Regular Article

Synthesis and Evaluation of Fatty Acid Amides on the *N*-Oleoylethanolamide-Like Activation of Peroxisome Proliferator Activated Receptor *α*

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A series of fatty acid amides were synthesized and their peroxisome proliferator-activated receptor *α* (PPAR-*α*) agonistic activities were evaluated in a normal rat liver cell line, clone 9. The mRNAs of the PPAR-*α* downstream genes, carnitine-palmitoyltransferase-1 and mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase, were determined by real-time reverse transcription-polymerase chain reaction (RT-PCR) as PPAR-*α* agonistic activities. We prepared nine oleic acid amides. Their PPAR-*α* agonistic activities were, in decreasing order, *N*-oleoylhistamine (OLHA), *N*-oleoylglycine, Oleamide, *N*-oleoyltyramine, *N*-oleoylsertonin, and Olvanil. The highest activity was found with OLHA. We prepared and evaluated nine *N*-acylhistamines (*N*-acyl-HAs). Of these, OLHA, C16:0-HA, and C18:1Δ^9-*trans*-HA showed similar activity. Activity due to the different chain length of the saturated fatty acid peaked at C16:0-HA. The PPAR-*α* antagonist, GW6471, inhibited the induction of the PPAR-*α* downstream genes by OLHA and *N*-oleoylethanolamide (OEA). These data suggest that *N*-acyl-HAs could be considered new PPAR-*α* agonists.

Key words *N*-oleoylhistamine; fatty acid amide; *N*-oleoylethanolamide; peroxisome proliferator-activated receptor-*α*; clone 9 cell

The obesity epidemic continues to spread throughout the world, increasing the need for efficient therapies to combat obesity. We are conducting ongoing investigations into new leads targeting peroxisome proliferator activated receptor *α* (PPAR-*α*). PPAR-*α* is a nuclear receptor and a key regulator of lipid metabolism and energy balance in mammals. Thus, PPAR-*α* agonists such as the ethanolamides of fatty acids[1–5] may have potential as antiobesity or antihyperlipidemic drugs.

The ethanolamides of different long-chain fatty acids constitute a class of naturally occurring lipid molecules that are collectively referred to as *N*-acylethanolamides (NAEs). NAEs exhibit a wide variety of biological activities, depending on their acyl chains, by binding to and activating specific receptors.[5] For example, *N*-palmitoylethanolamide (PEA) was reported to act as an anti-inflammatory and analgesic,[6] and *N*-oleoylethanolamide (OEA) to act as an appetite-suppressant[1]; both actions are believed to be due to PEA, OEA acting on PPAR-*α*.

In this study, fatty acid amides of endogenous fatty acids and various biogenic amines were synthesized and evaluated for their OEA-like activity to PPAR-*α*. To evaluate PPAR-*α* activation, we analyzed the mRNA levels of selected PPAR-*α* downstream genes such as mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (mHMG-CoA Syn) and carnitine-palmitoyltransferase-1 (CPT-1) in Clone 9 rat hepatocyte cells, according to the report[7] that these genes responded similarly against PPAR-*α* agonist in human and rat hepatocyte cells.

Results and Discussion

Chemical We prepared nine oleic acid amides (chemical structures as listed in Fig. 1): OEA, *N*-oleoylhistamine (OLHA), *N*-oleoyltyramine (OLTA), *N*-oleoylserotonine (OL-5-HT), *N*-oleoyldopamine (OLDA), *N*-oleoylputrescine (OLPut), *N*-oleoylspermine (OLSpm), *N*-oleoylglycine (OLGly) and *N*-oleoylvanillylamine (Olvanil). The compounds were synthesized by the condensation of oleoylchloride (**2**), derived from oleic acid (**1**) and oxalyl chloride, with the corresponding biogenic amines: histamine, tyramine, serotonine, dopamine, putrescine, spermine, glycine and vanillylamine, respectively (Chart 1). OLPut and OLSpm were synthesized by the condensation of the oleoylchloride with the Boc-protected polyamines (**3** and **4**) followed by a deprotection step. Satisfactory yields were obtained in all cases.

Evaluation of PPAR-*α* Agonist Activity Clone 9 cells, a normal rat liver cell line, were used for evaluating the candidate compounds as PPAR-*α* agonists. Cell viability of Clone 9 cells, determined by the trypan blue dye exclusion assay, was not reduced during 48 h incubation in the presence of OEA, up to a concentration of 25 μM; however, a 50 μM solution of OEA was cloudy. Incubation of Clone 9 cells with increasing amounts of OEA led to a concentration-dependent increase in mRNA levels of the PPAR-*α* downstream genes, CPT-1 and mHMG-CoA Syn (data not shown). Thus, the agonist activities of the candidate compounds were compared at 25 μM concentration. Under these conditions, *N*-arachidonoylethanolamide (AEA: anandamide) had no effect on the expression of the downstream genes, while PEA provided similar expression levels to OEA (Figs. 2A, B). These results were consistent with those reported previously using another assay system.[1,3] The synthesized oleic acid amides were examined for PPAR-*α* agonist activity by using our method (Figs. 3A, B). OLHA, OLGly, oleamide, OLTA, OL-5-HT, and Olvanil exhibited agonistic activities, with OLHA showing the most potent activity (Fig. 3B). In contrast, OLDA, OLPut, and OLSpm caused cell death (data not shown).

We further prepared nine OLHA analogs (*N*-acylhistamines: *N*-acyl-HAs, Fig. 1) : *N*-octanoylhistamine (C8:0-HA), *N*-caproylhistamine (C10:0-HA), *N*-didecylhitamine (C12:0-HA), *N*-

