Supporting Information

Identification of a New Type of Covalent PPARγ Agonist using a Ligand-Linking Strategy

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1. Identification and structural elucidation of compounds 1 and 2

General remarks. Organic solvents for fractionation were purchased from Nacalai Tesque. Flash column chromatography was performed using wako gel C-200 (Wako Pure Chemical Industries, Osaka, Japan) and Parallel FR-360 (Yamazen Corporation, Osaka, Japan). The following spectroscopic and analytical instruments were used: ^{1}H and ^{13}C NMR, Avance III 400 (reference TMS, Bruker, Germany), HR-ESI-TOF-MS, Waters Xevo G2-S QTof (Waters, Tokyo, Japan). All other chemicals and reagents were purchased from chemical companies and used without further purification.

Extraction, purification and identification of (*E*)-ethyl 3-(4-methoxyphenyl)acrylate (1) and (*E*)-ethyl 3-(3,4-dimethoxyphenyl)acrylate (2). Dried aerial parts (2 kg) of *Kaempferia galanga* were extracted with MeOH (10 L) for one week at room temperature. After filtration, the filtrate was evaporated to dryness *in vacuo* at 37 ºC to afford the MeOH extract (134.4 g). The MeOH extract (126.2 g) of *K. galanga* was partitioned between EtOAc (1.5 L) and H_2O (1.5 L). The EtOAc soluble portion (75 g) was subjected to silica gel column chromatography (ϕ50 x 500 mm, Hexane/EtOAc/TFA, 100:0:5 → 50:50:5 → Hexane/2-propanol, 50:50) to afford seven fractions (A1 ~ A7). A2 fraction (56 g) was recrystallized from Hexane/EtOAc to give (*E*)-ethyl 3-(4-methoxyphenyl)acrylate (**1**, 10 g). A4 fraction (3.9 g) was separated into eight fractions (B1 ~ B8) using silica gel column chromatography (ϕ20 x 500 mm, Hexane/EtOAc/TFA, 6:6:0.5). B2 fraction (1.2 g) was fractionated into nine fractions (D1 ~ D9) using ODS column chromatography (ϕ10 x 300, CH_3CN/H_2O/AcOH, 10:90:5 → 100:0:5). D7 fraction was purified by HPLC with ODS-gel column (Inertsil ODS3, 10 x 250 mm, CH_3CN/H_2O, 60:40) to give (*E*)-ethyl 3-(3,4-dimethoxyphenyl)acrylate (**2**, 15.4 mg). ^{1}H and ^{13}C NMR spectra of (*E*)-ethyl 3-(4-methoxyphenyl)acrylate and (*E*)-ethyl 3-(3,4-dimethoxyphenyl)acrylate were identical to those previously reported. (*E*)-ethyl 3-(4-methoxyphenyl)acrylate (**1**): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.33 (3H, t, *J* = 7.1 Hz), 3.83 (3H, s), 4.25 (2H, q, *J* = 7.1 Hz), 6.31 (1H, d, *J* = 16 Hz), 6.90 (2H, dd, *J* = 2.9, 8.8 Hz), 7.47 (2H, dd, *J* = 2.9, 8.8 Hz), 7.64 (1H, d, *J* = 16 Hz) ppm. ^{13}C NMR ($CDCl_3$, 400 MHz): δ 14.3, 55.3, 60.3, 114.3 (2C), 115.7, 127.1, 129.6 (2C), 144.2, 161.3, 167.3 ppm. (*E*)-ethyl 3-(3,4-dimethoxyphenyl)acrylate (**2**): ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.34 (3H, t,

J = 7.1 Hz), 3.92 (6H, s), 4.26 (2H, q, *J* = 7.1 Hz), 6.32 (1H, d, *J* = 15.9 Hz), 6.87 (1H, d, *J* = 8.3 Hz), 7.06 (1H, d, *J* = 1.9 Hz), 7.11 (1H, dd, *J* = 1.9, 8.3 Hz), 7.64 (1H, d, *J* = 15.9 Hz) ppm. ^{13}C NMR ($CDCl_3$, 400 MHz): δ 14.3, 55.8, 55.9, 60.3, 109.6, 111.0, 115.9, 122.5, 127.4, 144.5, 149.2, 151.0, 167.2 ppm.

2. Synthetic procedure of hybrid ligands 5 and 6

Synthesis of 8. Zn (785 mg, 12 mmol) and NH_4Cl (321 mg, 6 mmol) were added to a solution of **7** (200 mg, 1.2 mmol) in MeOH (10 mL) at room temperature. The reaction mixture was stirred at room temperature for 18 h. The solution was filtered and evaporated. After addition of water, the solution was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$, filtered, and the solvents were evaporated *in vacuo*. The residue was purified by silica gel chromatography (ϕ20×150 mm; $CHCl_3$/MeOH, 95:5) to afford **8** (137 mg, 1 mmol, 83 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.76 (2H, t, *J* = 6.5 Hz), 3.81 (2H, t, *J* = 6.5 Hz), 6.56 (2H, m), 6.62 (1H, d, *J* = 7.7 Hz), 7.10 (1H, m) ppm. ^{13}C NMR ($CDCl_3$, 400 MHz): δ 39.4, 63.7, 113.5, 116, 119.5, 129.7, 139.9, 146.8 ppm.

Synthesis of 9. $(Boc)_2O$ (218 μL, 1 mmol) was added dropwise to a solution of **8** (131 mg, 1 mmol) in dry THF (10 mL) at room temperature. The reaction mixture was stirred at room temperature for 18 h. Then, more dropwise $(Boc)_2O$ (220 μL, 1 mmol) was added to the reaction mixture at room temperature. After the solution was stirred at room temperature for 4 h, the solvents were evaporated *in vacuo*. The residue was purified by silica gel chromatography (ϕ20×150 mm; Hexane/EtOAc, 65:35) to afford **9** (217 mg, 0.9 mmol, 96%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.51 (9H, s), 2.43 (2H, t, *J* = 6.5 Hz), 3.84 (2H, t, *J* = 6.5 Hz), 6.57 (1H, s), 6.90 (1H, m), 7.17 (1H, m), 7.21 (1H, m), 7.30 (1H, s) ppm. ^{13}C NMR ($CDCl_3$, 400 MHz): δ 28.5 (3C), 39.4, 63.7, 80.7, 116.9, 119.3, 123.9, 129.3, 138.8, 139.8, 153 ppm.

