in THF (4.61 mL) afforded after chromatography (silica gel, 90:10 hexane/EtOAc) 102 mg (61%) of a yellow oil identified as 2,2-dimethyl-5-octyl-4*H*-benzo[*d*-1,3]dioxin-4-one (**15 a**). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 7.38 (t, J = 7.9 Hz, 1H), 6.91 (d, J = 7.5 Hz, 1H), 6.79 (d, J = 8.1 Hz, 1H), 3.08 (t, J = 7.7 Hz, 2H), 1.68 (s, 6H), 1.6–1.5 (m, 2H), 1.4–1.3 (m, 10H), 0.86 ppm (t, J = 6.6 Hz, 3H); ^{13}C NMR (100.62 MHz, $CDCl_3$): 160.1 (s), 157.0 (s), 148.4 (s), 135.0 (d), 125.0 (d), 115.0 (d), 112.0 (s), 104.8 (s), 34.3 (t), 31.8 (t), 31.1 (t), 29.6 (t), 29.4 (t), 29.2 (t), 25.6 (q, 2×), 22.6 (t), 14.0 ppm (q); MS (FAB$^+$): 292 (14, [M^++2]), 291 (69, [M^++1]), 290 (16, [M^+]), 289 (15, [M^+−1]), 234 (17), 233 (100), 232 (38); HRMS (FAB$^+$): calcd for $C_{18}H_{27}O_3$, 291.1960, found: 291.1960; IR (NaCl): $\tilde{\nu}$ = 3000–2800 (s), 1739 (s), 1605 (m), 1582 (m) cm^{-1}.

***N*-(4-Chloro-3-(trifluoromethyl)phenyl)-2-hydroxy-6-tetradecylbenzamide (17 ca)**; **general procedure for amidation**: To a solution of 4-chloro-3-(trifluoromethyl)aniline **16 a** (0.20 g, 1.05 mmol) in THF (4.3 mL) and DMPU (0.25 mL) at 0 °C was added *n*BuLi (0.77 mL, 1.26 M in hexane) and the reaction was stirred for 30 min at 25 °C. After this time, a solution of 2,2-dimethyl-5-tetradecyl-4*H*-benzo[*d*-1,3]dioxin-4-one (**15 c**) (0.08 g, 0.21 mmol) in THF (4.3 mL) was added and the reaction was stirred at 80 °C for 2 h. Water was added and the aqueous mixture was extracted with EtOAc (3×). The combined organic layers were washed with a 10% aqueous HCl solution (1×), water (2×) and brine (1×), dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by chromatography (silica gel, 85:15 hexane/EtOAc) to afford 96 mg (90%) of a yellow oil identified as *N*-(4-chloro-3-(trifluoromethyl)phenyl)-2-hydroxy-6-tetradecylbenzamide (**17 ca**). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 9.68 (bs, 1H), 8.43 (s, 1H), 8.10 (d, J = 8.7 Hz, 1H), 7.64 (d, J = 8.7 Hz, 1H), 7.17 (t, J = 7.8 Hz, 1H), 6.78 (d, J = 8.2 Hz, 2H), 2.85 (bs, 1H), 2.67 (t, J = 7.8 Hz, 2H), 1.6–1.5 (m, 2H), 1.3–1.2 (m, 22H), 0.88 ppm (t, J = 6.5 Hz, 3H); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): δ = 168.6 (s), 155.9 (s), 143.8 (s), 140.9 (d), 133.9 (d), 129.6 (q, $^2J_{C\text{-}F}$ = 31.0 Hz), 126.8 (s), 126.7 (s), 125.6 (d), 124.9 (q, $^1J_{C\text{-}F}$ = 272.5 Hz), 122.5 (d), 120.0 (q, $^3J_{C\text{-}F}$ = 5.7 Hz), 115.1 (d), 34.8 (t), 33.6 (t), 33.1 (t), 31.4 (t), 31.3 (t), 31.2 (t), 31.1 (t), 31.0 (t), 30.8 (t), 30.6 (t), 30.4 (t), 30.2 (t), 24.3 (t), 15.3 ppm (t); MS (FAB$^+$): 515 (10), 514 (36), 513 (34), 512 ([M^++1], 100), 511 (M^+, 11), 510 (12), 318 (10), 317 (43), 179 (14); HRMS (FAB$^+$): calcd for $C_{28}H_{38}ClF_3NO_2$, 512.2543, found: 512.2555; IR (NaCl): $\tilde{\nu}$ = 3000–2800 (br), 1739 (s), 1605 (m), 1582 (m) cm^{-1}.