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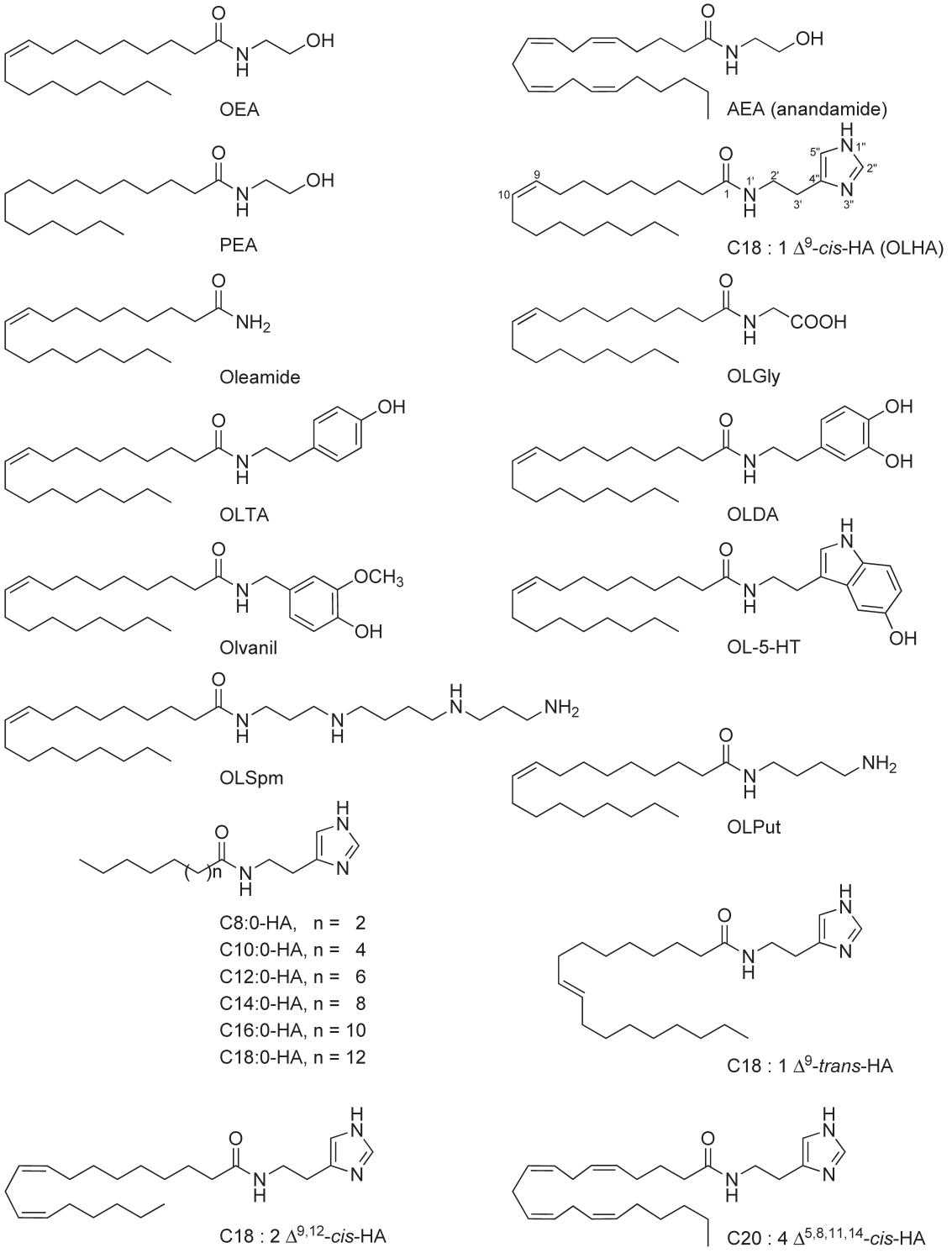
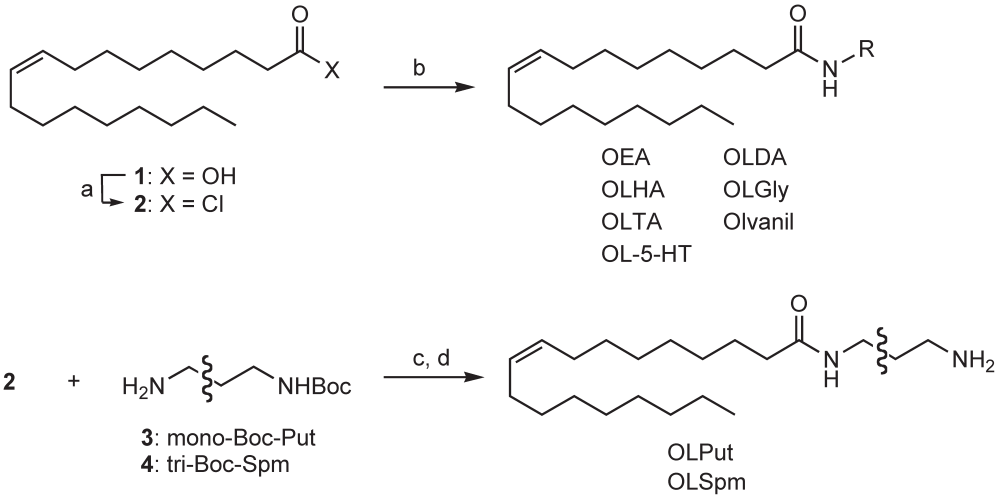


Fig. 1. Chemical Structures of the Fatty Acid Amides Used in This Study

tetradecylhistamine (C14:0-HA), *N*-palmitoylhistamine (C16:0-HA), *N*-stearoylhistamine (C18:0-HA), *N*-elaidoylhistamine (C18:1Δ^9-*trans*-HA), *N*-linoleylhistamine (C18:2$\Delta^{9,12}$-*cis*-HA), and *N*-arachidonoylhistamine (C20:4$\Delta^{5,8,11,14}$-*cis*-HA) and evaluated PPAR-α agonistic activity. Saturated fatty acid histamine amides resulted in the expression of PPAR-α downstream genes, depending on acyl chain length (from C8:0 to C18:0), with the maximum expression occurring in the presence of C16:0 (Figs. 4A, B). Unsaturated fatty acid amides, OLHA and C18:1Δ^9-*trans*-HA, showed a high level of activity, similar to C16:0-HA, whereas the activities of the linoleoyl and arachidonoyl derivatives were weak. These results were consistent with the reports of *N*-acylethanolamides.[1,8] Histamine showed no activity, equivalent to the DMSO blank.

We then examined the effect of a PPAR-α antagonist (GW6471)[9] on the levels of OLHA-induced PPAR-α downstream gene mRNA levels in Clone 9 cells. As shown in Figs. 5A and B, GW6471 significantly and incrementally inhibited the levels of CPT-1A and mHMG-CoA Syn mRNA, indicating competition of OLHA or OEA with GW6471 at PPAR-α.



Reagents and conditions: (a) $(COCl)_2$, CH_2Cl_2; (b) Biogenic amine (RNH_2), base; (c) Et_3N, CH_2Cl_2; (d) TFA and then 2 M HCl–MeOH (1 : 1).

Chart 1. Protocol for the Synthesis of Oleic Acid Amides

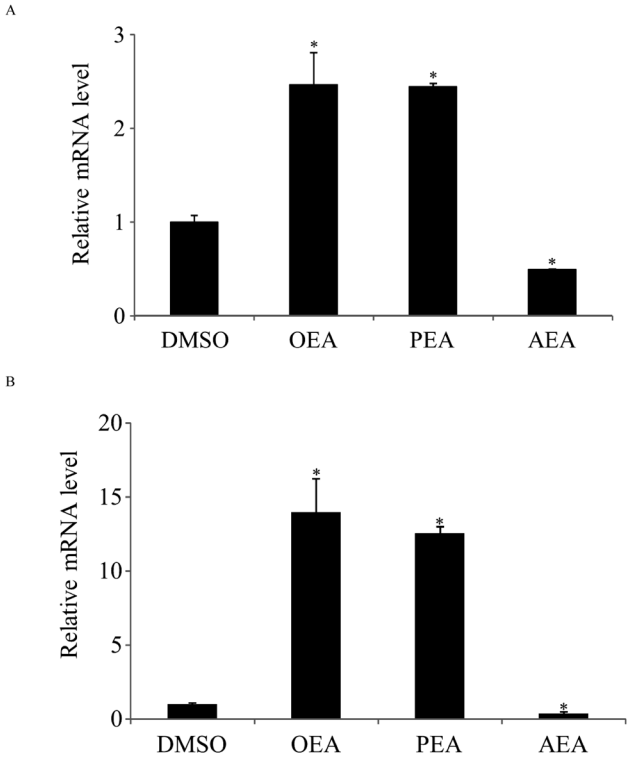


Fig. 2. Effects of NAEs on the mRNA Levels of CPT-1A (A) and mHMG-CoA Syn (B) in Clone 9 Cells

Clone 9 cells were treated with 25 μM NAE. The mRNA levels were determined by real-time RT-PCR analysis using the β-actin mRNA level for normalization. Values are the mean and range calculated by the $\Delta\Delta$Ct method (n=3–6). *p<0.01 was compared with the DMSO control.