Synthesis of 10. The solution of **9** (217 mg, 0.9 mmol) in dry CH_2Cl_2 (5 mL) was added to *p*-toluensulfonyl chloride (191 mg, 1 mmol) with DMAP (12 mg, 0.1 mmol) and Et_3N (279 μL, 2 mmol) in dry CH_2CL_2 (7 mL) at 0 ºC. Then, the reaction mixture was stirred at room temperature for 18 h. After addition of water, the solution was extracted

with CH_2Cl_2. The organic layer was washed with brine and dried over $MgSO_4$, filtered, and the solvents were evaporated *in vacuo*. The residue was purified by silica gel chromatography (ϕ20×150 mm; Hexane/EtOAc, 90:10) to afford **10** (318 mg, 0.8 mmol, 89%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.52 (9H, s), 2.43 (3H, s), 2.91 (2H, t, *J* = 7.0 Hz), 4.19 (2H, t, *J* = 7.0 Hz), 6.44 (1H, s), 6.79 (1H, m), 7.16 (3H, m), 7.28 (2H, d, *J* = 8.5 Hz), 7.69 (2H, d, *J* = 8.5 Hz) ppm. ^{13}C NMR ($CDCl_3$, 400 MHz): δ 21.8, 28.5 (3C), 35.5, 70.7, 80.8, 117.2, 119, 123.8, 128.1 (2C), 129.4, 130 (2C), 133.2, 137.4, 138.8, 144.8, 152.8 ppm. HR ESI-MS (positive ion) *m/z*: 430.1115 $(M + K)^+$ (Calcd for $C_{20}H_{25}NO_5SK$: 430.1091).

Synthesis of 11. K_2CO_3 (161 mg, 1.17 mmol) was added to a solution of **10** (306 mg, 0.78 mmol) in dry CH_3CN (12 mL) at room temperature. The reaction mixture was stirred at room temperature for 20 min. *p*-hydroxybenzaldehyde (114 mg, 0.94 mmol) was added to the reaction mixture at room temperature. The reaction mixture was refluxed for 18 h and cooled to room temperature. The solution was added H_2O, and extracted with $CHCl_3$. The organic layer was washed with brine and dried over $MgSO_4$, filtered, and the solvents were evaporated *in vacuo*. The residue was purified by silica gel chromatography (ϕ20×150 mm ; Hexane/EtOAc, 93:7) to afford **11** (192 mg, 0.56 mmol, 72 %).^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.52 (9H, s), 3.10 (2H, t, *J* = 7.0 Hz), 4.25 (2H, t, *J* = 7.0 Hz), 6.50 (1H, s), 6.96 (1H, m), 7.00 (2H, d, *J* = 8.8 Hz), 7.14 (1H, m), 7.24 (1H, m), 7.43 (1H, s), 7.82 (2H, d, *J* = 8.8 Hz), 9.87 (1H,s) ppm. ^{13}C NMR ($CDCl_3$, 400 MHz): δ 28.5 (3C), 35.8, 69.1, 80.7, 115 (2C), 117, 119.2, 123.8, 129.3, 130.1, 132.2 (2C), 138.8, 138.9, 153, 164.1, 191 ppm. HR ESI-MS (negative ion) *m/z*: 340.1559 $(M - H)^-$ (Calcd for $C_{20}H_{22}NO_4$: 340.1549).

Synthesis of 12. $(EtO)_2P(O)CH_2COOEt$ (132 μL, 0.66 mmol) was added to LiCl (29 mg, 0.66 mmol) and DBU (200 μL) in dry CH_3CN (4 mL), and stirred at room temperature for 1 h. Then **11** (149 mg, 0.44 mmol) in dry CH_3CN (1 mL) was added to the solution and stirred at room temperature for 18 h. After addition of water, the solution was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$, filtered, and the solvents were evaporated *in vacuo*. The residue was purified by silica gel chromatography (ϕ20×150 mm; Hexane/EtOAc, 95:5) to afford **12** (160 mg, 0.39 mmol, 89 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.33 (3H, t, *J* = 7.1 Hz),

1.52 (9H, s), 3.07 (2H, t, *J* = 7.0 Hz), 4.18 (2H, t, *J* = 7.0 Hz), 4.25 (2H, q, *J* = 7.1 Hz), 6.30 (1H, d, *J* = 15.9 Hz), 6.52 (1H, s), 6.88 (2H, d, *J* = 8.8 Hz), 6.95 (1H, m), 7.17 (1H, m), 7.22 (1H, m), 7.39 (1H, s), 7.44 (2H, d, *J* = 8.8 Hz), 7.63 (1H, d, *J* = 15.9 Hz) ppm. ^{13}C NMR ($CDCl_3$, 400 MHz): δ 14.7, 28.7 (3C), 36, 60.6, 69, 80.8, 115.2 (2C), 116, 117.1, 119.3, 124, 127.5, 129.4, 130 (2C), 138.9, 139.3, 144.6, 153.1, 160.9, 167.7 ppm. HR ESI-MS (positive ion) *m/z*: 434.1971 $(M + Na)^+$ (Calcd for $C_{24}H_{29}NO_5Na$: 434.1943).