***N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-6-tetradecylbenzamide (17 cb)**: Following the general procedure for amidation, the reaction of 4-amino-2-(trifluoromethyl)benzonitrile **16 b** (0.12 g, 0.625 mmol) in THF (2.6 mL) and DMPU (0.15 mL) with 5-decyl-2,2-dimethyl-4*H*-benzo[*d*-1,3]dioxin-4-one (**15 c**) (0.04 g, 0.13 mmol) in THF (2.6 mL) afforded after purification by column chromatography (silica gel, 90:10 hexane/EtOAc) 96 mg, (90%) of a white solid identified as *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-6-tetradecylbenzamide (**17 cb**); mp: 118 °C (hexane/acetone). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 8.10 (bs, 1H), 8.05 (s, 1H), 7.97 (d, J = 8.5 Hz, 1H), 7.81 (d, J = 8.5 Hz, 1H), 7.17 (t, J = 8.0 Hz, 1H), 6.84 (d, J = 7.6 Hz, 1H), 6.79 (d, J = 8.2 Hz, 1H), 2.80 (t, J = 7.9 Hz, 2H), 1.7–1.6 (m, 2H), 1.3–1.2 (m, 22H), 0.89 ppm (t, J = 6.6 Hz, 3H); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): δ = 167.6 (s), 154.8 (s), 142.7 (s), 139.9 (s), 132.9 (d), 130.9 (d), 128.6 (q, $^2J_{C\text{-}F}$ = 31.0 Hz), 125.8 (s), 124.6 (d), 123.8 (q, $^1J_{C\text{-}F}$ = 272.0 Hz), 121.5 (d), 118.9 (d), 114.1 (d), 33.8 (t), 32.6 (t), 32.1 (t), 30.4 (t), 30.3 (t), 30.2 (t), 30.1 (t, 2×), 30.0 (t), 29.8 (t), 29.6 (t), 29.4 (t), 29.3 (t), 23.3 (t), 14.3 ppm (q); MS (FAB$^+$): 504 (33, [M^++2]), 503 (100, [M^++1]), 502 (13, [M^+]), 501 (12), 317 (32), 154 (12); HRMS (FAB$^+$): calcd for $C_{29}H_{38}F_3N_2O_2$, 503.2885, found: 503.2885; IR (NaCl): $\tilde{\nu}$ = 3200–3000 (br), 3000–2800 (br), 1649 (s), 1588 (s), 1536 (s) cm^{-1}; Elemental analysis: calcd for $C_{29}H_{37}F_3N_2O_2$: C 69.30, H 7.42; found C, 69.08, H 7.44.

***N*-(4-Chloro-3-(trifluoromethyl)phenyl)-2-decyl-6-hydroxybenzamide (17 ba)**: Following the general procedure for amidation, the reaction of 4-chloro-3-(trifluoromethyl)benzenamine **16 a** (0.12 g,

0.63 mmol) in THF (2.6 mL) and DMPU (0.15 mL) with 5-decyl-2,2-dimethyl-4*H*-benzo[*d*-1,3]dioxin-4-one (**15b**) (0.04 g, 0.12 mmol) in THF (2.6 mL) afforded, after purification by chromatography (silica gel, 90:10 hexane/EtOAc) 54 mg (94%) of a white solid identified as *N*-(4-chloro-3-(trifluoromethyl)phenyl)-2-decyl-6-hydroxybenzamide (**17ba**); mp: 148 °C (hexane/acetone). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 8.61 (s, 1H), 7.88 (s, 1H), 7.81 (d, J = 8.7 Hz, 1H), 7.68 (s, 1H), 7.52 (d, J = 8.7 Hz, 1H), 7.28 (t, J = 7.8 Hz, 1H), 6.85 (m, 2H), 2.83 (t, J = 7.9 Hz, 2H), 1.7–1.6 (m, 2H), 1.4–1.2 (m, 14H), 0.87 ppm (t, J = 6.7 Hz, 3H); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): δ = 167.0 (s), 154.8 (s), 142.7 (s), 139.9 (s), 132.9 (d), 130.9 (d), 128.6 (q, $^2J_{C\text{-}F}$ = 32.3 Hz), 125.8 (s), 125.4 (q, $^1J_{C\text{-}F}$ = 272 Hz), 124.6 (d), 122.5 (s), 121.5 (d), 118.9 (d), 114.1 (d), 33.8 (t), 32.6 (t), 32.1 (t), 30.3 (t), 30.2 (t), 30.1 (t), 30.0 (t), 29.8 (t), 23.3 (t), 14.3 ppm (q); MS (FAB$^+$): 458 (34), 457 (30, [M^++2]), 456 (100, [M^++1]), 455 (15, [M^+]), 261 (44), 154 (18); HRMS (FAB$^+$): calcd for $C_{24}H_{30}ClF_3NO_2$, 456.1917, found: 456.1914; IR (NaCl): $\tilde{\nu}$ = 3200–3000 (br), 3000–2800 (br), 1639 (s), 1591 (s), 1540 (s) cm^{-1}; Elemental analysis: calcd for $C_{24}H_{29}ClF_3NO_2$: C 63.22, H 6.41, found: C 62.34, H 6.38.