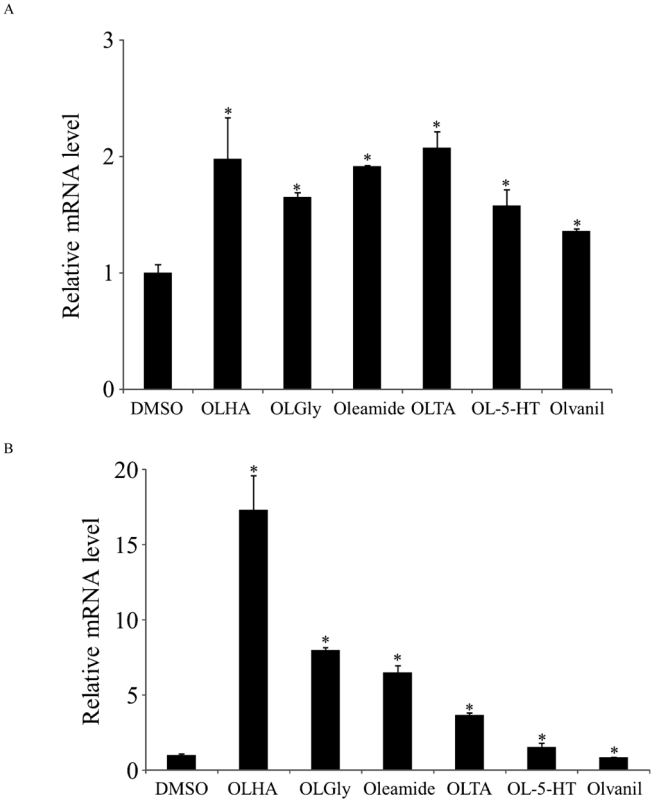


Fig. 3. Effects of Fatty Acid Amides on the mRNA Levels of CPT-1A (A) and mHMG-CoA Syn (B) in Clone 9 Cells

Clone 9 cells were treated with 25 μM fatty acid amide. The mRNA levels were determined by real-time RT-PCR analysis using the β-actin mRNA level for normalization. Values are the mean and range calculated by the $\Delta\Delta$Ct method (n=3–6). *p<0.01 was compared with the DMSO control.

This is the first time to report OLHA has PPAR-α agonistic activity.

This study suggested that *N*-acyl-HAs could be as a new member of the PPAR-α agonist. Many groups are attempting to develop OEA-like compounds to be used as new antiobesity and antihyperlipidemic drugs. *N*-Acyl-HAs, such as OLHA, have potential as a lead compound in these efforts. However, further investigation on another species PPAR-α agonistic activity, as well as proper *in vivo* model needed to evaluate as potential drug.

Experimental

Chemistry All reagents and solvents were purchased from commercial sources. Analytical thin-layer chromatography was performed on silica-coated plates (silica gel 60 F-254, Merck) and visualized under UV light. Column chromatography was carried out using silica gel (Wakogel C-200, Wako Pure Chemical Industries, Ltd., Osaka, Japan). All melting points were determined using a Yanagimoto micro-hot

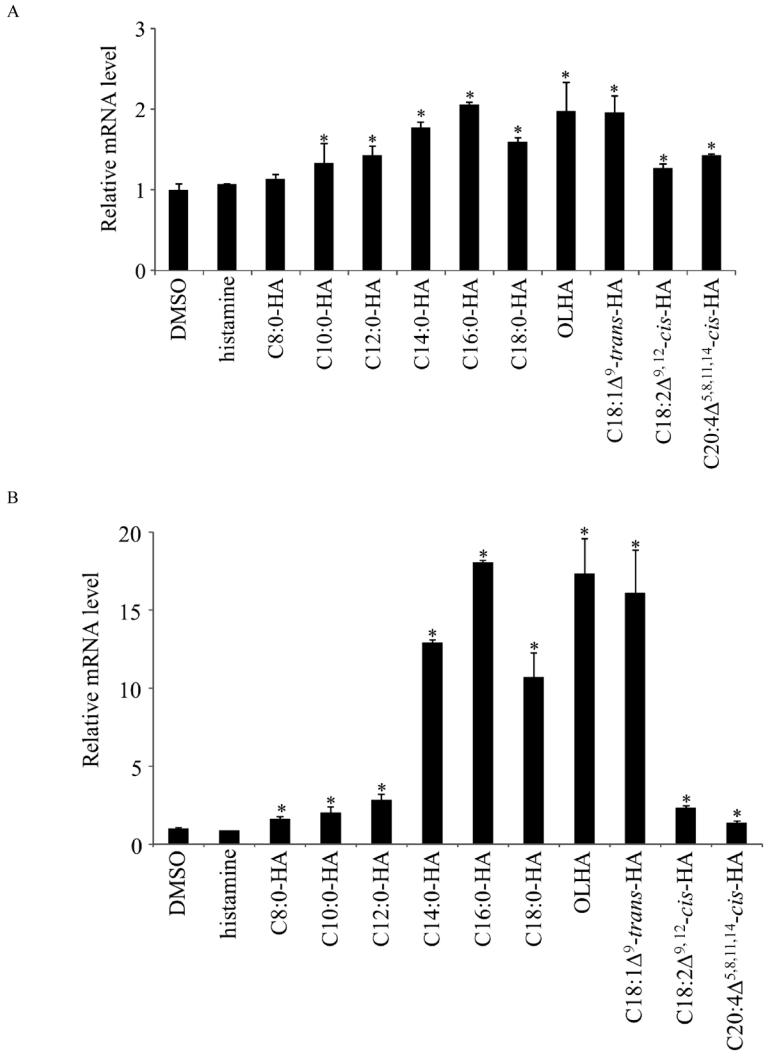


Fig. 4. Effects of *N*-Acylhistamines on the mRNA Levels of CPT-1A (A) and mHMG-CoA Syn (B) in Clone 9 Cells

Clone 9 cells were treated with 25 μM fatty acid histamine amide. The mRNA levels were determined by real-time RT-PCR analysis using the β-actin mRNA level for normalization. Values are the mean and range calculated by the ΔΔCt method (n=3–6). * $p<0.01$ was compared with the DMSO control.

stage and are uncorrected. ^{1}H-NMR spectra were recorded on a Varian 400-MR spectrometer using tetramethylsilane as the internal standard (s=singlet, d=doublet, t=triplet, m=multiplates and br=broad). MS spectra were measured using a JEOL JMS-700 spectrometer.

General Procedure for Preparation of *N*-Acylhistamines (*N*-Acyl-HAs) A solution of fatty acid acyl chloride (1.0 mmol), purchased from Tokyo Chemical Industory (Tokyo, Japan), in *N*,*N*-dimethylformamide (DMF) (2 mL) was added dropwise to a suspension of histamine dihydrochlolide (2.0 mmol) and Et_3N (8 mmol) in DMF (5 mL) cooled in an ice bath. In some cases (*i.e.*, for the C18:1, C18:2 and C20:4 analogues), the acyl chlorides were prepared by reacting the free fatty acids with oxalyl chloride (5 eq, CH_2Cl_2, r.t., 3 h). The reaction mixture was stirred for 5 h at room temperature. Ice-water was added to the mixture and the reaction mix was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH : aq. NH_3=20 : 1 : 0.5) to give the corresponding *N*-acylhistamine.

N-[2-(1*H*-Imidazol-4-yl)ethyl]octanamide (C8:0-HA) Yield 82%; Colorless amorphous; mp 130–132°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.58 (1H, d, J=1.0 Hz, H-2″), 6.82 (1H, d, J=1.0 Hz, H-5″), 6.37 (1H, br s, NH), 3.54 (2H, q, J=6.4 Hz, H-2′), 2.82 (2H, t, J=6.4 Hz, H-3′), 2.17 (2H, t, J=7.6 Hz, H-2), 1.60 (2H, m, H-3), 1.34–1.20 (8H, m, CH_2), 0.87 (3H, t, J=7.0 Hz, H-8); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 173.6 (C, C-1), 134.7 (CH, C-2″), 39.1 (CH_2, C-2′), 36.9 (CH_2, C-2), 31.7 (CH_2), 29.2 (CH_2), 29.0 (CH_2), 27.0 (CH_2, C-3′), 25.9 (CH_2, C-3), 22.6 (CH_2), 14.0 (CH_3, C-18); high resolution-mass spectrum (HR-MS) m/z Calcd for $C_{13}H_{23}N_3O$ (M^+): 237.1841; Found: 237.1833.