Synthesis of 13. 4N HCl / EtOAc (2 mL) was added to a solution of **12** (130 mg, 0.32 mmol) in EtOAc (1 mL) at room temperature. The reaction mixture was stirred at room temperature for 18 h. Then, the solvents were evaporated *in vacuo*. The residue was purified by silica gel chromatography (ϕ10×300 mm; Hexane/EtOAc, 95:5) to afford ethyl **13** (94.8 mg, 0.27 mmol, 86 %). ^{1}H NMR (CD_3OD, 400 MHz): δ 1.30 (3H, t, *J* = 7.1 Hz), 3.16 (2H, t, *J* = 6.3 Hz), 4.21 (2H, q, *J* = 7.1 Hz), 4.27 (2H, t, *J* = 6.3 Hz), 4.88 (2H, s), 6.35 (1H, d, *J* = 16.0 Hz), 6.94 (2H, d, *J* = 8.8 Hz), 7.29 (1H, m), 7.40 (1H, m), 7.46 (2H, m), 7.52 (2H, d, *J* = 8.8 Hz), 7.61 (1H, d, *J* = 16.0 Hz) ppm. ^{13}C NMR (CD_3OD, 400 MHz): δ 14.8, 36.3, 61.6, 69.4, 116.2 (2C), 116.6, 122.2, 124.8, 128.7, 131.0, 131.1 (2C), 131.4, 132.2, 143.1, 145.9, 162.3, 169.2 ppm. HR ESI-MS (positive ion) *m/z*: 312.1621 $(M + H)^+$ (Calcd for $C_{19}H_{22}NO_3$: 312.1600).

Synthesis of 5. Et_3N (50 μL) in dry CH_2Cl_2 (2 mL) was added to the solution of **13** (40 mg, 0.12 mmol), and stirred at room temperature until dissolved. Then, 5-chloro-2-nitrobenzoyl chloride (27.8 mg, 0.13 mmol) was added to the solution and stirred at room temperature for 18 h. After addition of water, the solution was extracted with $CHCl_3$. The organic layer was washed with brine and dried over $MgSO_4$, filtered, and the solvents were evaporated *in vacuo*. The residue was purified by silica gel chromatography (ϕ10×300 mm; Hexane/EtOAc, 70:30) to afford **5** (42.7 mg, 0.086 mmol, 75 %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.31 (3H, t, *J* = 7.1 Hz), 3.10 (2H, t, *J* = 6.8 Hz), 4.20 (2H, t, *J* = 6.8 Hz), 4.21 (2H, q, *J* = 7.1 Hz), 6.24 (1H, d, *J* = 15.8 Hz), 6.87 (2H, d, *J* = 8.7 Hz), 7.12 (1H, d, *J* = 7.9 Hz), 7.32 (1H, t, *J* = 7.9 Hz), 7.41(2H, d, *J* = 8.8 Hz), 7.48 (1H, d, *J* = 7.9 Hz), 7.56 (1H, m), 7.57 (1H, d, *J* = 15.8 Hz), 7.63 (1H, m), 8.16 (1H, dd, *J* = 2.7, 8.8 Hz), 8.38 (1H, s), 8.48 (1H, d, *J* = 2.7 Hz) ppm. ^{13}C NMR ($CDCl_3$, 400 MHz): δ 14.4, 35.8, 60.6, 68.6, 115.1 (2C), 115.8, 118.7, 121, 125.1, 125.9,

126.2, 127.3, 129.5, 129.9 (2C), 131.6, 136.9, 137.5, 137.9, 139.6, 144.5, 146.6, 160.7, 162.7, 167.7 ppm. HR ESI-MS (negative ion) *m/z*: 493.1168 $(M - H)^-$ (Calcd for $C_{26}H_{22}N_2O_6Cl$: 493.1166).

Synthesis of 6. Et_3N (37.5 μL) in dry CH_2Cl_2 (2 mL) was added to the solution of **13** (30 mg, 0.086 mmol), and stirred at room temperature until dissolved. Then 3-nitrobenzoyl chloride (17.5 mg, 0.094 mmol) was added to the solution and stirred at room temperature for 18 h. After addition of water, the solution was extracted with $CHCl_3$. The organic layer was washed with brine and dried over $MgSO_4$, filtered, and the solvents were evaporated *in vacuo*. The residue was purified by silica gel chromatography (ϕ10×300 mm; Hexane/EtOAc, 65:35) to afford **6** (32.0 mg, 0.070 mmol, 81 %). ^{1}H NMR (DMSO-d_6, 400 MHz): δ 1.24 (3H, t, *J* = 7.1 Hz), 3.06 (2H, t, *J* = 6.7 Hz), 4.16 (2H, q, *J* = 7.1 Hz), 4.27 (2H, t, *J* = 6.7 Hz), 6.46 (1H, d, *J* = 16.0 Hz), 6.99 (2H, d, *J* = 8.8 Hz), 7.12 (1H, d, *J* = 7.7 Hz), 7.32 (1H, t, *J* = 7.7 Hz), 7.59 (1H, d, *J* = 16.0 Hz), 7.65 (2H, d, *J* = 8.8 Hz), 7.66 (1H, m), 7.75 (1H, m), 7.84 (1H, m), 8.43 (2H, m), 8.80 (1H, m), 10.58 (1H, s) ppm, ^{13}C NMR (DMSO-d_6, 400 MHz): δ 14.2, 34.9, 59.8, 68.2, 114.8 (2C), 115.4, 118.7, 121.1, 122.4, 124.8, 126.1, 126.7, 128.6, 130.1 (2C), 130.2, 134.2, 136.3, 138.7, 138.8, 144.1, 147.7, 160.3, 163.3, 166.4 ppm. HR ESI-MS (negative ion) *m/z*: 459.1583 $(M - H)^-$ (Calcd for $C_{26}H_{23}N_2O_6$: 459.1556).

3. Method of MTT assay

HepG2 or pre-differentiated 3T3-L1 cells were seeded in 100 μL of medium at a density of 1×10^4 cells /well in a 96 well micro-plate. After a 24 h incubation, the cells were treated with test samples for 24 or 48 h. Subsequently, 10 μL of 3-(4,5-dimethyl -2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT; DOJINDO, Kumamoto, Japan) dissolved in PBS at 5 mg/mL was added. After 4 h incubation, the colored formazan was dissolved in 100 μL of 10% SDS in PBS. The absorbance at 570 nm was determined using a multi-detection micro plate reader (Powerscan HT, Dainippon Pharmaceutical, Osaka, Japan).

