under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:40) to yield 89 mg of **19** as yellow crystals (7.9%). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.47 (d, J = 2.0 Hz, 1H), 7.88 (dd, J = 9.0, 2.0 Hz, 1H), 7.28 (s, 1H), 7.25 (d, J = 3.0 Hz, 1H), 7.23 (s, 1H), 7.09 (d, J = 9.0 Hz, 1H), 6.75 (d, J = 3.0 Hz, 1H), 3.95 (s, 3H), 2.00 (s, 3H), 1.74 (s, 1H), 1.36 (s, 6H), 1.28 (s, 6H).

1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-1H-indole-5-carboxylic Acid (7). To a solution of **19** (66 mg, 0.18 mmol) in MeOH (2.0 mL) and THF (1.0 mL) was added 2 N NaOH (2.0 mL). The reaction mixture was stirred at 60 °C for 1.5 h, poured into 2 N HCl (2.0 mL), and extracted with EtOAc (3 × 40 mL). The organic layer was collected, washed with brine (20 mL), dried over $MgSO_4$, and evaporated under reduced pressure to yield 58 mg of **7** (92%). The residue was recrystallized from EtOAc/*n*-hexane to yield 10 mg of **7** as a colorless powder. Mp: 244.0–245.0 °C. HPLC: 33 min; >95% purity (MeOH:$AcONH_4$(aq) = 80:20). ^{1}H NMR (300 MHz, DMSO) δ: 12.5 (s, 1H), 8.47 (d, J = 2.0 Hz, 1H), 7.74 (dd, J = 9.0, 2.0 Hz, 1H), 7.58 (d, J = 3.0 Hz, 1H), 7.41 (s, 1H), 7.26 (s, 1H), 7.09 (d, J = 9.0 Hz, 1H), 6.79 (d, J = 3.0 Hz, 1H), 1.94 (s, 3H), 1.68 (s, 4H), 1.31 (s, 6H), 1.24 (s, 6H). FAB-MS m/z: 362 $[M + H]^+$. Anal. Calcd for $C_{24}H_{27}NO_2 \cdot 1/4H_2O$: C, 78.76; H, 7.57; N, 3.83. Found: C, 78.60; H, 7.36; N, 3.93.

Luciferase Reporter Gene Assay. *Culture of COS-1 Cells.* COS-1 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FBS, $NaHCO_3$ (1.0 g), L-glutamine (0.292 g), penicillin (25 000 units), and streptomycin (25 000 μg) in a humidified atmosphere of 5% CO_2 in air at 37 °C.

Luciferase Reporter Gene Assay. Luciferase reporter gene assays were performed using COS-1 cells transfected with three kinds of vectors: each receptor subtype, a luciferase reporter gene under the control of the appropriate RXR response element, and secreted alkaline phosphatase (SEAP) gene[33] as a background. CRBPII-tk-Luc, tk-TREPII-Luc, tk-PPREx3-Luc, and tk-rBARx3-Luc reporters were used as RXR, RAR, PPAR, and LXR response elements, respectively. The amounts of each receptor subtype and response element were 1.0 and 4.0 μg, respectively. Transfection was performed with QIA Effectene transfection reagent according to the supplier's protocol. In the case of heterodimer assay, RXRα (0.5 μg), each partner receptor (PPARγ or LXRα, 0.5 μg), and the partner response element (4.0 μg) were transfected into COS-1 cells as described above. Test compound solutions (DMSO concentration below 1%) were added to the suspension of transfected cells, which were seeded at about 2.0×10^4 cells/well in 96-well white plates. After incubation in a humidified atmosphere of 5% CO_2 at 37 °C for 18 h, 25 μL of the medium was used for analyzing SEAP activities, and the remaining cells were used for luciferase reporter gene assays with a Steady-Glo luciferase assay system (Promega) according to the supplier's protocol. The luciferase activities were normalized using SEAP activities. The assays were carried out in triplicate three times.

Production and Purification of RXRα Protein. Production and purification of recombinant RXRα protein were done using GATEWAY technology.[34,35] Destination vectors were generated by insertion of human RXRα DNA (Ultimate Human ORF Clone, Invitrogen)[36] into a pDEST17 vector (Invitrogen) and were transformed into *Escherichia coli* BL21-AI cells (Invitrogen) by means of LR reaction. These cells were used as expression clones. The expression clones were cultured in LB medium containing 100 μg/mL ampicillin at 37 °C with shaking until the OD_{600} reached 0.6–1.0 and then were diluted to OD 0.1 at 600 nm. After addition of 0.2% L-arabinose and 0.1% glucose to the culture during the exponential phase of growth (OD 0.4 at 600 nm), cells were cultured for 2 h and then harvested. The cell pellets were resuspended in Lysis buffer (50 mM Tris-HCl, pH 7.5, 0.15 M NaCl, 0.1% SDS, 1.0% Triton X-100, 1 mM PMSF) at 4 °C. The RXR protein was purified using a His GraviTrap column (GE Healthcare)[37] to give 2.2 mg/L pure protein in the culture medium.