N-[2-(1*H*-Imidazol-4-yl)ethyl]decanamide (C10:0-HA) Yield 97%; Colorless amorphous; mp 127–129°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.58 (1H, d, J=1.0 Hz, H-2″), 6.83 (1H, d, J=1.0 Hz, H-5″), 6.35 (1H, br s, NH), 3.55 (2H, q, J=6.4 Hz, H-2′), 2.81 (2H, t, J=6.4 Hz, H-3′), 2.18 (2H, t, J=7.6 Hz, H-2), 1.60 (2H, m, H-3), 1.34–1.20 (12H, m, CH_2), 0.87 (3H, t, J=6.9 Hz, H-10); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 173.6 (C, C-1), 134.7 (CH, C-2″), 39.1 (CH_2, C-2′), 36.9 (CH_2, C-2), 31.8 (CH_2), 29.4 (CH_2), 29.3 (CH_2), 29.3 (2C, CH_2), 27.0 (CH_2, C-3′), 25.8 (CH_2, C-3), 22.7 (CH_2), 14.1 (CH_3, C-18); HR-MS m/z Calcd for $C_{15}H_{27}N_3O$ (M^+): 265.2154; Found: 265.2146.

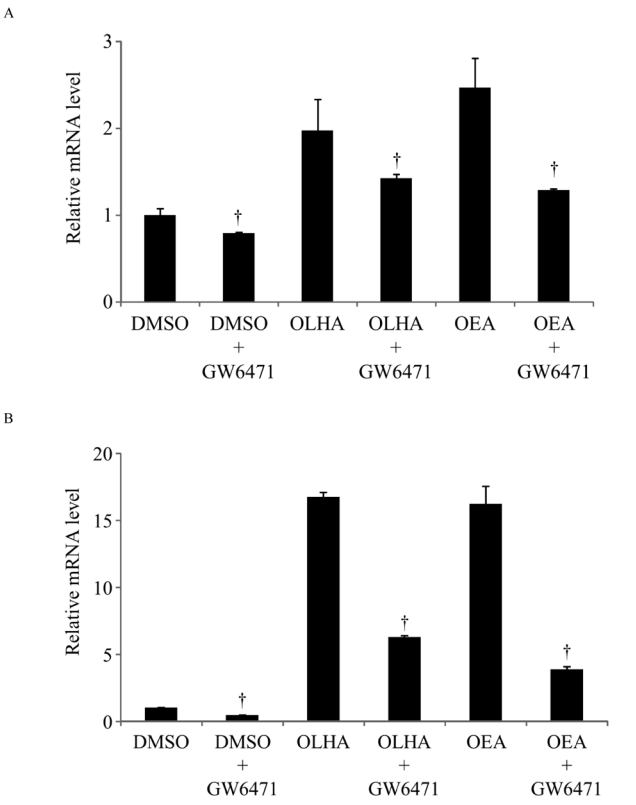


Fig. 5. Effects of GW6471 (PPAR-α Antagonist) on OEA- or OLHA-Induced mRNA Levels of CPT-1A (A) and mHMG-CoA Syn (B) in Clone 9 Cells

Clone 9 cells were treated with 25 μM OEA or OLHA in the absence or presence of 10 μM GW6471. The mRNA levels were determined by real-time RT-PCR analysis using the β-actin mRNA level for normalization. Values are the mean and range calculated by the $\Delta\Delta$Ct method (n=3–6). $^{\dagger}p<0.01$ was compared with GW6471 treated cells and untreated cells.

N-[2-(1*H*-Imidazol-4-yl)ethyl]dodecanamide (C12:0-HA) Yield 71%; Colorless amorphous; mp 118–120°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.58 (1H, d, J=1.0 Hz, H-2″), 6.83 (1H, d, J=1.0 Hz, H-5″), 6.36 (1H, brs, NH), 3.55 (2H, q, J=6.3 Hz, H-2′), 2.82 (2H, t, J=6.3 Hz, H-3′), 2.16 (2H, t, J=7.6 Hz, H-2), 1.60 (2H, m, H-3), 1.34–1.20 (16H, m, CH_2), 0.88 (3H, t, J=6.9 Hz, H-12); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 173.6 (C, C-1), 134.7 (CH, C-2″), 39.1 (CH_2, C-2′), 36.9 (CH_2, C-2), 31.9 (CH_2), 29.60 (CH_2), 29.59 (CH_2), 29.5 (CH_2), 29.34 (CH_2), 29.32 (CH_2), 29.27 (CH_2), 27.0 (CH_2, C-3′), 25.8 (CH_2, C-3), 22.7 (CH_2), 14.1 (CH_3, C-18); HR-MS *m/z* Calcd for $C_{17}H_{31}N_3O$ (M^+): 293.2467; Found: 239.2462.

N-[2-(1*H*-Imidazol-4-yl)ethyl]tetradecanamide (C14:0-HA) Yield 71%; Colorless amorphous; mp 124–126°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.58 (1H, d, J=1.0 Hz, H-2″), 6.83 (1H, d, J=1.0 Hz, H-5″), 6.32 (1H, brs, NH), 3.55 (2H, q, J=6.2 Hz, H-2′), 2.82 (2H, t, J=6.2 Hz, H-3′), 2.16 (2H, t, J=7.6 Hz, H-2), 1.60 (2H, m, H-3), 1.34–1.20 (20H, m, CH_2), 0.88 (3H, t, J=7.0 Hz, H-14); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 173.6 (C, C-1), 134.6 (CH, C-2″), 39.1 (CH_2, C-2′), 36.9 (CH_2, C-2), 31.9 (CH_2), 29.67 (CH_2), 29.64 (2C, CH_2), 29.61 (CH_2), 29.5 (CH_2), 29.4 (2C, CH_2), 29.3 (CH_2), 26.9 (CH_2, C-3′), 25.8 (CH_2), 22.7 (CH_2), 14.1 (CH_3, C-18); HR-MS *m/z* Calcd for $C_{19}H_{35}N_3O$ (M^+): 321.2780; Found: 321.2782.

N-[2-(1*H*-Imidazol-4-yl)ethyl]hexadecanamide (C16:0-HA) Yield 74%; Colorless amorphous; mp 127–129°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.58 (1H, s, H-2″), 6.83 (1H, s, H-5″), 6.32 (1H, brs, NH), 3.55 (2H, q, J=6.3 Hz, H-2′), 2.81 (2H, t, J=6.3 Hz, H-3′), 2.16 (2H, t, J=7.6 Hz, H-2), 1.60 (2H, m, H-3), 1.34–1.20 (24H, m, CH_2), 0.88 (3H, t, J=7.0 Hz, H-16); ^{13}C-NMR ($CDCl_3$-CD_3OD, 100 MHz) δ: 174.3 (C, C-1), 134.6 (CH, C-2″), 39.2 (CH_2, C-2′), 36.6 (CH_2, C-2), 31.9 (CH_2), 29.62 (3C, CH_2), 29.59 (2C, CH_2), 29.57 (CH_2), 29.5 (CH_2), 29.3 (2C, CH_2), 29.2 (CH_2), 26.6 (CH_2, C-3′), 25.7 (CH_2, C-3), 22.6 (CH_2), 14.0 (CH_3, C-18); HR-MS *m/z* Calcd for $C_{21}H_{39}N_3O$ (M^+): 349.3093; Found: 349.3081.