4. Supplementary Table

Table S1. Primer pairs for real time RT-PCR

	Forward primer	Reverse primer
β-actin	5'- GGCCAACCGTGAAAAGATGA-3'	5'- CAGCCTGGATGGCTACGTACA-3'
adiponectin	5'- CACCTACGACCAGTATCAG -3'	5'-GCCAGTAAATGTAGAGTCGT-3'
aP2	5'- GTCACCATCCGGTCAGAGAG -3'	5'- CTTGTGGAAGTCACGCC -3'

5. Supplementary Figures

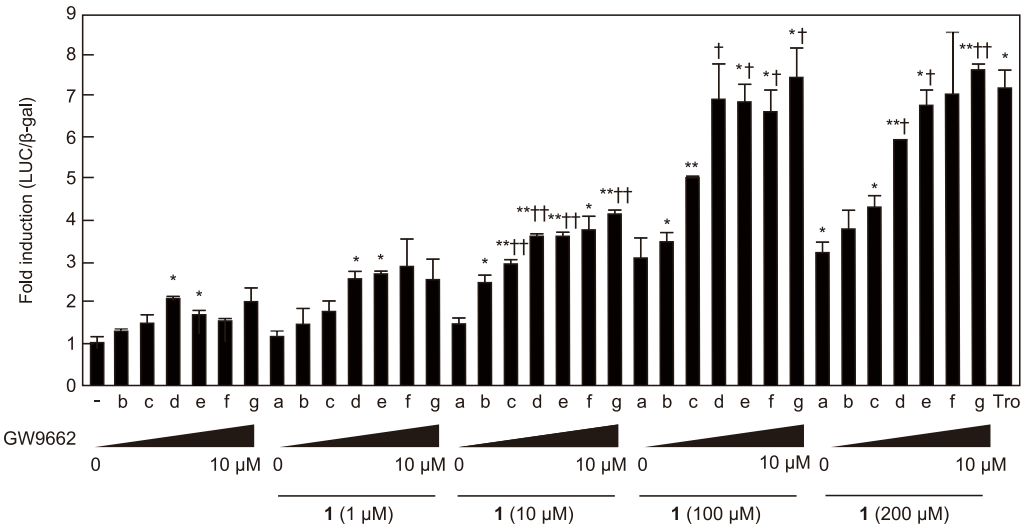

Figure S1. Dose–response effect of GW9662 on the cooperative activation of PPARγ in combination with **1**. The luciferase assay was performed in HepG2 cells transiently co-transfected with pGal4-PPARγLBD, pUAS-tk-Luc reporter and pact-βGal plasmids. Relative luciferase activities normalized to β-galactosidase activity are indicated. HepG2 cells were treated with vehicle (shown as -; 0.1% DMSO), troglitazone (Tro, 10 μM), a synthetic PPARγ agonist as a positive control, and **1** (0, 1, 10, 100, and 200 μM) with or without GW9662 (0.1, 1, 10, 100 nM, 1, and 10 μM) for 6 h. Results are presented as the mean ± SD (n = 2). **P* < 0.05, ***P* < 0.01, compared with vehicle control. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, compared with cells treated without GW9662.