***N*-(4-Cyano-3-(trifluoromethyl)phenyl)-2-decyl-6-hydroxybenzamide (17bb)**: Following the general procedure for amidation, the reaction of 4-amino-2-(trifluoromethyl)benzonitrile **16b** (0.12 g, 0.625 mmol) in THF (2.6 mL) and DMPU (0.15 mL) with 5-decyl-2,2-dimethyl-4*H*-benzo[*d*-1,3]dioxin-4-one (**15b**) (40 mg, 0.125 mmol) in THF (2.6 mL) afforded, after purification by chromatography (silica gel, 90:10 hexane/EtOAc), 54 mg (97%) of a white solid identified as *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-decyl-6-hydroxybenzamide (**17bb**); mp: 118 °C (hexane/acetone). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 8.04 (s, 1H), 7.98 (d, J = 8.6 Hz, 1H), 7.83 (d, J = 8.4 Hz, 1H), 7.28 (t, J = 7.8 Hz, 1H), 7.26 (s, 1H), 6.9–6.8 (m, 2H), 2.81 (t, J = 7.7 Hz, 2H), 1.7–1.6 (m, 2H), 1.3–1.2 (m, 14H), 0.87 ppm (t, J = 6.6 Hz, 3H); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): δ = 169.1 (s), 155.9 (s), 145.8 (s), 143.9 (s), 138.1 (d), 134.6 (q, $^2J_{C\text{-}F}$ = 32.3 Hz), 132.2 (d), 126.4 (s), 124.6 (q, $^1J_{C\text{-}F}$ = 272 Hz), 123.8 (d), 122.6 (d), 118.5 (d), 117.4 (s), 115.1 (d), 104.7 (s), 34.7 (t), 33.6 (t), 33.3 (t), 33.1 (t), 31.3 (t), 31.2 (t), 31.0 (t), 30.8 (t), 24.3 (t), 15.3 ppm (q); MS (FAB$^+$): 448 (23, [M^++1]), 447 (83, [M^+]), 446 (20), 445 (10), 307 (25), 289 (15), 281 (11), 261 (40), 166 (11), 155 (33), 154 (100); HRMS (FAB$^+$): calcd for $C_{25}H_{29}F_3N_2O_2$, 447.2259, found: 447.2246; IR (NaCl): $\tilde{\nu}$ = 3200–3000 (br), 3000–2800 (br), 1651 (s), 1588 (s), 1525 (s) cm^{-1}; Elemental analysis: calcd for $C_{25}H_{29}F_3N_2O_2$: C 67.25, H 6.55, found: C 66.90, H 6.54.

***N*-(4-Chloro-3-(trifluoromethyl)phenyl)-2-hydroxy-6-octylbenzamide (17aa)**: Following the general procedure for amidation, the reaction of 4-chloro-3-(trifluoromethyl)aniline **16a** (0.13 g, 0.69 mmol) in THF (2.6 mL) and DMPU (0.17 mL) with 6-octyl-2,2-dimethyl-4*H*-benzo[*d*-1,3]dioxin-4-one (**15a**) (40 mg, 0.138 mmol) in THF (2.6 mL) afforded, after purification by chromatography (silica gel, 90:10 hexane/EtOAc), 51 mg (86%) of a white solid identified as *N*-(4-chloro-3-(trifluoromethyl)phenyl)-2-hydroxy-6-octylbenzamide (**17aa**); mp: 156 °C (hexane/acetone). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 8.62 (s, 1H), 7.88 (s, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.68 (s, 1H), 7.52 (d, J = 8.7 Hz, 1H), 7.28 (t, J = 7.9 Hz, 1H), 6.9–6.8 (m, 2H), 2.83 (t, J = 7.8 Hz, 2H), 1.7–1.6 (m, 2H), 1.3–1.2 (m, 10H), 0.87 ppm (t, J = 6.2 Hz, 3H); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): δ = 167.7 (s), 155.0 (s), 142.9 (s), 140.0 (s), 132.9 (d), 131.0 (d), 128.8 (q, $^2J_{C\text{-}F}$ = 31 Hz), 125.9 (s), 125.8 (s), 124.7 (d), 123.0 (q, $^1J_{C\text{-}F}$ = 272 Hz), 121.6 (d), 119.1 (d), 114.2 (d), 33.9 (t), 32.6 (t), 32.2 (t), 30.2 (t), 30.1 (t), 29.9 (t), 23.3 (t), 14.4 ppm (q). MS: (FAB$^+$): 430 (35), 429 (30, [M^++2]), 428 (100, [M^++1]), 427 (18, [M^+]), 233 (42), 154 (24); HRMS: (FAB$^+$): calcd for $C_{22}H_{26}ClF_3NO_2$, 428.1604, found: 428.1600; IR (NaCl): $\tilde{\nu}$ = 3200–3000 (br), 3000–2800 (br), 1641 (s), 1590 (s), 1541 (s) cm^{-1}; Elemental analysis: calcd for $C_{22}H_{25}ClF_3NO_2$: C 61.75, H 5.89, found: C 61.19, H 5.89.