N-[2-(1*H*-Imidazol-4-yl)ethyl]octadecanamide (C18:0-HA) Yield 72%; Colorless amorphous; mp 128–129°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.58 (1H, d, J=1.2 Hz, H-2″), 6.83 (1H, d, J=1.0 Hz, H-5″), 6.32 (1H, brs, NH), 3.55 (2H, q, J=6.3 Hz, H-2′), 2.81 (2H, t, J=6.2 Hz, H-3′), 2.16 (2H, t, J=7.6 Hz, H-2), 1.60 (2H, m, H-3), 1.34–1.20 (28H, m, CH_2), 0.88 (3H, t, J=6.9 Hz, H-18); ^{13}C-NMR ($CDCl_3$-CD_3OD, 100 MHz) δ: 174.3 (C, C-1), 134.7 (CH, C-2″), 39.3 (CH_2, C-2′), 36.6 (CH_2, C-2), 31.9 (CH_2), 29.64 (5C, CH_2), 29.60 (2C, CH_2), 29.58 (CH_2), 29.5 (CH_2), 29.3 (2C, CH_2), 29.2 (CH_2), 26.7 (CH_2, C-3′), 25.7 (CH_2, C-3), 22.6 (CH_2), 14.0 (CH_3, C-18); HR-MS *m/z* Calcd for $C_{23}H_{43}N_3O$ (M^+): 377.3406; Found: 377.3397.

(*Z*)-*N*-[2-(1*H*-Imidazol-4-yl)ethyl]-9-octadecenamide (C18:1-Δ^9-*cis*-HA: OLHA) Yield 84%; Colorless amorphous; mp 93–95°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.58 (1H, d, J=1.0 Hz, H-2″), 6.83 (1H, d, J=1.0 Hz, H-5″), 6.32 (1H, brs, NH), 5.34 (2H, m, H-9, -10), 3.55 (2H, q, J=6.4 Hz, H-2′), 2.82 (2H, t, J=6.4 Hz, H-3′), 2.16 (2H, t, J=7.6 Hz, H-2), 2.01 (4H, m, H-8, -11), 1.61 (2H, m, H-3), 1.38–1.20 (20H, m, CH_2), 0.88 (3H, t, J=7.0 Hz, H-18); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 173.6 (C, C-1), 134.7 (CH, C-2″), 130.0 (CH, C-9 or -10), 129.7 (CH, C-9 or -10), 39.2 (CH_2, C-2′), 36.9 (CH_2, C-2), 31.9 (CH_2), 29.75 (CH_2), 29.70 (CH_2), 29.5 (CH_2), 29.30 (2C, CH_2), 29.26 (CH_2), 29.24 (CH_2), 29.1 (CH_2), 27.21 (CH_2, C-8 or -11), 27.17 (CH_2, C-8 or -11), 26.9 (CH_2, C-3′), 25.8 (CH_2, C-3), 22.7 (CH_2), 14.1 (CH_3, C-18); HR-MS *m/z* Calcd for $C_{23}H_{41}N_3O$ (M^+): 375.3250; Found: 375.3240.

(*E*)-*N*-[2-(1*H*-Imidazol-4-yl)ethyl]-9-octadecenamide (C18:1-Δ^9-*trans*-HA) Yield 88%; Colorless amorphous; mp 117–119°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.58 (1H, d, J=1.0 Hz, H-2″), 6.83 (1H, d, J=1.0 Hz, H-5″), 6.31 (1H, brs, NH), 5.38 (2H, m, H-9, -10), 3.55 (2H, q, J=6.4 Hz, H-2′), 2.81 (2H, t, J=6.4 Hz, H-3′), 2.16 (2H, t, J=7.6 Hz, H-2), 1.95 (4H, m, H-8, -11), 1.60 (2H, m, H-3), 1.38–1.20 (20H, m, CH_2), 0.88 (3H, t, J=7.0 Hz, H-18); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 173.5 (C, C-1), 134.7 (CH, C-2″), 130.4 (CH, C-9 or -10), 130.2 (CH, C-9 or -10), 39.1 (CH_2, C-2′), 36.9 (CH_2, C-2), 32.6 (CH_2), 32.5 (CH_2), 31.9 (CH_2), 29.64 (CH_2), 29.58 (CH_2), 29.47 (CH_2), 29.30 (CH_2), 29.28 (CH_2), 29.22 (CH_2), 29.20 (CH_2), 29.18 (CH_2), 29.0 (CH_2), 27.0, (CH_2, C-3′) 25.7 (CH_2, C-3), 22.7 (CH_2), 14.1 (CH_3, C-18); HR-MS *m/z* Calcd for $C_{23}H_{41}N_3O$ (M^+): 375.3250; Found: 375.3248.

(9*Z*,12*Z*)-*N*-[2-(1*H*-Imidazol-4-yl)ethyl]-9,12-octadecadienamide (C18:2-$\Delta^{9,12}$-*cis*-HA) Yield 94%; pale yellow oily solid; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.58 (1H, d, J=1.0 Hz, H-2″), 6.82 (1H, d, J=1.0 Hz, H-5″), 6.34 (1H, brs, NH), 5.35 (4H, m, H-9, -10, -12, -13), 3.54 (2H, q, J=6.1 Hz, H-2′), 2.82 (2H, t, J=6.1 Hz, H-3′), 2.77 (2H, m, H-11), 2.16 (2H, t, J=7.6 Hz, H-2), 2.05 (4H, m, H-8, -14), 1.60 (2H, m, H-3), 1.40–1.20 (16H, m, CH_2), 0.89 (3H, t, J=7.0 Hz, H-18); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 173.6 (C, C-1), 136.1 (C, br, C-4″), 134.7 (CH, C-2″), 130.2 (CH, C-9 or -10), 130.0 (CH,

C-9 or -10), 128.0 (CH, C-12 or -13), 127.9 (CH, C-12 or -13), 116.1 (CH, br, C-5″), 39.2 (CH_2, C-2′), 36.9 (CH_2, C-2), 31.5 (CH_2), 29.6 (CH_2), 29.3 (CH_2), 29.26 (CH_2), 29.23 (CH_2), 29.1 (CH_2), 27.2 (2C, CH_2, C-8, -14), 26.9 (CH_2, C-3′), 25.7 (CH_2, C-3), 25.6 (CH_2, C-11), 22.6 (CH_2), 14.1 (CH_3, C-18); HR-MS *m/z* Calcd for $C_{23}H_{39}N_3O$ (M^+): 373.3093; Found: 373.3089.

(5*Z*,8*Z*,11*Z*,14*Z*)-*N*-[2-(1*H*-Imidazol-4-yl)ethyl]-5,8,11,14-eicosatetraenamide (C20:4-$\Delta^{5,8,11,14}$-*cis*-HA) Yield 74%; pale yellow oily solid; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.58 (1H, d, *J*=1.0 Hz, H-2″), 6.82 (1H, d, *J*=1.0 Hz, H-5″), 6.37 (1H, brs, NH), 5.37 (8H, m, H-5, -6, -8, -9, -11, -12, -14, -15), 3.54 (2H, q, *J*=6.1 Hz, H-2′), 2.86–2.75 (8H, m, H-7, -10, -13, -3′), 2.18 (2H, t, *J*=7.6 Hz, H-2), 2.14–2.00 (4H, m, H-4, -16), 1.70 (2H, m, H-3), 1.40–1.22 (6H, m, H-17, -18, -19), 0.89 (3H, t, *J*=7.0 Hz, H-20); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 173.4 (C, C-1), 135.5 (C, br, C-4″), 134.6 (CH, C-2″), 130.5, 129.1, 128.7, 128.6, 128.2, 128.1, 127.8, 127.5 (CH, C-5, -6, -8, -9, -11, -12, -14 or -15), 116.1 (CH, br, C-5″), 39.2 (CH_2, C-2′), 36.1 (CH_2, C-2), 31.5 (CH_2), 29.3 (CH_2), 27.2 (CH_2), 26.8 (CH_2), 26.7 (CH_2, C-3′), 25.62 (CH_2, C-3), 25.60 (2C, CH_2), 25.57 (CH_2), 22.6 (CH_2), 14.1 (CH_3, C-18); HR-MS *m/z* Calcd for $C_{25}H_{39}N_3O$ (M^+): 397.3093; Found: 397.3087.