Fluorescence Polarization Assay. Fluorescein-labeled cofactor peptides were purchased from Life Technologies. Assays were performed in 96-well half-area black plates (Greiner) in a final volume of 40 μL. All reagents were diluted in phosphate buffer (50 mM sodium phosphate pH 7.2, 154 mM NaCl, 1 mM dithiothreitol, 1 mM EDTA, 0.01% NP40), and the final DMSO concentration in the assay mixtures was adjusted to 1%. The mixtures containing fluorescein-labeled cofactor peptide, RXRα, and various RXR ligands in phosphate buffer were incubated for 1 h at 25 °C. The fluorescence polarization of the mixtures was measured at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Fluorescence polarization measurements were made with a TECAN Polarion. Fluorescence polarization is the ratio of the difference between the intensities of parallel and perpendicularly polarized fluorescent light to the total light intensity.

Electrostatic Potential Fields and Molecular Docking. The crystal structure of human RXRα–ligand binding domain was retrieved from the Brookhaven Protein Data Bank (http://www.rcsb.org/pdb/Welcome.do). Polar hydrogen atoms were added to both the protein and the ligand. United atom Kollman charges were assigned for the protein. The 3D structures of ligands used for the docking study were constructed by using Spartan (Wave function, Inc.). After semi-empirical pm3 calculations, 6-31G* ab initio calculations were performed to find the lowest energy conformers. The electrostatic potential fields (ESP) were drawn with Spartan. The AutoDock4.2 molecular docking program[32] was employed by using a genetic algorithm with local search (GALS). One hundred individual GA runs, 150 chromosomes, a crossover ratio of 0.80, a rate of gene mutation of 0.02, and an elitism ratio of 0.10 were used for each ligand. The grid box was created with dimensions of 40 × 40 × 40 Å^3, which encloses the original ligand. Molfeat (FiatLux Co., Tokyo, Japan) was used for molecular modeling.

In Vivo Assays. *General.* All experiments were conducted in accordance with the Guidelines for Animal Experiments at Okayama University Advanced Science Research Center, and all procedures were approved by the Animal Research Control Committee of Okayama University.

Measurement of Serum Concentration of Test Compounds after Oral Administration at 30 mg/kg to Mice. Groups of six-week-old ICR male mice (n = 6–9 in each) were treated with solutions of test compound 30 mg/kg (1% ethanol and 0.5% CMC in distilled water) at a volume of 10 mL/kg of body weight by oral administration. At the indicated times, 0.6 mL of blood was taken from the inferior vena cava under diethyl ether anesthesia. Each blood sample was centrifuged at 1900*g* for 5 min at rt. To 100 μL of the resulting plasma were added 100 μL of ice-cold 5 mM ammonium acetate solution (adjusted with acetic acid to pH 5.0) and 1 mL of ice-cold ethyl acetate. The resulting mixture was vortexed for 30 s, kept at room temperature for 10 min, and centrifuged at 1900*g* for 30 s at room temperature. An 800 μL aliquot of the ethyl acetate phase was removed and concentrated to dryness in a centrifugal evaporator. To the resulting residue was added 100 μL of HPLC-grade methanol. This solution was directly subjected to HPLC analysis, and the concentration of each compound was determined from the peak area of the sample with reference to a calibration plot obtained with the authentic compound.

HPLC Conditions. The HPLC system used in this study was a Shimadzu liquid chromatographic system (Kyoto, Japan) consisting of an SCL-10A system controller, LC-10AD pump, SPD-10AV UV–vis spectrophotometric detector, SIL-10AD autoinjector, CTO-10A column oven, DGU-14A degasser, and C-R7A Chromatopac. The samples (each 20 μL) were injected using a refrigerated autosampler kept at 10 °C. The chromatographic analyses were carried out on an Inertsil ODS-3 (4.6 i.d. × 250 mm, 5 μm, GL Sciences, Tokyo, Japan) kept at 40 °C, using methanol:33.3 mM ammonium acetate (adjusted with acetic acid to pH 5.0) (85:15, v/v) as the mobile phase. The flow rate was 0.7 mL/min and the absorbance was monitored at 280 nm.

Observation of Side Effects after Once-Daily Oral Administration at 30 mg/kg for 7 Consecutive Days in Male ICR Mice. Six- to seven-week-old male ICR mice were purchased from Charles River Laboratories Japan, Inc. After arrival of the animals, all were group-housed and acclimated to the colony for 1 or 2 days before the experiment. Before the experiment, they were housed with four mice per cage, with free access to water and chow pellets in a light (12 h on, 8:00 a.m. /12 h off, 8:00 p.m.), temperature (23 ± 1 °C), and relative

humidity (50 ± 20%) controlled environment. Before experiments, mice were assigned to experimental groups so as to minimize the variance between groups based on the measured weight [four per cage (17 × 33 × 15 cm^3)]. Body weight was measured at approximately 10:00 a.m. every day for 7 days before dosing. Mice were administered orally with a solution of test compound at a dose of 30 mg/kg or with the vehicle [1% ethanol and 0.5% carboxymethyl cellulose (CMC) in distilled water] at a volume of 10 mL/kg of animal at approximately 10:00 a.m. every day for 7 days. On the final day of dosing, animals were fasted from 17:00 p.m. and given water ad libitum. On the next day, at approximately 10:00 a.m., animals were weighed and anesthetized with diethyl ether. Blood and liver were removed immediately, and the liver was weighed. Approximately 1 mL of blood in an Eppendorf sample tube was centrifuged to afford a serum sample. Each blood sample was centrifuged at 1900*g* for 5 min at rt.