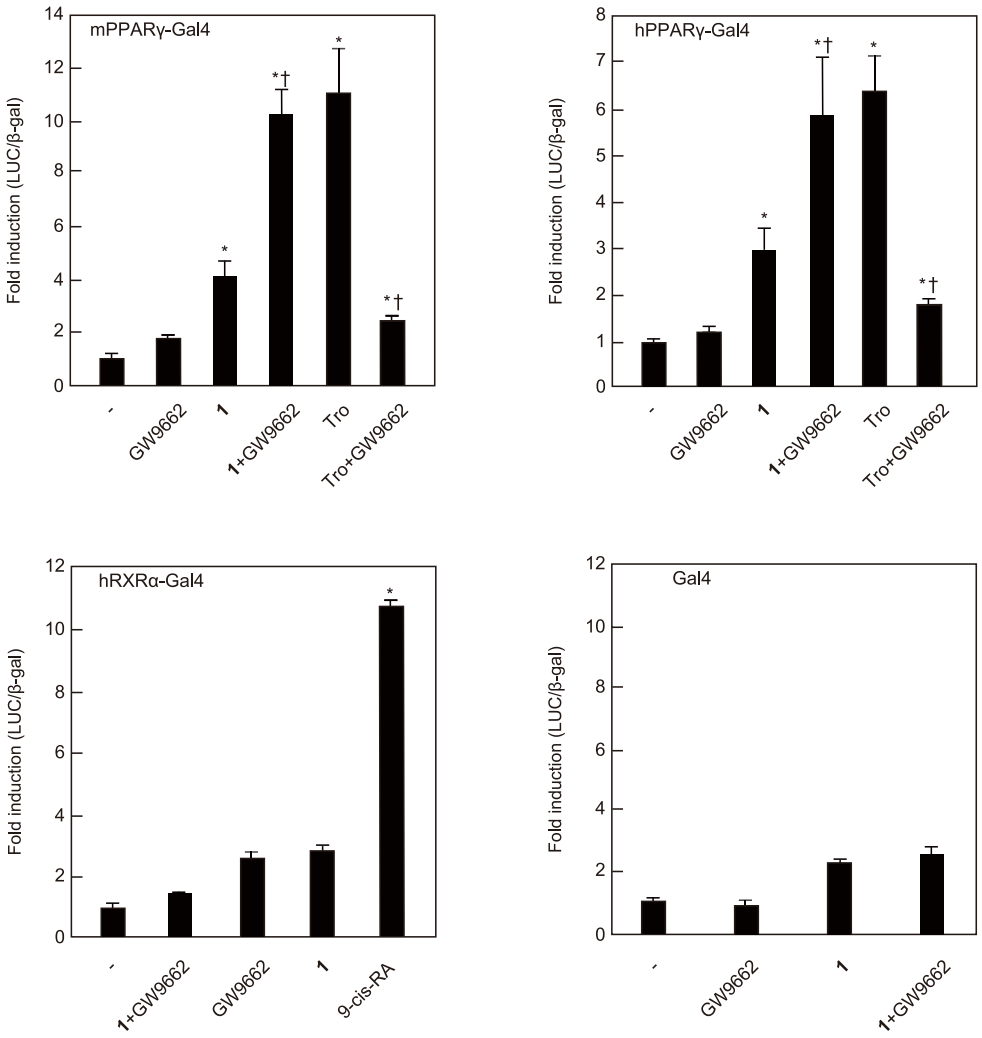

Figure S2. Cooperative effects of GW9662 and **1** on PPARγ, RXRα, and Gal4 transcriptional activities. The luciferase assay was performed in HepG2 cells transiently co-transfected with pUAS-tk-Luc reporter, pact-β Gal plasmids, and pGal4-mousePPARγLBD, pGal4-humanPPARγLBD, pGal4-humanRXRαLBD or pGal4. Relative luciferase activities normalized to β-galactosidase activity are indicated. HepG2 cells were treated by vehicle (shown as -; 0.1% DMSO), troglitazone (Tro, 10 μM), a synthetic PPARγ agonist as a positive control for the PPARγ agonist, 9-cis-retinoic acid (9-cis-RA, 10 μM) as a positive control for the RXRα agonist, and **1** with or without GW9662 (10 μM) for 6 h. Results are presented as the mean ± SD (n = 3) of three independent experiments. **P* < 0.01, compared with vehicle control. †*P* < 0.01, compared with cells treated without GW9662.

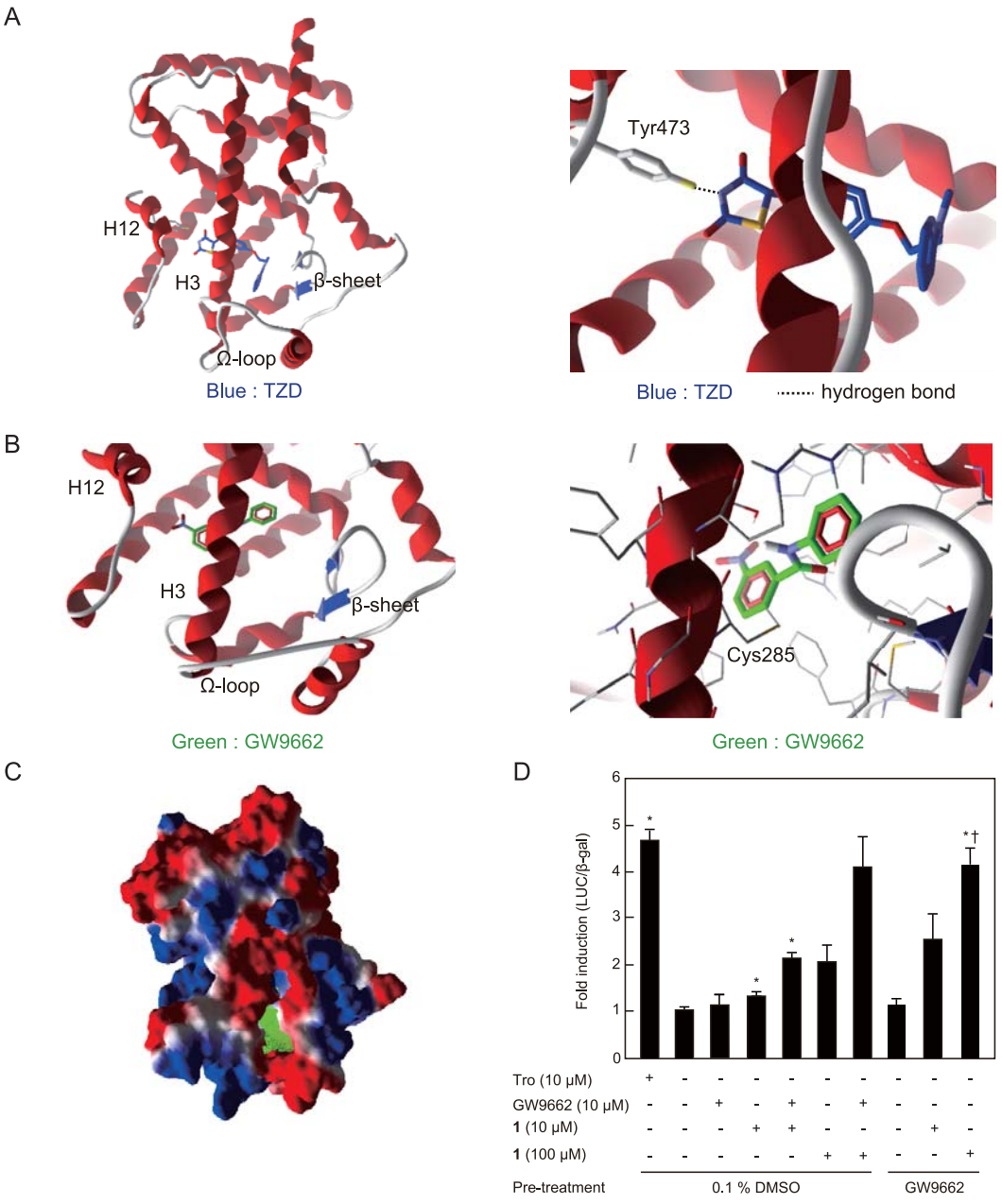

Figure S3. (A) Structure of the complex between PPARγ LBD and rosiglitazone (PDB code 2PRG). Rosiglitazone exhibits a "U-shaped" conformation, located near the Cys285 residue with its central benzene ring directly behind helix 3 (*left*) and the TZD head group extending toward helix 12 to form a direct hydrogen bond with the hydroxyl moiety of Tyr473 (*right*) (B) Structure of the complex between PPARγ LBD and GW9662 (PDB code3B0R) (*left*). GW9662 forms a covalent bond with the Cys285 residue in the PPARγ LBD (*right*). (C) Open cavity in the GW9662-bound PPARγ LBD. The potential ligand-binding site is detected by the Molegro cavity detection algorithm and is displayed as a green cavity. (D) Effect of treatment with **1** alone after pre-exposure of GW9662 on the transactivation of PPARγ. The luciferase assay was performed in HepG2 cells transiently co-transfected with the pUAS-tk-Luc reporter, pact-βGal plasmids, and pGal4-humanPPARγLBD. Relative luciferase activities normalized to the β-galactosidase activity are indicated. After pre-exposure of vehicle (0.1% DMSO) or GW9662 for 1 h, HepG2 cells were treated with vehicle (shown as -; 0.1% DMSO), troglitazone (Tro, 10 μM), and **1** with or without GW9662 (10 μM) for 6 h. Results are presented as the mean ± SD (n = 3) of three independent experiments. **P* < 0.05, compared with vehicle control. †*P* < 0.05, compared with cells treated without GW9662.