General Procedure for the Preparation of *N*-Acylethanolamides (NAEs) A solution of fatty acid oleoyl chloride (1.0 mmol) in CH_2Cl_2 (2 mL) was added dropwise to a solution of ethanolamine (10 mmol) in CH_2Cl_2 (10 mL) cooled in an ice bath. The reaction mixture was stirred for 1 h at room temperature, then extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography ($CHCl_3$: MeOH=30 : 1) or by recrystallization (AcOEt–hexane) to give *N*-acylethanolamide.

(5*Z*,8*Z*,11*Z*,14*Z*)-*N*-(2-Hydroxyethyl)-5,8,11,14-eicosatetraenamide (AEA) Yield 92%; Colorless oil; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.90 (1H, brs, NH), 5.37 (8H, m, H-5, -6, -8, -9, -11, -12, -14, -15), 3.73 (2H, t, *J*=4.6 Hz, OCH_2), 3.43 (2H, dt, *J*=5.7, 4.6 Hz, NCH_2), 2.86–2.76 (6H, m, H-7, -10, -13), 2.60 (1H, brs, OH), 2.22 (2H, t, *J*=7.6 Hz, H-2), 2.16–2.02 (4H, m, H-4, -16), 1.73 (2H, m, H-3), 1.40–1.22 (6H, m, H-17, -18, -19), 0.88 (3H, t, *J*=7.0 Hz, H-20); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 174.3 (C, C-1), 130.5, 129.0, 128.8, 128.6, 128.2, 128.1, 127.8, 127.5 (CH, C-5, -6, -8, -9, -11, -12, -14 or -15), 62.4 (CH_2, OCH_2), 42.4 (CH_2, NCH_2), 35.9 (CH_2, C-2), 31.5 (CH_2), 29.3 (CH_2), 27.2 (CH_2), 26.6 (CH_2), 25.6 (2C, CH_2), 25.5 (CH_2), 22.6 (CH_2), 14.1 (CH_3, C-18); HR-MS *m/z* Calcd for $C_{22}H_{37}NO_2$ (M^+): 347.2824; Found: 347.2824. The ^{1}H-NMR spectrum was similar to that previously reported.[10]

(*Z*)-*N*-(2-Hydroxyethyl)-9-octadecenamide (OEA) Yield 96%; Colorless amorphous; mp 64–65°C (lit. 75–76°C[10]); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.92 (1H, brs, NH), 5.34 (2H, m, H-9, -10), 3.73 (2H, q, *J*=4.6 Hz, OCH_2), 3.43 (2H, dt, *J*=5.6, 4.6 Hz, NCH_2), 2.66 (1H, m, OH), 2.21 (2H, t, *J*=7.6 Hz, H-2), 2.01 (4H, m, H-8, -11), 1.68–1.58 (4H, m, CH_2), 1.38–1.20 (18H, m, CH_2), 0.88 (3H, t, *J*=7.0 Hz, H-18); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 174.6 (C, C-1), 130.0 (CH, C-9 or -10), 129.7 (CH, C-9 or -10), 62.4 (CH_2, OCH_2), 42.4 (CH_2, NCH_2), 36.7 (CH_2, C-2), 31.9 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.3 (2C, CH_2), 29.2 (2C, CH_2), 29.1 (CH_2), 27.2 (CH_2, C-8 or -11), 27.1 (CH_2, C-8 or -11), 25.7 (CH_2, C-3), 22.7 (CH_2), 14.1 (CH_3, C-18); HR-MS *m/z* Calcd for $C_{20}H_{39}NO_2$ (M^+): 325.2981; Found: 325.2972. The ^{1}H-NMR spectrum was similar to that previously reported.[10]

N-(2-Hydroxyethyl)hexadecanamide (PEA) Yield 95%; Colorless amorphous; mp 102–103°C (lit. 99–100°C[10]); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.94 (1H, brs, NH), 3.73 (2H, q, *J*=4.7 Hz, OCH_2), 3.43 (2H, dt, *J*=5.7, 4.7 Hz, NCH_2), 2.73 (1H, m, OH), 2.20 (2H, t, *J*=7.6 Hz, H-2), 1.68–1.58 (4H, m, CH_2), 1.35–1.20 (22H, m, CH_2), 0.88 (3H, t, *J*=7.0 Hz, H-16); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 174.6, (C, C-1), 62.6 (CH_2, OCH_2), 42.5 (CH_2, NCH_2), 36.7 (CH_2, C-2), 31.9 (CH_2), 29.68 (2C, CH_2), 29.66 (CH_2), 29.64 (2C, CH_2), 29.61 (CH_2), 29.5 (CH_2), 29.34 (2C, CH_2), 29.27 (CH_2), 25.7 (CH_2), 22.7 (CH_2), 14.1 (CH_3, C-18); HR-MS *m/z* Calcd for $C_{18}H_{37}NO_2$ (M^+): 299.2824; Found: 299.2836. The ^{1}H-NMR spectrum was similar to that previously reported.[10]

Synthesis of Oleic Acid Amides (OLDA, OLTA, OL-5-HT and Olvanil) According to the general procedure for the preparation of *N*-acylhistamines, oleoyl chloride and the corresponding amine (1.2 eq) were treated with Et_3N (4 eq), and the crude product was purified by silica gel column chromatography (hexane–AcOEt) to give the corresponding oleic acid amide.

(*Z*)-*N*-[2-(3,4-Dihydroxyphenyl)ethyl]-9-octadecenamide (OLDA) Yield 97%; Colorless amorphous; mp 58–61°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.66 (1H, brs, OH), 6.81 (1H, d, *J*=8.0 Hz, H-5″), 6.75 (1H, d, *J*=2.0 Hz, H-2″), 6.57 (1H, dd, *J*=8.0, 2.0 Hz, H-6″), 6.02 (1H, brs, OH), 5.63 (1H, brt, *J*=5.8 Hz, NH), 5.34 (2H, m, H-9, -10), 3.48 (2H, td, *J*=7.1, 5.8 Hz, H-2′), 2.70 (2H, t, *J*=7.1 Hz, H-3′), 2.15 (2H, t, *J*=7.6 Hz, H-2), 1.99 (4H, m, H-8, -11), 1.58 (2H, m, H-3), 1.38–1.20 (20H, m, CH_2), 0.88 (3H, t, *J*=6.9 Hz, H-18); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 174.5, (C, C-1), 144.3 (C, C-3″ or -4″), 143.2 (C, C-3″ or -4″), 130.4 (C, C-1″), 130.0 (CH, C-9 or -10), 129.7 (CH, C-9 or -10), 120.4 (CH, C-6″), 115.3 (CH, C-2″ or -5″), 115.1 (CH, C-2″ or -5″), 41.0 (CH_2, C-2′), 36.8 (CH_2, C-2), 34.9 (CH_2, C-3′), 31.9 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.4 (CH_2), 29.32 (CH_2), 29.31 (CH_2), 29.19 (CH_2), 29.15 (CH_2), 29.10 (CH_2), 27.2 (CH_2, C-8 or -11), 27.1 (CH_2, C-8 or -11), 25.7 (CH_2, C-3), 22.7 (CH_2), 14.1 (CH_3, C-18); HR-MS *m/z* Calcd for $C_{26}H_{43}NO_3$ (M^+): 417.3234; Found: 417.3230.