Observation of Side Effects after Once-Daily Oral Administration at 30 mg/kg for 28 Consecutive Days in SD Rats. Four-week-old male and female SD rats were purchased from Charles River Laboratories Japan, Inc. After arrival of the rats, all were group-housed and acclimated to the colony for 6 (male) or 7 (female) days before the experiment. Before the experiment, they were housed with two rats per cage, with free access to water and chow pellets in a light (12 h on, 8:00 a.m./12 h off, 8:00 p.m.), temperature (23 ± 1 °C), and relative humidity (50 ± 20%)-controlled environment. Before experiments, rats were assigned to experimental groups so as to minimize the variance between groups based on the measured weight [two per cage (25.0 × 41.5 × 19.0 cm^3)]. Body weight was measured at approximately 10:00 a.m. every day for 28 days before dosing. Rats were administered orally with a solution of test compound at a dose of 30 mg/kg or with the vehicle (1% ethanol and 0.5% CMC in distilled water) at a volume of 5 mL/kg of animal at approximately 10:00 a.m. every day for 28 days. On the final day of dosing, animals were fasted from 17:00 p.m. and given water ad libitum. On the next day, at approximately 10:00 a.m., animals were weighed and anesthetized with isoflurane. Blood, liver, brain, kidney, spleen, and testis (male only) were removed immediately. The liver, brain, kidney, spleen, and testis were weighed and frozen with liquid nitrogen. Approximately 10 mL of blood in a centrifuge tube was centrifuged at 2000*g* for 10 min at 4 °C to obtain a serum sample.

Evaluation of Blood Glucose-Lowering Activities in KK-A^y Mice. Type 2 diabetic KK-A^y mice, in which the A^y allele at the agouti locus had been transferred to the inbred KK strain by repetitive backcrossing, were used as the congenic strain. The introduction of the A^y allele caused DM and massive hereditary obesity. Four-week-old male KK-A^y mice were purchased from CLEA Japan Inc. The KK-A^y mice were allowed free access to solid food and tap water. After arrival of the animals, all were group-housed and acclimated to the colony for 6 weeks before the experiment. Before the experiment, they were housed with one mouse per cage, with free access to water and chow pellets in a light (12 h on, 8:00 a.m./12 h off, 8:00 p.m.), temperature (23 ± 1 °C), and relative humidity (50 ± 20%) controlled environment. Before experiments, mice were assigned to experimental groups so as to minimize the variance between groups based on the blood glucose level [one per cage (17 × 33 × 15 cm^3)]. Body weight was measured at approximately 10:00 a.m. every day for 14 days before dosing. Mice were administered orally with a solution of test compound at a dose of 10 mg/kg or with the vehicle (1% ethanol and 0.5% CMC in distilled water) at a volume of 10 mL/kg of animal at approximately 10:00 a.m. every day for 14 days. At day 15, an oral glucose tolerance test (OGTT) was performed, and animals were fasted from 17:00 p.m. and given water ad libitum. On the next day, at approximately 10:00 a.m., animals were weighed and anesthetized with diethyl ether. Blood was removed immediately and centrifuged in an Eppendorf sample tube to obtain serum. Each blood sample was centrifuged at 1900*g* for 5 min at rt. Samples for measurements of fed blood glucose level were taken from the tail vein of the mice, and glucose was measured by using the glucose oxidase method (Medisafe-mini, TERUMO, Tokyo, Japan).

Measurements of Blood Parameters. Aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase (γ-GTP), alkaline phosphatase (ALP), creatinine (CRE), blood urea nitrogen (BUN), triglyceride (TG), total cholesterol (TCHO), and glucose (GLU) were measured by using a Fuji Dry Chem system (Dry Chem 4000 V, Fuji Medical Co., Tokyo, Japan).

Oral Glucose Tolerance Test. KK-A^y mice treated with each compound for 14 days were fasted for 17 h and orally given glucose solution (100 mg glucose in 1 mL distilled water) at a dose of 1 g/kg body weight. At 0, 15, 30, 45, 60, 90, and 120 min after the glucose loading, blood glucose level was measured as described above.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

HPLC charts and in vitro and in vivo data are described. This information is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Tel/Fax: +81-(0)86-251-7963. E-mail: kakuta@pharm.okayama-u.ac.jp.

Author Contributions

H.K. conceived and designed the project. F.O., N.Y., R.S. and M.H. synthesized compounds. F.O., S.Y., and Y.O. performed reporter gene assays. M.M. prepared plasmids. H.N. prepared RXR proteins. S.Y. performed cofactor recruitment assays. A.T. and H.K. performed HPLC analysis. F.O., M.N., K.K., T.K., K.K., Y.F., C.F., Y.Y., H.Y., and H.K. performed in vivo experiments. The manuscript was written by F.O., A.T., and H.K.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

RXR, retinoid X receptor; RAR, retinoic acid receptor; LXR, liver X receptor; PPAR, peroxisome proliferator-activated receptors; qy, quantitative yield; TFAA, trifluoroacetic anhydride; TFA, trifluoroacetic acid; TEA, triethylamine; DCE, 1,2-dichloroethane; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; DMEDA, *N,N′*-dimethylethylenediamine; Asn, asparagine; Gly, glycine; TG, triglyceride; TCHO, total cholesterol; AST, alanine aminotransferase; ALT, aspartate aminotransferase; γ-GTP, γ-glutamyltranspeptidase; ALP, alkaline phosphatase; CRE, creatinine; BUN, blood urea nitrogen.