***N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-6-octylbenzamide (17ab)**: Following the general procedure for amidation, the reaction of 4-amino-2-(trifluoromethyl)benzonitrile **16b** (0.13 g, 0.69 mmol) in THF (2.7 mL) and DMPU (0.17 mL) with 5-octyl-2,2-dimethyl-4*H*-benzo[*d*-1,3]dioxin-4-one (**15a**) (40 mg, 0.125 mmol) in THF (2.7 mL) afforded, after purification by column chromatography (silica gel, 90:10 hexane/EtOAc), 52 mg (87%) of a white solid identified as *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-6-octylbenzamide (**17ab**); mp: 131 °C (hexane/acetone). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 8.04 (s, 1H), 8.0–7.9 (m, 3H), 7.83 (d, J = 8.4 Hz, 1H), 7.29 (t, J = 7.7 Hz, 1H), 6.9–6.8 (m, 2H), 2.81 (t, J = 7.6 Hz, 2H), 1.7–1.6 (m, 2H), 1.4–1.2 (m, 10H), 0.85 ppm (t, J = 6.7 Hz, 3H); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): δ = 167.1 (s), 153.9 (s), 143.8 (s), 141.9 (s), 136.1 (d), 132.7 (q, $^2J_{C\text{-}F}$ = 31 Hz), 130.2 (d), 124.4 (s), 122.6 (q, $^1J_{C\text{-}F}$ = 272 Hz), 121.8 (d), 120.6 (s), 116.6 (d), 115.4 (s), 113.1 (d), 102.7 (d), 32.7 (t), 31.5 (t), 29.0 (t), 28.8 (t), 28.6 (t), 28.4 (t), 22.2 (t), 13.3 ppm (q); MS (FAB$^+$): 420 (24, [M^++2]), 419 (93, [M^++1]), 418 (24, [M^+]), 307 (33), 289 (17), 233 (32), 155 (31), 154 (100); HRMS (FAB$^+$): calcd for $C_{23}H_{26}F_3N_2O_2$, 419.1946, found: 419.1956; IR (NaCl): $\tilde{\nu}$ = 3200–3000 (br), 3000–2800 (br), 1651 (s), 1588 (s), 1535 (s) cm^{-1}; Elemental analysis: calcd for $C_{23}H_{25}F_3N_2O_2$: C 66.02, H 6.02, found: C 65.50, H 6.01.