(*Z*)-*N*-[2-(4-Hydroxyphenyl)ethyl]-9-octadecenamide (OLTA) Yield 90%; Colorless amorphous; mp 72–74°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.03 (2H, d, *J*=8.4 Hz, H-2″, -6″), 6.78 (2H, d, *J*=8.4 Hz, H-3″, -5″), 5.60 (1H, brs, OH), 5.44 (1H, brt, *J*=5.9 Hz, NH), 5.34 (2H, m, H-9, -10), 3.48 (2H, td, *J*=6.9, 5.9 Hz, H-2′), 2.74 (2H, t, *J*=6.9 Hz, H-3′), 2.12 (2H, t, *J*=7.6 Hz, H-2), 2.00 (4H, m, H-8, -11), 1.58 (2H, m, H-3), 1.38–1.20 (20H, m, CH_2), 0.88 (3H, t, *J*=7.0 Hz, H-18); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 173.7 (C, C-1), 155.0 (C, C-4″), 130.0 (CH, C-9 or -10), 129.73 (CH, C-9 or -10), 129.70 (C, C-1″), 129.70 (CH, C-2″, -6″), 115.6 (CH, C-3″, -5″), 40.8 (CH_2, C-2′), 36.8 (CH_2, C-2), 34.8 (CH_2, C-3′), 31.9 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.3 (CH_2), 29.22 (CH_2), 29.20 (CH_2), 29.1 (CH_2), 27.2 (CH_2, C-8 or -11), 27.1 (CH_2, C-8 or -11), 25.7 (CH_2, C-3), 22.7 (CH_2), 14.1 (CH_3, C-18); HR-MS *m/z* Calcd for $C_{26}H_{43}NO_2$ (M^+): 401.3294; Found: 401.3292.

(*Z*)-*N*-[2-(5-Hydroxy-1*H*-indol-3-yl)ethyl]-9-octadecenamide (OL-5-HT) Yield 82%; Colorless amorphous; mp 80–82°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 7.93 (1H, brs, OH), 7.23 (1H, d, *J*=8.7 Hz, H-7″), 7.04 (1H, d, *J*=2.4 Hz, H-4″), 7.00 (1H, d, *J*=2.3 Hz, H-2″), 6.80 (1H, dd, *J*=8.7, 2.4 Hz, H-6″), 5.56

(1H, br t, J=5.7 Hz, NH), 5.34 (2H, m, H-9, -10), 3.58 (2H, td, J=6.8, 5.7 Hz, H-2′), 2.90 (2H, t, J=6.8 Hz, H-3′), 2.12 (2H, t, J=7.6 Hz, H-2), 2.00 (4H, m, H-8, -11), 1.58 (2H, m, H-3), 1.38–120 (20H, m, CH_2), 0.88 (3H, t, J=6.9 Hz, H-18); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 173.8, (C, C-1), 150.1 (C, C-5″), 131.4 (C, C-3a″), 130.0 (CH, C-9 or -10), 129.8 (CH, C-9 or -10), 128.0 (C, C-7a″), 123.0 (CH, C-2″), 112.3 (CH, C-4″ or -7″), 112.1 (C, C-3″), 111.9 (CH, C-4″ or -7″), 103.2 (CH, C-6″), 39.7 (CH_2, C-2′), 36.8 (CH_2, C-2), 31.9 (CH_2), 29.76 (CH_2), 29.71 (CH_2), 29.5 (CH_2), 29.32 (CH_2), 29.31 (CH_2), 29.25 (2C, CH_2), 29.15 (CH_2), 27.22 (CH_2, C-8 or -11), 27.18 (CH_2, C-8 or -11), 25.8 (CH_2, C-3), 25.4 (CH_2, C-3′), 22.7 (CH_2), 14.1 (CH_3, C-18); HR-MS m/z Calcd for $C_{28}H_{44}N_2O_2$ (M^+): 440.3403; Found: 440.3388.

(*Z*)-*N*-[2-(4-Hydroxy-3-methoxyphenyl)methyl]-9-octadecenamide (Olvanil) Yield 89%; Colorless amorphous; mp 42–43°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.87 (1H, d, J=8.0 Hz, H-5″), 6.82 (1H, d, J=1.9 Hz, H-2″), 6.77 (1H, dd, J=8.0, 1.9 Hz, H-6″), 5.64 (1H, brs, NH), 5.60 (1H, brs, OH), 5.34 (2H, m, H-9, -10), 4.36 (2H, d, J=5.6 Hz, NCH_2), 2.19 (2H, t, J=7.6 Hz, H-2), 2.00 (4H, m, H-8, -11), 1.66 (2H, m, H-3), 1.38–1.20 (20H, m, CH_2), 0.88 (3H, t, J=7.0 Hz, H-18); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 172.9, (C, C-1), 146.7 (C, C-3″ or -4″), 145.1 (C, C-3″ or -4″), 130.3 (C, C-1″), 130.0 (CH, C-9 or -10), 129.7 (CH, C-9 or -10), 120.8 (CH, C-6″), 114.4 (CH, C-5″), 110.7 (CH, C-2″), 55.9 (CH_3, OMe), 43.5 (CH_2, C-2′), 36.8 (CH_2, C-2), 31.9 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.31 (CH_2), 29.28 (CH_2), 29.25 (CH_2), 29.1 (CH_2), 27.2 (CH_2, C-8 or -11), 27.1 (CH_2, C-8 or -11), 25.8 (CH_2, C-3), 22.7 (CH_2), 14.1 (CH_3, C-18); HR-MS m/z Calcd for $C_{26}H_{43}NO_3$ (M^+): 417.3243; Found: 417.3241.

Synthesis of *N*-[(9*Z*)-1-Oxo-9-octadecenyl]glycine (OLGly) Oleoyl chloride (2.0 mmol) was added dropwise to an aqueous solution containing glycine sodium salt. The solution was kept between pH 9–12.5 by the simultaneous addition of a 10% aqueous NaOH solution, and the temperature was maintained below 35°C. After stirring for 1 h, the solution was acidified with 30% H_2SO_4 to below pH 4.5 and extracted with AcOEt. The organic layer was washed with water and then dried over Na_2SO_4. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : AcOEt=1 : 1) to give the title compound in 57% yield.