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Supporting Information

Mechanism of RXR partial agonistic action of CBt-PMN and structural development to increase potency

Fuminori Ohsawa[†], *Shoya Yamada*[†‡], *Nobumasa Yakushiji*[†], *Ryosuke Shinozaki*[†], *Mariko Nakayama*[†], *Kohei Kawata*[†], *Manabu Hagaya*[†], *Toshiki Kobayashi*[†], *Kazutaka Kohara*[†], *Yuuki Furusawa*[†], *Chisa Fujiwara*[†], *Yui Ohta*[†], *Makoto Makishima*[§], *Hirotaka Naitou*[¶], *Akihiro Tai*[#], *Yutaka Yoshikawa*[◊], *Hiroyuki Yasui*[◊], *and Hiroki Kakuta*[†]*

†Division of Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 1-1-1, Tsushima-Naka, Okayama 700-8530, Japan.

‡Research Fellowship Division, Japan Society for the Promotion of Science, Sumitomo-Ichibancho FS Bldg., 8 Ichibancho, Chiyoda-ku, Tokyo 102-8472, Japan.

§Division of Biochemistry, Department of Biomedical Sciences, Nihon University School of Medicine, 30-1 Oyaguchi-kamicho, Itabashi-ku, Tokyo 173-8610, Japan.

¶Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan.

#Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima, 562 Nanatsuka-Cho, Shobara, Hiroshima 727-0023, Japan.

◊Kyoto Pharmaceutical University, 5 Nakauchi-cho, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan.

E-mail: kakuta@pharm.okayama-u.ac.jp

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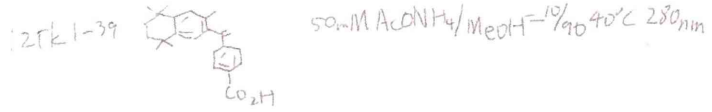
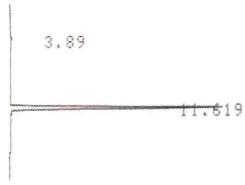
1. *HPLC charts*

1:



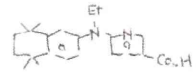


CHROMATOGRAM 1 MEMORIZED

C-R5A CHROMATOPAC
CHANNEL NO 1 FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 3

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.89	662			0.7596	
2	11.619	86471			99.2404	
	TOTAL	87133			100	

2:



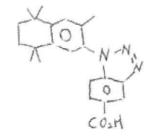
07Mo5-37
(NEt-TMN)
(AcONH4:MeOH=20:80)

CHROMATOGRAM 1 MEMORIZED

C-R5A CHROMATOPAC
CHANNEL NO 1 FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 1

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	24.918	86773			100	
	TOTAL	86773			100	

4a:



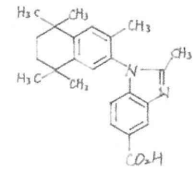
07H06-19
AcONH4 aq. MeOH · 20 80

C-R5A CHROMATOPAC
CHANNEL NO 1 FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 4

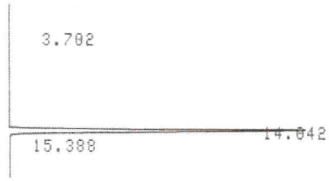
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	13.83	3290078			100	
	TOTAL	3290078			100	

4b:

11MHA 1-25 1次晶



AcONH4 : MeOH
= 20 : 80

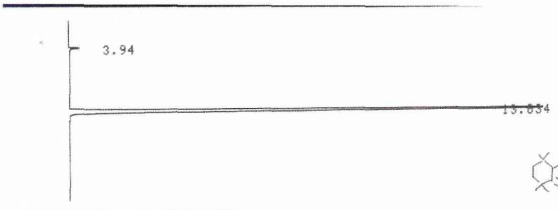


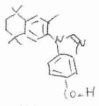
CHROMATOGRAM 1 MEMORIZED

C-R5A CHROMATOPAC
CHANNEL NO 1 FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 5

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.702	535			0.022	
2	14.042	2423895			99.7381	
3	15.388	5829	V		0.2399	
	TOTAL	2430259			100	

6a:





CHROMATOGRAM 1 MEMORIZED

C-R5A CHROMATOPAC
CHANNEL NO 1 FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 2

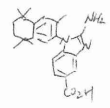
PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.94	3863			1.0982	
2	13.034	347856			98.9018	
TOTAL		351719			100	

09RS2-16

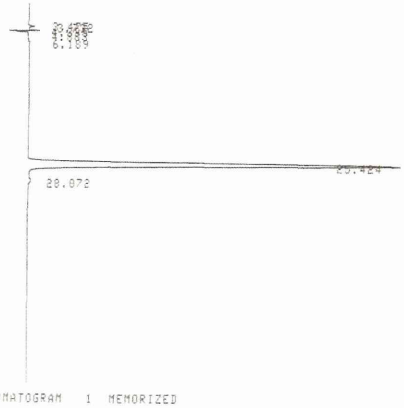
溶離液アセニトリル.MeOH
20 : 80

6b:

09RS2-60



AcONH4 : MeOH
30 : 70



CHROMATOGRAM 1 MEMORIZED

C-R5A CHROMATOPAC
CHANNEL NO 1 FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 2

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.475	388			0.0696	
2	3.732	2724	V		0.4884	
3	4.395	6530			1.1708	
4	6.883	13123			2.3532	
5	6.189	411			0.0737	
6	25.424	530061			95.0499	
7	28.072	4415			0.7943	
TOTAL		557666			100	

2. Supplementary Data

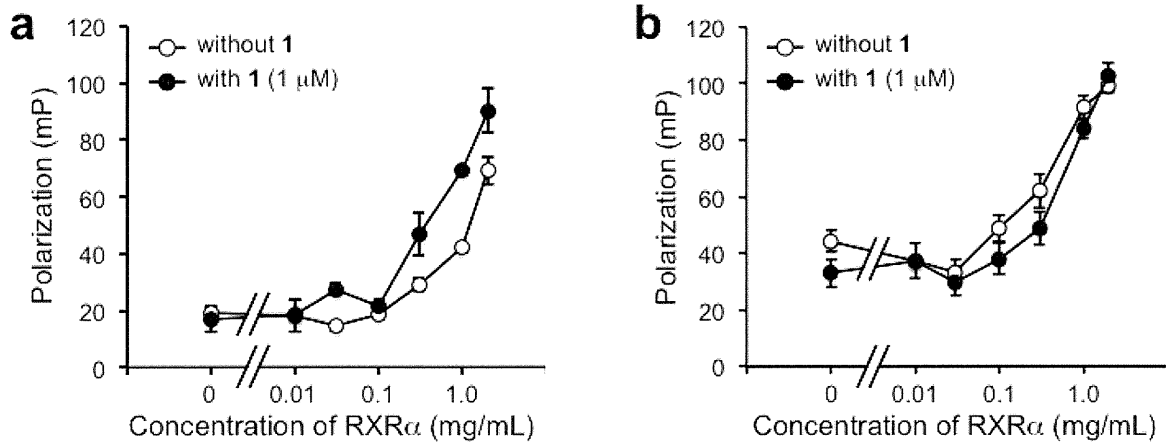


Figure S1. Changes in fluorescence polarization values at various RXR concentrations in the absence (open circles) or presence of 1 μM **1** (closed circles). a) Fluorescein-labeled co-activator peptide D22 (5 nM). b) Fluorescein-labeled co-repressor peptide SMRT-ID2 (5 nM). Fluorescence polarization values, expressed in mP, are the mean ± SEM of measurements obtained from triplicate wells.

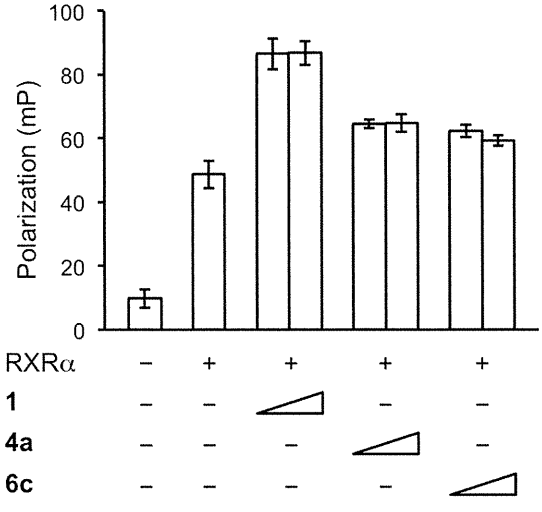


Figure S2. Dose dependency of the effect of RXR agonists at 10 or 33 μM on fluorescence polarization of fluorescein-labeled D22 (5 nM). Fluorescence polarization values, expressed in mP (n = 3–4), are the mean ± SEM. Arrow symbols opposite compound numbers indicate 10 μM (left side/column) and 33 μM (right side/column).