***N*-(4-Chloro-3-(trifluoromethyl)phenyl)-2-ethoxy-6-tetradecylbenzamide (8ca)**: General procedure for the alkylation of the phenol. To a solution of *N*-(4-chloro-3-(trifluoromethyl)phenyl)-2-hydroxy-6-tetradecylbenzamide (**17ca**) (0.015 g, 0.029 mmol) in acetone (0.9 mL) was added K_2CO_3 (0.01 g, 0.073 mmol) and Et_2SO_4 (0.005 mL, 0.032 mmol) and the reaction was stirred for 3 h. After this time, an aqueous saturated NH_4Cl solution was added (1.5 mL) and the aqueous mixture was extracted with Et_2O (3×). The combined organic layers were washed with brine (2×), dried over Na_2SO_4 and the solvent was evaporated. The residue was purified by chromatography (silica gel, 90:10 hexane/EtOAc) to afford 15 mg (99%) of a yellow oil identified as *N*-(4-chloro-3-(trifluoromethyl)phenyl)-2-ethoxy-6-tetradecylbenzamide (**8ca**). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 9.72 (s, 1H), 8.41 (s, 1H), 8.09 (d, J = 8.7 Hz, 1H), 7.64 (d, J = 8.7 Hz, 1H), 7.29 (t, J = 7.9 Hz, 1H), 6.9–6.8 (m, 2H), 4.06 (q, J = 7.0 Hz, 2H), 2.66 (t, J = 7.7 Hz, 2H), 1.62 (t, J = 7.0 Hz, 2H), 1.6–1.2 (m, 25H), 0.85 ppm (t, J = 6.6 Hz, 3H); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): δ = 168.3 (s), 157.6 (s), 143.6 (s), 140.9 (s), 134.0 (d), 132.0 (d), 129.6 (q, $^2J_{C\text{-}F}$ = 31.0 Hz), 128.7 (s), 126.7 (s), 124.9 (q, $^1J_{C\text{-}F}$ = 272.3 Hz), 123.4 (d), 119.9 (d), 111.5 (d), 65.8 (t), 34.7 (t, 2×), 33.6 (t), 33.1 (t, 2×), 31.4 (t), 31.3 (t, 3×), 31.2 (t, 2×), 31.1 (t), 24.3 (t), 16.1 (q), 15.4 ppm (q); MS (FAB$^+$): 542 (23, [M^++2]), 541 (24, [M^++1]), 540 (67, [M^+]), 539 (12), 538 (17), 346 (25), 345 (100); HRMS (FAB$^+$): calcd for $C_{30}H_{42}ClF_3NO_2$, 540.2856, found: 540.2846; IR (NaCl): $\tilde{\nu}$ = 3200–3000 (br), 3000–2800 (br), 1659 (s), 1592 (s), 1535 (s) cm^{-1}.

***N*-(4-Cyano-3-(trifluoromethyl)phenyl)-2-ethoxy-6-tetradecylbenzamide (8cb)**: Following the general procedure for alkylation, the reaction of *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-6-tetradecylbenzamide (**17cb**) (0.05 g, 0.099 mmol) with K_2CO_3 (0.034 g, 0.248 mmol) and Et_2SO_4 (0.014 mL, 0.109 mmol) in acetone (3.1 mL) afforded, after chromatography (silica gel, 95:5 hexane/EtOAc), 22 mg (42%) of a white solid identified as *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-ethoxy-6-tetradecylbenzamide (**8cb**); mp: 101 °C (hexane/acetone). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 10.05 (s, 1H), 8.51 (s, 1H), 8.27 (d, J = 8.5 Hz, 1H), 8.07 (d, J = 8.5 Hz, 1H), 7.33 (t, J = 8.1 Hz, 1H), 7.0–6.9 (m, 2H), 4.08 (q, J = 7.0 Hz, 2H), 2.68 (t, J = 7.8 Hz, 2H), 1.7–1.6 (m, 2H), 1.3–1.2 (m, 25H), 0.88 ppm (t, J =

6.5 Hz, 3H); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): δ = 168.9 (s), 157.6 (s), 145.8 (s), 143.7 (s), 138.2 (d), 134.7 (q, $^2J_{C\text{-}F}$ = 31 Hz), 132.3 (d), 128.1 (s), 124.6 (q, $^1J_{C\text{-}F}$ = 272 Hz), 123.7 (d), 123.5 (d), 118.5 (d), 117.4 (s), 111.4 (d), 104.8 (s), 65.8 (t), 34.6 (t, 2×), 33.6 (t), 33.1 (t, 2×), 31.5 (t, 3×), 31.4 (t, 2×), 31.3 (t, 2×), 24.3 (t), 16.0 (q), 15.4 ppm (q); MS (FAB$^+$): 532 (21, [M^++2]), 531 (55, [M^++1]), 530 (10, [M^+]), 529 (19), 508 (16), 480 (11), 346 (26), 345 (100), 322 (11), 281 (11), 207 (11); HRMS (FAB$^+$): calcd for $C_{31}H_{42}F_3N_2O_2$, 531.3198, found: 531.3195; IR (NaCl): $\tilde{\nu}$ = 3200–3000 (br), 3000–2800 (br), 1698 (s), 1588 (s), 1524 (s) cm^{-1}; Elemental analysis: calcd for $C_{31}H_{41}F_3N_2O_2$: C 70.16, H 7.79, found: C 69.61, H 7.77.