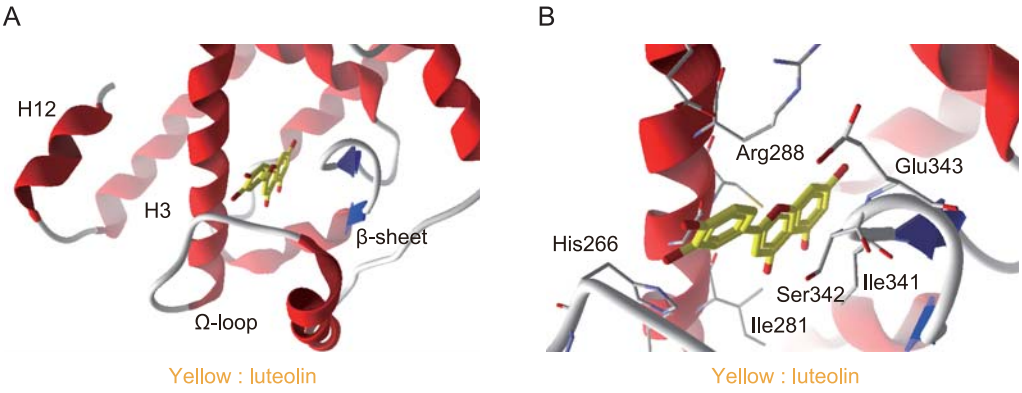

Figure S4. Structure of the complex between PPARγ LBD and luteolin (PDB code 3SZ1). Luteolin occupies the region near helix 3, the β-sheet, the Ω-loop (*left*) and interacts with the residue in these regions (*right*).

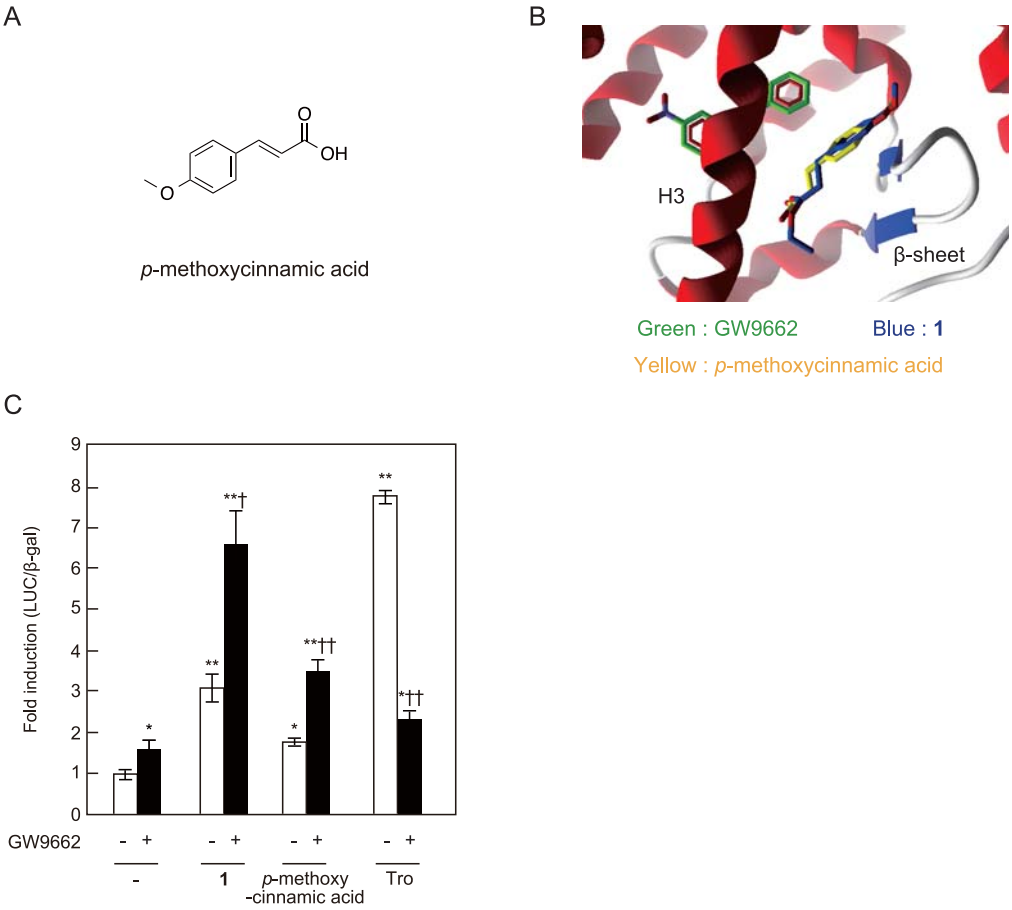

Figure S5. Comparison of the putative binding mode and cooperative transcriptional activity of the hydrolysis product of **1**, *p*-methoxycinnamic acid, in combination with GW9662. (A) Structure of *p*-methoxycinnamic acid, a hydrolyzed product of **1**. (B) Superposition of the docking poses of **1** and *p*-methoxycinnamic acid in complex with human PPARγ LBD and GW9662. The crystal structure of human PPARγ LBD and GW9662 was retrieved from the RCSB Protein Data Bank (PDB code: 3B0R). After conversion of GW9662 to a cofactor, **1** or *p*-methoxycinnamic acid were docked as a ligand to the complex of human PPARγ LBD and GW9662. (C) Effect of *p*-methoxycinnamic acid (100 μM) on the cooperative activation of PPARγ in combination with GW9662 (10 μM). - indicates vehicle (0.1% DMSO), and Tro indicates troglitazone (10 μM). Results are presented as the mean ± SD (n = 3) of three independent experiments. **P* < 0.05, ***P* < 0.01, compared with vehicle control. $^{\dagger}P < 0.05$, $^{\dagger\dagger}P < 0.01$, compared with cells treated without GW9662.