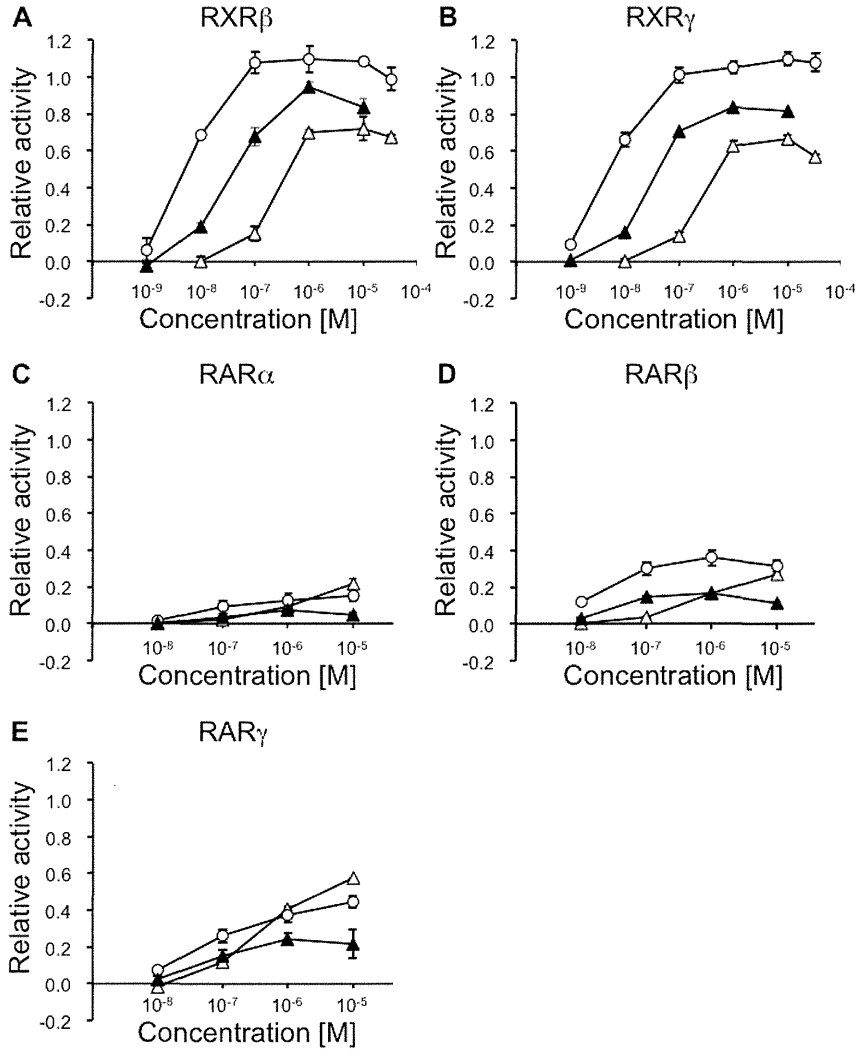


Figure S3. Relative transactivation activities toward RXRβ, RXRγ, RARα, RARβ and RARγ by **2**, **4a** and **6c**. Open circles, triangle and closed triangle indicate **2, 4a** and **6c**, respectively.

A) Relative transactivation data based on the luciferase activity of 1 μM LGD1069 taken as 1.0 toward RXRβ. B) Relative transactivation data based on the luciferase activity of 1 μM LGD1069 taken as 1.0 toward RXRγ. C) Relative transactivation data based on the luciferase activity of 1 μM Am80 (RARα/β selective agonist) taken as 1.0 toward RARα. D) Relative transactivation data based on the luciferase activity of 1 μM Am80 (RARα/β selective agonist) taken as 1.0 toward RARβ. E) Relative transactivation data based on the luciferase activity of 1 μM All-trans retinoic acid (RAR pan agonist) taken as 1.0 toward RARγ.