***N*-(4-Chloro-3-(trifluoromethyl)phenyl)-2-decyl-6-ethoxybenzamide (8ba)**: Following the general procedure for alkylation, the reaction of *N*-(4-chloro-3-(trifluoromethyl)phenyl)-2-hydroxy-6-decylbenzamide (**17ba**) (0.02 g, 0.043 mmol) with K_2CO_3 (0.015 g, 0.108 mmol) and Et_2SO_4 (0.007 mL, 0.048 mmol) in acetone (2.5 mL) afforded, after chromatography (silica gel, 95:5 hexane/EtOAc), 20 mg (91%) of a yellow oil identified as *N*-(4-chloro-3-(trifluoromethyl)phenyl)-2-decyl-6-ethoxybenzamide (**8ba**) ^{1}H NMR (400.13 MHz, $CDCl_3$): δ 9.71 (s, 1H), 8.41 (s, 1H), 8.08 (d, J = 8.7 Hz, 1H), 7.64 (d, J = 8.8 Hz, 1H), 7.29 (t, J = 8.3 Hz, 1H), 6.9–6.8 (m, 2H), 4.05 (q, J = 6.7 Hz, 2H), 2.66 (t, J = 7.8 Hz, 2H), 1.7–1.6 (m, 2H), 1.29 (t, J = 6.9 Hz, 3H), 1.3–1.2 (m, 14H), 0.86 ppm (t, J = 6.8 Hz, 3H); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): δ = 168.3 (s), 157.6 (s), 143.6 (s), 140.9 (s), 134.0 (d), 132.0 (d), 129.7 (q, $^2J_{C\text{-}F}$ = 31 Hz), 128.7 (s), 126.7 (s), 125.6 (d), 124.9 (q, $^2J_{C\text{-}F}$ = 272 Hz), 123.4 (d), 119.9 (d), 111.5 (d), 65.8 (t), 34.7 (t), 33.6 (t), 33.1 (t), 31.3 (t), 31.2 (t), 31.1 (t), 31.0 (t), 30.8 (t), 24.3 (t), 16.1 (q), 15.3 ppm (q); MS (FAB$^+$): 485 (23, [M^++2]), 484 (71, [M^++1]), 483 (10, [M^+]), 482 (12), 290 (21), 289 (100), 154 (15); HRMS (FAB$^+$): calcd for $C_{26}H_{34}ClF_3NO_2$, 484.2230, found: 484.2207; IR (NaCl): $\tilde{\nu}$ = 3200–3000 (br), 3000–2800 (br), 1658 (s), 1592 (s), 1532 (s) cm^{-1}.

***N*-(4-Cyano-3-(trifluoromethyl)phenyl)-2-decyl-6-ethoxybenzamide (8bb)**: Following the general procedure for alkylation, the reaction of *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-6-decylbenzamide (**17bb**) (0.03 g, 0.067 mmol) with K_2CO_3 (0.013 g, 0.168 mmol) and Et_2SO_4 (0.01 mL, 0.074 mmol) in acetone (2.8 mL) afforded, after chromatography (silica gel, 95:5 hexane/EtOAc), 23 mg (72%) of a white solid identified as *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-decyl-6-ethoxybenzamide (**8bb**); mp: 88 °C (hexane/acetone). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 10.06 (s, 1H), 8.51 (s, 1H), 8.26 (d, J = 8.5 Hz, 1H), 8.07 (d, J = 8.5 Hz, 1H), 7.32 (t, J = 7.9 Hz, 1H), 7.0–6.9 (m, 2H), 4.07 (q, J = 7.0 Hz, 2H), 2.66 (t, J = 7.7 Hz, 2H), 1.7–1.6 (m, 2H), 1.29 (t, J = 7.0 Hz, 3H), 1.3–1.2 (m, 14H), 0.86 ppm (t, J = 6.9 Hz, 3H); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): δ = 168.9 (s), 157.6 (s), 145.8 (s), 143.8 (s), 138.2 (d), 134.7 (q, $^2J_{C\text{-}F}$ = 31 Hz), 132.3 (d), 128.2 (s), 124.6 (q, $^1J_{C\text{-}F}$ = 272 Hz), 123.8 (d), 123.5 (d), 118.5 (d), 117.4 (s), 111.5 (d), 104.8 (s), 65.8 (t), 34.6 (t), 33.6 (t), 33.1 (t), 31.3 (t), 31.2 (t), 31.1 (t), 31.0 (t), 30.8 (t), 24.3 (t), 16.0 (q), 15.3 ppm (q); MS (FAB$^+$): 476 (14, [M^++2]), 475 (43, [M^++1]), 473 (13), 322 (13), 290 (22), 289 (100), 219 (17), 154 (13); HRMS (FAB$^+$): calcd for $C_{27}H_{34}F_3N_2O_2$, 475.2572, found: 475.2570; IR (NaCl): $\tilde{\nu}$ = 3200–3000 (br), 3000–2800 (br), 1671 (s), 1587 (s), 1529 (s) cm^{-1}; Elemental analysis: calcd for $C_{27}H_{33}F_3N_2O_2$: C 68.33, H 7.01, found: C 68.06, H 6.97.