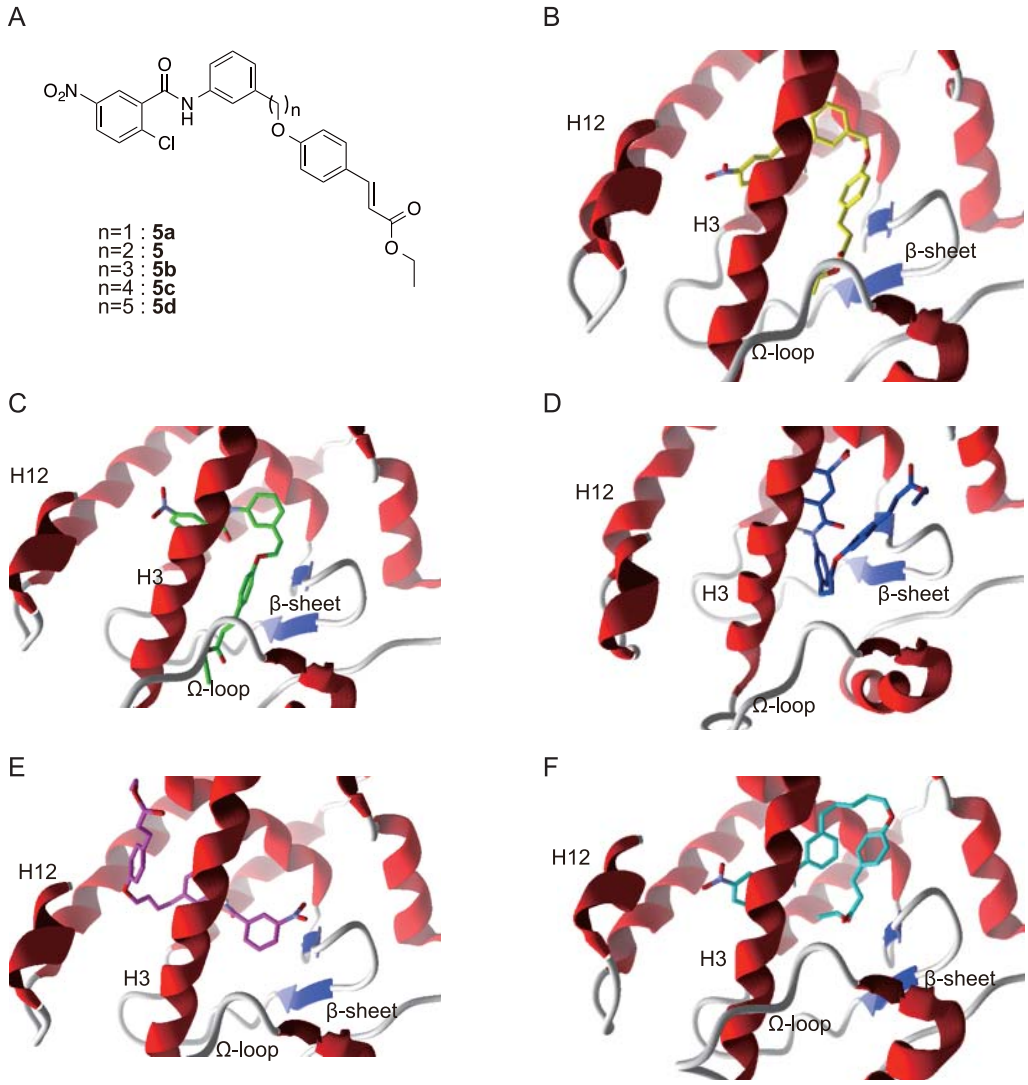

Figure S6. Comparison of the putative binding pose of the designed ligands. (A) Structure of the designed ligand in which carbon number of alkyl chain linker is in a range of one to five (**5a**, **5**, **5b**, **5c**, and **5d**). (B) Docking pose of **5a** in a crystal structure of the PPARγ LBD (PDB code 2ZK4). (C) Docking pose of **5**. (D) Docking pose of **5b**. (E) Docking pose of **5c**. (F) Docking pose of **5d**.

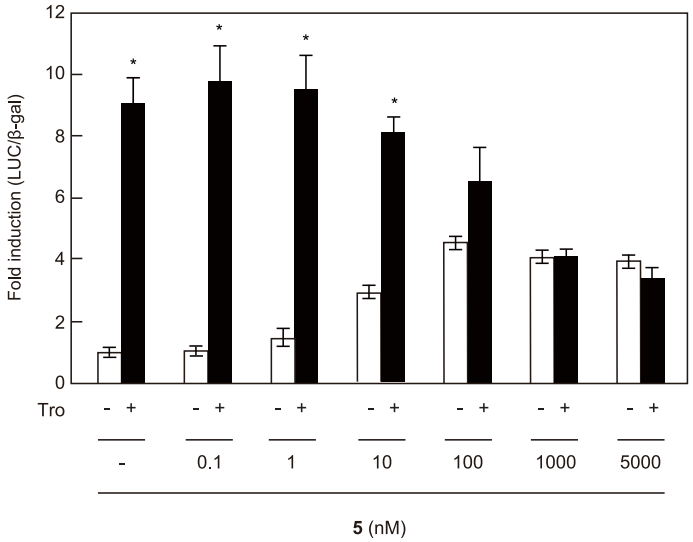

Figure S7. Competitive inhibition by **5** against the PPARγ agonist activity of troglitazone. The full agonist activity of troglitazone was partially inhibited by the co-treatment with **5**. The luciferase assay was performed according to the method described in the Figure S1 legend. HepG2 cells were treated with the vehicle (shown as -; 0.1% DMSO), troglitazone (10 μM), or **5**. Results are presented as the mean ± SD (n = 3) of three independent experiments. **P* < 0.01, compared with cells treated without **5**.