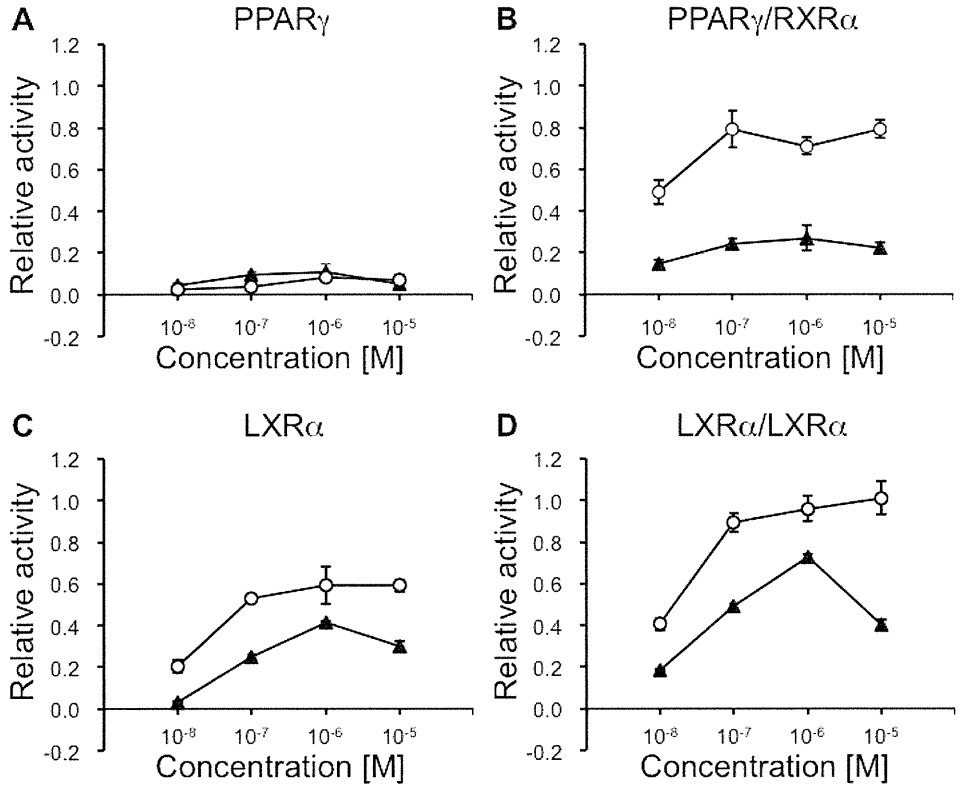


Figure S4. Relative transactivation activities toward PPARγ, PPARγ/RXRα, LXRα and LXRα/RXRα by **2** and **6c**. Open circles and closed triangles indicate **2** and **6c**, respectively. A) Relative transactivation data based on the luciferase activity of 1 μM TIPP703 taken as 1.0 toward PPARγ. B) Relative transactivation data based on the luciferase activity of 1 μM TIPP703 taken as 1.0 toward PPARγ/RXRα. C) Relative transactivation data based on the luciferase activity of 1 μM carba-T0901317 taken as 1.0 toward LXRα. D) Relative transactivation data based on the luciferase activity of 1 μM carba-T0901317 taken as 1.0 toward LXRα/RXRα.

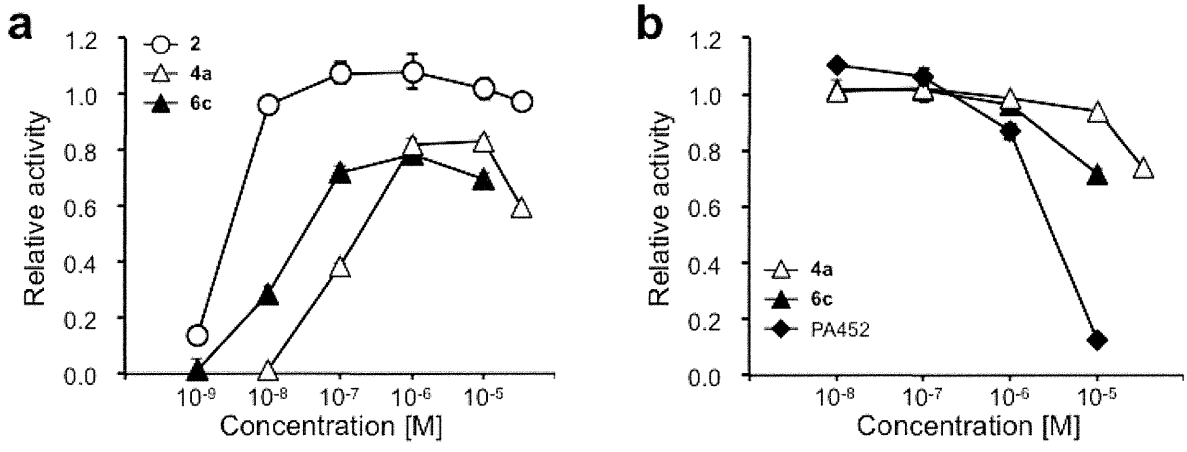


Figure S5. Relative transactivation activities of **2**, **4a** and **6c** toward mouse RXRα. a) Dose-dependence of RXRα agonist activities of **2** (open circles), **4a** (open triangles), and **6c** (closed triangles). b) Dose-dependence of RXRα antagonist activities of **4a** (open triangles), **6c** (closed squares) and RXR antagonist PA452 (closed diamonds) in the presence of 1 μM **1**. COS-1 cells were transfected with three kinds of vectors: mouse RXRα receptor subtype, a luciferase reporter gene under the control of the appropriate RXR response element (CRBPII-tk-Luc), and secreted alkaline phosphatase (SEAP) gene as a background. The transactivation activity is shown as relative activity based on the luciferase activity of 1 μM **1** taken as 1.0. Error bars are SEM.

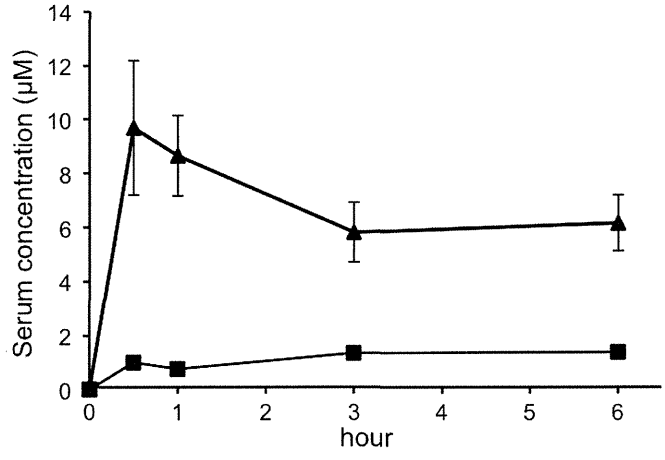


Figure S6. Plasma concentrations of **2** and **6c** in ICR mice after single oral administration of 30 mg/kg. Closed Squares and triangles indicate **2** and **6c**, respectively. The data (n = 5–9) represent the mean ± SEM.