***N*-(4-Chloro-3-(trifluoromethyl)phenyl)-2-ethoxy-6-octylbenzamide (8aa)**: Following the general procedure for alkylation, the reaction of *N*-(4-chloro-3-(trifluoromethyl)phenyl)-2-hydroxy-6-octylbenzamide (**17aa**) (0.025 g, 0.099 mmol) with K_2CO_3 (0.02 g, 0.145 mmol) and Et_2SO_4 (0.008 mL, 0.064 mmol) in acetone (2.5 mL) afforded, after chromatography (silica gel, 95:5 hexane/EtOAc), 25 mg (94%) of a yellow oil identified as *N*-(4-chloro-3-(trifluoromethyl)phenyl)-2-ethoxy-6-octylbenzamide (**8aa**). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 9.70 (s, 1H), 8.42 (s, 1H), 8.09 (d, J = 8.7 Hz, 1H), 7.66 (d, J = 8.7 Hz, 1H), 7.31 (t, J = 8.1 Hz, 1H), 7.0–6.9 (m, 2H), 4.07 (q, J = 6.9 Hz, 2H), 2.67 (t, J = 7.8 Hz, 2H), 1.7–1.6 (m, 2H), 1.3–1.2 (m, 13H), 0.85 ppm (t, J = 6.3 Hz, 3H); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): δ = 166.3 (s), 155.6 (s), 141.6 (s), 138.9 (s), 131.9 (d), 129.9 (d), 127.6 (q, $^2J_{C\text{-}F}$ = 31 Hz), 126.7 (s), 124.7 (s), 123.6 (d), 122.9 (q, $^1J_{C\text{-}F}$ = 269 Hz), 121.4 (d), 117.9 (d), 109.5 (d), 63.8 (t), 31.5 (t), 31.08 (t), 29.1 (t), 28.9 (t), 28.8 (t), 28.6 (t), 22.2 (t), 14.0 (q), 13.3 ppm (q); MS (FAB$^+$): 457 (14, [M^++2]), 456 (47, [M^++1]), 262 (20), 261 (100), 154 (18); HRMS (FAB$^+$): calcd for $C_{24}H_{30}ClF_3NO_2$, 456.1917, found: 456.1904; IR (NaCl): $\tilde{\nu}$ = 3200–3000 (br), 3000–2800 (br), 1659 (s), 1591 (s), 1532 (s) cm^{-1}.

***N*-(4-Cyano-3-(trifluoromethyl)phenyl)-2-ethoxy-6-octylbenzamide (8ab)**: Following the general procedure for alkylation, the reaction of *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-hydroxy-6-octylbenzamide (**17ab**) (0.03 g, 0.072 mmol) with K_2CO_3 (0.025 g, 0.18 mmol) and Et_2SO_4 (0.01 mL, 0.08 mmol) in acetone (2.8 mL) afforded, after chromatography (silica gel, 95:5 hexane/EtOAc), 20 mg (62%) of a white solid identified as *N*-(4-cyano-3-(trifluoromethyl)phenyl)-2-ethoxy-6-octylbenzamide (**8ab**); mp: 93 °C (hexane/acetone). ^{1}H NMR (400.13 MHz, $CDCl_3$): δ = 10.06 (s, 1H), 8.51 (s, 1H), 8.26 (d, J = 8.5 Hz, 1H), 8.05 (d, J = 8.5 Hz, 1H), 7.32 (t, J = 7.9 Hz, 1H), 7.0–6.9 (m, 2H), 4.07 (q, J = 7.0 Hz, 2H), 2.66 (t, J = 7.8 Hz, 2H), 1.7–1.6 (m, 2H), 1.29 (t, J = 7.0 Hz, 3H), 1.2–1.1 (m, 10H), 0.83 ppm (t, J = 6.8 Hz, 3H); ^{13}C NMR (100.62 MHz, $(CD_3)_2CO$): δ = 168.9 (s), 157.6 (s), 145.8 (s), 143.7 (s), 138.2 (d), 134.7 (q, $^2J_{C\text{-}F}$ = 31 Hz), 132.3 (d), 128.2 (s), 124.6 (q, $^1J_{C\text{-}F}$ = 272 Hz), 123.8 (d), 123.5 (d), 118.5 (d), 117.4 (s), 111.5 (d), 104.8 (s), 65.8 (t), 34.6 (t), 33.6 (t), 33.1 (t), 31.1 (t), 31.0 (t), 30.8 (t), 24.2 (t), 16.0 (q), 15.3 ppm (q); MS (FAB$^+$): 448 (18, [M^++2]), 447 (53, [M^++1]), 394 (22), 393 (29), 322 (42), 262 (28), 261 (100), 219 (33); HRMS (FAB$^+$): calcd for $C_{25}H_{30}F_3N_2O_2$, 447.2259, found: 447.2265; IR (NaCl): $\tilde{\nu}$ = 3200–3000 (br), 3000–2800 (br), 1671 (s), 1587 (s), 1528 (s) cm^{-1}; Elemental analysis: calcd for $C_{25}H_{29}F_3N_2O_2$: C 67.25, H 6.55, found: C 66.98, H 6.49.