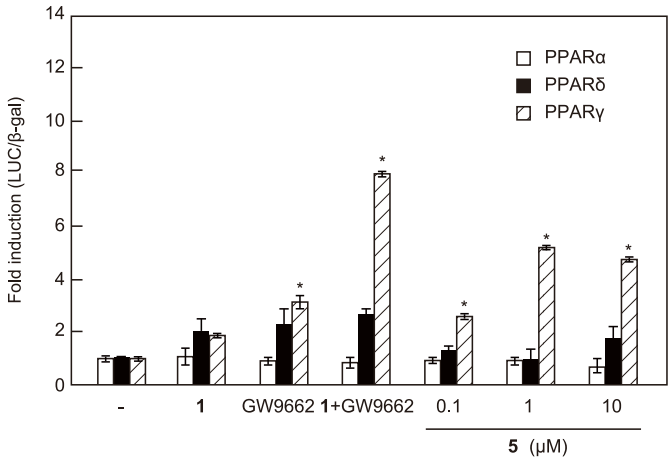

Figure S8. Ligand **5** and the combination of GW9662 and **1** specifically activate PPARγ in the luciferase reporter assay, while they did not significantly activate other subtypes of the PPAR family. The luciferase reporter assay was performed using pGal4-PPARα, PPARδ and PPARγ LBD plasmids. - indicates the vehicle (0.1% DMSO). Results are presented as the mean ± SD (n = 3) of three independent experiments. *$P < 0.01$, compared with vehicle control.

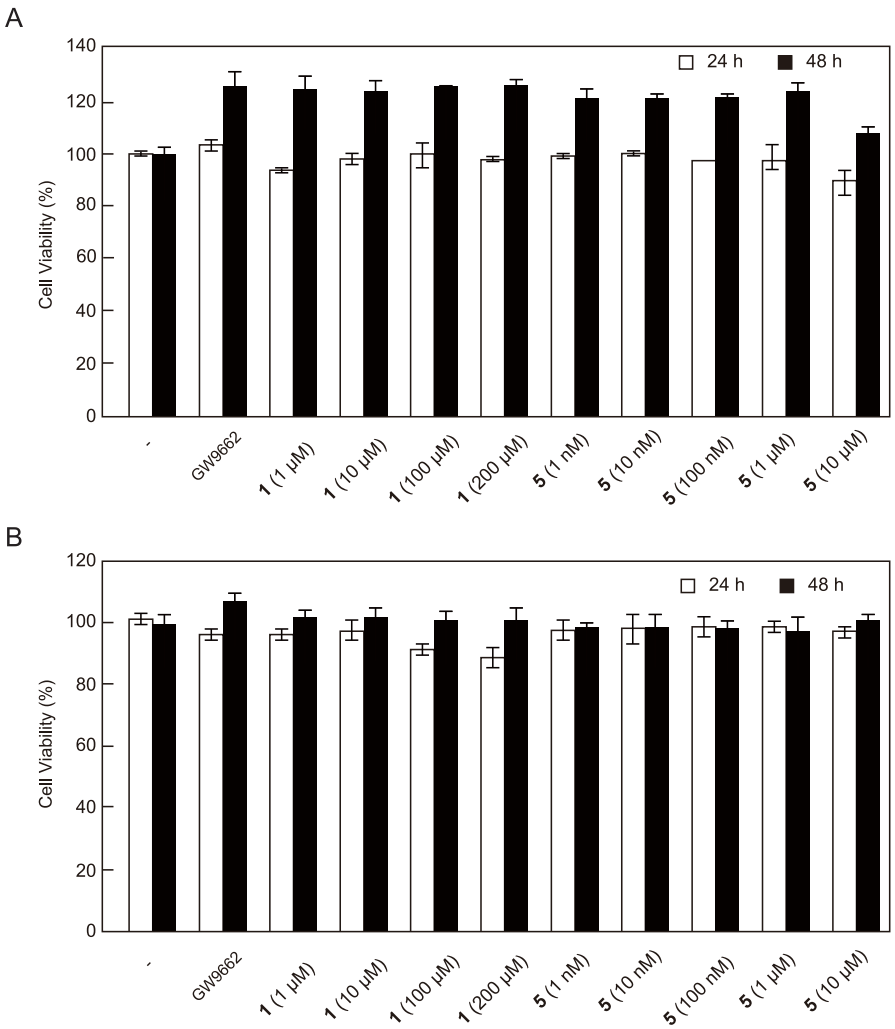

Figure S9. Effect of **1** and **5** on the cell viability of HepG2 (A) and 3T3-L1 (B) cells. HepG2 or 3T3-L1 cells were treated with vehicle (0.1% DMSO), GW9662 (10 μM), **1** (1, 10, 100, or 200 μM), or **5** (1, 10, 100 nM, 1, or 10 μM), and then incubated for 24 or 48 hours. Relative cell viability was assessed using MTT assay. All results are presented as the mean ± SD (n = 3) of three independent experiments.

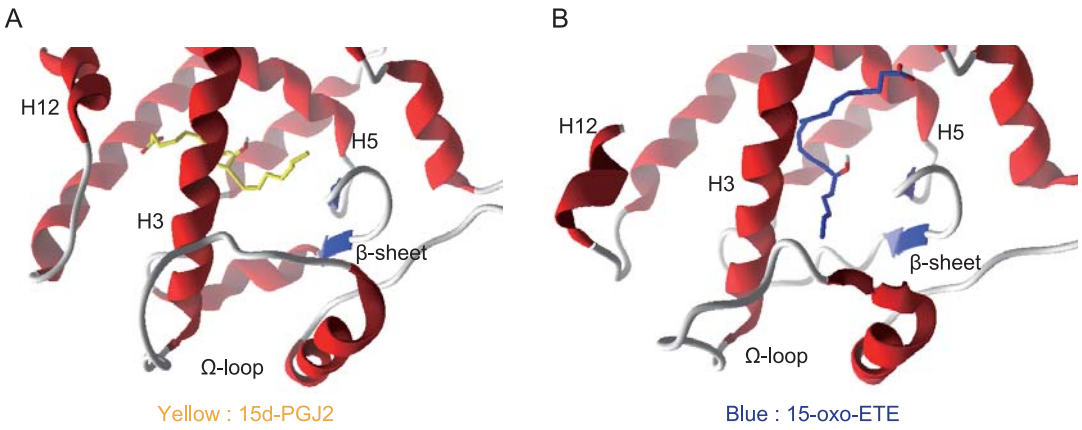

Figure S10. Structure of the complex of PPARγ LBD and 15d-PGJ_2 (PDB code 2ZK1) (A) or 15-oxo-ETE (PDB code 2ZK4) (B).