Colorless amorphous; mp 93–94°C (lit. 92–93°C[11]); ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 6.15 (1H, brt, J=5.2 Hz, NH), 5.35 (2H, m, H-9, -10), 4.08 (2H, d, J=5.2 Hz, NCH_2), 2.27 (2H, t, J=7.6 Hz, H-2), 2.01 (4H, m, H-8, H-11), 1.63 (2H, m, CH_2), 1.38–1.20 (20H, m, CH_2), 0.88 (3H, t, J=6.9 Hz, H-18); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 174.7, (C, C=O), 172.7 (C, C=O), 130.0 (CH, C-9 or -10), 129.7 (CH, C-9 or -10), 41.5 (CH_2, NCH_2), 36.3 (CH_2, C-2), 31.9 (CH_2), 29.8 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.3 (2C, CH_2), 29.2 (CH_2), 29.16 (CH_2), 29.11 (CH_2), 27.2 (CH_2, C-8 or -11), 27.1 (CH_2, C-8 or -11), 25.5 (CH_2, C-3), 22.7 (CH_2), 14.1 (CH_3, C-18); HR-MS m/z Calcd for $C_{20}H_{37}NO_3$ (M^+): 339.2773; Found: 339.2758. The ^{1}H- and ^{13}C-NMR spectra were similar to that previously reported.[11]

Procedure for Preparation of OLSPm and OLPut A solution of fatty acid oleoyl chloride (2.4 mmol) in CH_2Cl_2 (2 mL) was added dropwise to a solution of Boc-protected polyamine[12,13] (2.0 mmol) and Et_3N (8 mmol) in CH_2Cl_2 (10 mL) cooled in an ice bath. The reaction mixture was stirred for 3 h at room temperature. Ice-water was added to the mixture and the mixture was extracted with $CHCl_3$. The organic layer was dried over Na_2SO_4 and the solvent was evaporated under reduced pressure. The residue was passed once through a short silica gel column (hexane : AcOEt=2 : 1) and the solvent was evaporated. The residue was treated with TFA followed by 2 M HCl–MeOH (1 : 1) to give the corresponding crude oleic acid amide hydrochloride salt.

(*Z*)-*N*-(4-Aminobutyl)-9-octadecenamide (OLPut) The crude compound obtained by general procedure was purified by silica gel colum chromatography ($CHCl_3$–MeOH–aq. NH_3=20 : 1 : 0.5) to give the title compound.

Yield 68% (for 2 steps); Colorless solid; mp 90–92°C; ^{1}H-NMR ($CDCl_3$, 400 MHz) δ: 5.82 (1H, brs, NH), 5.34 (2H, m, H-9, -10), 3.26 (2H, q, J=6.4 Hz, NCH_2), 2.72 (2H, t, J=6.7 Hz, NCH_2), 2.15 (2H, t, J=7.6 Hz, H-2), 2.00 (4H, m, H-8 and H-11), 1.65–1.45 (6H, m, CH_2), 1.38–1.20 (20H, m, CH_2), 0.88 (3H, t, J=7.0 Hz, H-18); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 173.1 (C, C-1), 130.0 (CH, C-9 or -10), 129.7 (CH, C-9 or -10), 41.6 (CH_2, NH_2CH_2), 39.2 (CH_2, $NHCH_2$), 36.9 (CH_2, C-2), 31.9 (CH_2), 30.6 (CH_2), 29.7 (CH_2), 29.5 (CH_2), 29.3 (3C, CH_2), 29.2 (CH_2), 29.17 (CH_2), 29.13 (CH_2), 27.2 (CH_2, C-8 or -11), 27.1 (CH_2, C-8 or -11), 27.0 (CH_2), 25.8 (CH_2, C-3), 22.7 (CH_2), 14.1 (CH_3, C-18); HR-MS m/z Calcd for $C_{22}H_{44}N_2O$ (M^+): 352.3454; Found: 352.3447.

(*Z*)-*N*-[3-[[4-[(3-Aminopropyl)amino]butyl]amino]propyl]-9-octadecenamide (OLSPm) The crude compound obtained by general procedure was recrystallized from aqueous MeOH to give the title compound (trihydrochloride salt).

Yield 64% (for 2 steps); Colorless amorphous; ^{1}H-NMR (D_2O, 400 MHz) δ: 5.21 (2H, m, H-9, -10), 3.12 (2H, t, J=6.7 Hz, NCH_2), 3.08–2.90 (10H, m, NCH_2), 2.10 (2H, t, J=7.6 Hz, H-2), 2.00–1.62 (12H, m, CH_2), 1.42 (2H, m, CH_2), 1.24–1.05 (20H, m, CH_2), 0.72 (3H, t, J=7.0 Hz, H-18); ^{13}C-NMR (D_2O, 100 MHz) δ: 176.0 (C, C-1), 129.8 (CH, C-9 or -10), 129.4 (CH, C-9 or -10), 47.1 (CH_2), 45.4 (CH_2), 44.6 (CH_2), 36.5 (CH_2), 36.2 (CH_2), 35.9 (CH_2), 31.8 (CH_2), 29.69 (CH_2), 29.67 (CH_2), 29.5 (CH_2), 29.27 (CH_2), 29.23 (CH_2), 29.1 (CH_2), 27.2 (CH_2), 27.1 (CH_2), 25.8 (CH_2), 25.6 (CH_2), 23.8 (CH_2), 23.0 (CH_2), 22.9 (CH_2), 22.5 (CH_2), 13.8 (CH_2, C-18); HR-MS m/z Calcd for $C_{28}H_{58}N_4O$ (M^+): 466.4611; Found: 466.4621. The ^{1}H-NMR spectrum was similar to that previously reported.[12]

Evaluation of PPAR-α Agonist Activity Clone 9 cells and Ham's F12 medium were purchased from DS Pharma Biomedical. Penicillin–streptomycin stabilized solution, trypsin, ethylenediamine tetraacetic acid disodium salt, oleic acid, and GW6471 were purchased from Sigma-Aldrich Japan. Oleamide was purchased from KANTO Chemicals. Fetal bovine serum was purchased from Nichirei Bioscience. An RNeasy Mini Kit was purchased from QIAGEN. A Prime Script RT Reagent Kit (Perfect Real Time), SYBR Premix Ex Taq (Tli RNaseH Plus), and loading buffer were purchased from TaKaRa Bio (Shiga, Japan). All other reagents were of research grade.

Cell Culture and Treatment of the Tested Compounds Clone-9 rat hepatocytes were routinely cultured in Ham's F12 medium in 10 cm dishes at 37°C and 5% CO_2 in the presence of 1% penicillin/streptomycin with 10% fetal bovine serum. The cells used for experiments were passages 25–55. For the experiments, the Clone 9 cells (10^5 cells/10 cm dish) were cul-

tured for 72 h, then the sub-confluent cells were incubated in serum-free medium at 37°C for 24 h before the addition of the test compounds. For treatment with GW6471, a PPAR-α antagonist,[9] 10 μM GW6471 was added with the tested compounds. After 48 h, the treated cells were harvested, counted, and used for total RNA extraction. Oleoylethanolamide and the test compounds were dissolved in DMSO. The final concentration of DMSO in both the control and treatment medium was identical in all studies, with a maximum level of 1% (v/v).[14]

Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) Analysis The total RNA from the Clone 9 cells was isolated using an RNeasy Mini Prep Kit, according to the manufacturer's protocol. The mRNA expression of genes was measured using an ABI PRISM 7500 Real-Time PCR system, a Prime Script RT Reagent Kit (Perfect Real Time), and SYBR Premix Ex Taq (Tli RNaseH Plus). Relative mRNA expression was calculated using the $\Delta\Delta$Ct method.[15] The results were expressed as mean and range. The housekeeping gene, β-actin, was used for normalization. The primers, CPT-1 (forward primer: 5′-CGC TCA TGG TCA ACA GCA ACT AC-3′, reverse primer: 5′-TCA CGG TCT AAT GTG CGA CGA-3′), mHMG-CoA Syn (forward primer: 5′-CAC TTG GTA CCT TGA ACG AGT GGA-3′, reverse primer: 5′-CCG TTT GGG ATT CGG CTC TG-3′), β-actin (forward primer: 5′-TGA CAG GAT GCA GAA GGA GA-3′, and reverse primer: 5′-TAG AGC CAC CAA TCC ACA CA-3′) used in the experiments were purchased from TaKaRa Bio.

Statistical Analysis The statistical significance of data was determined by the Student's *t*-test. A *p*-value less than 0.01 was considered significant.

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Conflict of Interest The authors declare no conflict of interest.

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