Western blot analysis; histone extraction protocol: Cells were harvested and washed twice with ice-cold PBS 1x. Cells were then lysed in Triton Extraction Buffer (TEB: PBS containing 0.5% Triton X-100 (*v*/*v*), 2 μM phenylmethylsulfonyl fluoride (PMSF), 0.02% (*w*/*v*) NaN_3) at a cellular density of 10^7 cells per mL for 10 min on ice, with gentle stirring. After a brief centrifugation at 2000 rpm at 4 °C, the supernatant was removed and the pellet was washed in half the volume of TEB and centrifuged as before. The pellet was resuspended in 0.2 M HCl at a cell density of 4×10^7cells per mL and acid extraction was maintained overnight at 4 °C on a rolling table. The day after the samples were centrifuged at 2000 rpm for 10 min at 4 °C, the supernatant was removed and its protein content was determined using the Bradford assay.

Immunoblot protocol for detection of histones: About 10 μg of acid-extracted proteins were loaded on 15% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to nitrocellulose. The blotted nitrocellulose was washed twice with water and then incubated in freshly prepared PBS containing 3% nonfat dry milk (PBS-MLK) for one hour at room temperature with constant stirring. The nitrocellulose was incubated with 1:500 dilution of anti acetylH3 (Upstate), anti acetylH4 (Upstate) and anti histone H4 and H3 (Abcam) antibodies in freshly prepared PBS-MLK, overnight at 4 °C with stirring. The nitrocellulose was then washed three times with water and was incubated with the second reagent of choice in PBS-MLK for 1.5 h at room temperature under stirring. The nitrocellulose was washed three times with water, and one time with PBS-

0.05% Tween 20 for 5 min and then rinsed for 4–5 times with water. The ECL method (Amersham) was used for detection.

p300/CBP HAT assay: HAT inhibition assay was performed as recommended by the suppliers (Upstate). Briefly, an indirect ELISA assay was used for the detection of acetyl residues on histone H3 substrate using human recombinant p300 as source of HAT activity. The incubations with DMSO alone (control) or with selected compounds AA, **8bb** and **8ab**, (all at 100 μM) were carried out for 60 min, after pre-incubation of the compounds and enzyme for 15 min. Data is expressed as a percentage of HAT activity. Experiments have been performed in triplicate.

Cell lines and cultures: The U937 cell line was cultured in RPMI with 10% fetal calf serum, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 250 ng mL^{-1} amphotericin-B, 10 mM HEPES and 2 mM glutamine. U937 cells were kept at the constant concentration of 200 000 cells per milliliter of culture medium. MCF-7 cells were cultured in DMEM with 10% fetal calf serum, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 250 ng mL^{-1} amphotericin-B, 10 mM HEPES and 2 mM glutamine. HEK-TE cells (human embryonic kidney cells immortalized by the addition of LT and hTERT) were cultured in αMEM medium with 10% fetal calf serum, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 250 ng mL^{-1} amphotericin-B, 10 mM HEPES and 2 mM glutamine.

Cell cycle analysis on U937 cells: 2.5×10^5 cells were collected and re-suspended in 500 μL of a hypotonic buffer (0.1% Triton X-100, 0.1% sodium citrate, 50 μg mL^{-1} propidium iodide, RNase A). Cells were incubated in the dark for 30 min. Samples were run 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) as previously reported.[19] All the experiments were performed in triplicate.

FACS analysis of apoptosis in U937 cells: Apoptosis was measured by caspase 3 activation detection (B-BRIDGE) as recommended by the suppliers; samples were analyzed by FACS with Cell Quest technology (Becton Dickinson).

Granulocytic differentiation in U937 cells: Granulocytic differentiation was carried out as previously described.[40] Briefly, U937 cells were harvested and re-suspended in 10 μL phycoerythrin-conjugated CD11c (CD11c-PE). Control samples were incubated with 10 μL PE conjugated mouse IgG1, incubated for 30 min at 4 °C in the dark, washed in PBS and re-suspended in 500 μL PBS containing PI (0.25 μg mL^{-1}). Samples were analyzed by FACS with Cell Quest technology (Becton Dickinson). PI-positive cells have been excluded from the analysis.

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