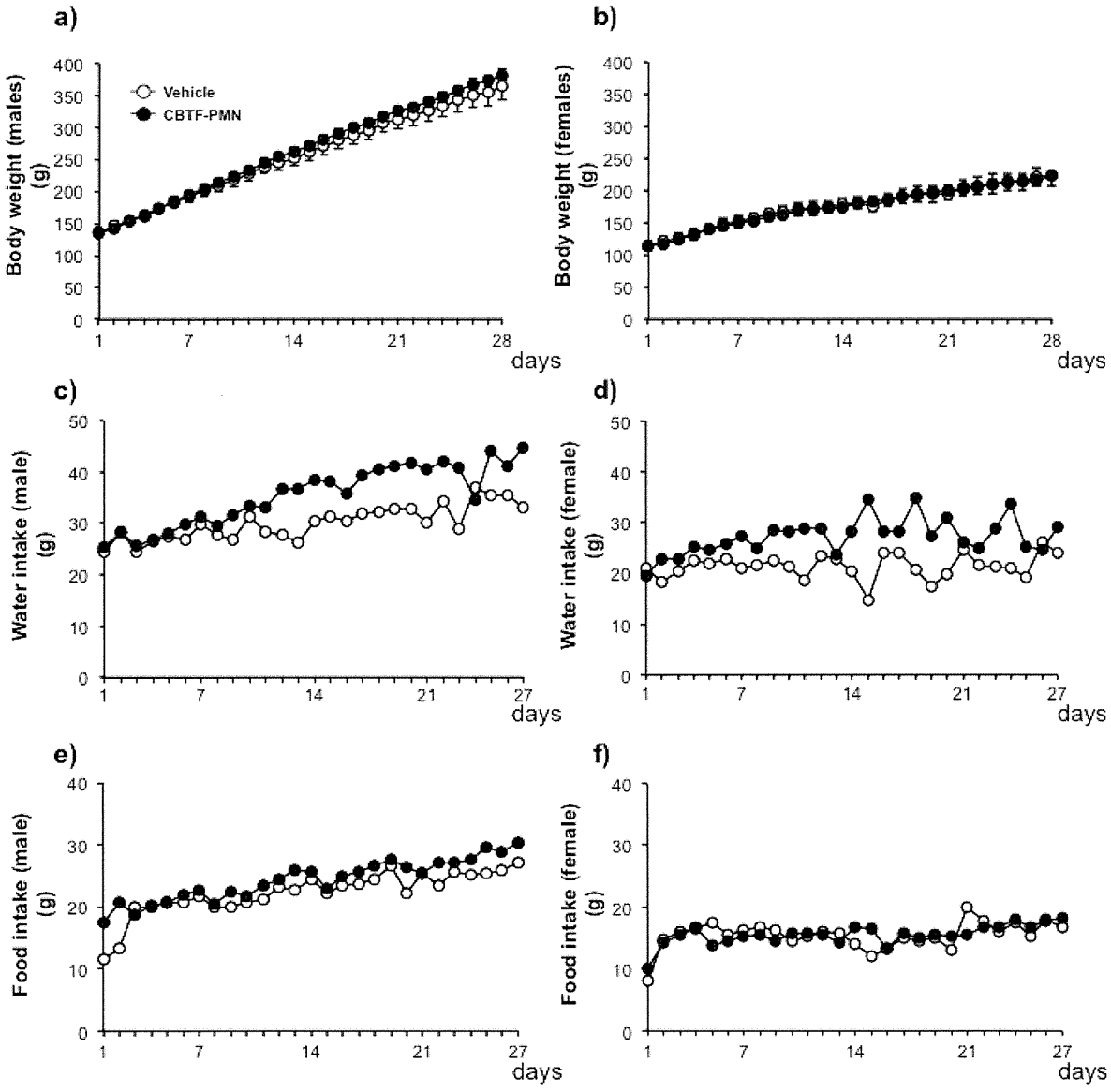


Figure S7. Changes in body weight gain, water intake, and food intake of male or female SD rats treated with oral administration of vehicle or **6c** at 30 mg/kg/day for 28 consecutive days (n = 3–6). a–b) Body weight gain. c–d) Water intake change. e–f) Food intake change. Males: a, c, and e. Females: b, d, and f. Open and closed circles indicate vehicle and **6c** treatment, respectively.

Table S1. Plasma parameters of male ICR mice after oral administration of vehicle, **2**, **4b** or **6c** at 30 mg/kg/day for 7 consecutive days (n = 7–16)

	Vehicle	**2**	**4b**	**6c**
AST (U/I)	54.6 ± 3.8	87.9 ± 18.9**	51.3 ± 3.9	70.1 ± 6.5
ALT (U/I)	22.3 ± 1.5	43.5 ± 6.0**	32.7 ± 6.4*	26.5 ± 2.1
γ-GTP (U/I)	6.1 ± 0.7	7.1 ± 0.3	6.4 ± 0.3	4.5 ± 0.6
ALP (U/I)	281.3 ± 15.7	968.9 ± 115.0**	456.0 ± 51.5*	381.6 ± 58.9
CRE (mg/dL)	D.L.[a]	D.L.	D.L.	D.L.
BUN (mg/dL)	24.1 ± 1.0	26.0 ± 1.8	24.1 ± 2.1	24.8 ± 2.1

a) D.L. means below the detection limit (0.2 mg/dL).

AST : aspartate aminotransferase, ALT : alanine aminotransferase, γ-GTP : γ-glutamyltranspeptidase, ALP : alkaline Phosphatase, CRE : creatinine, BUN : blood urea nitrogen.

Data are mean ± SEM. Statistical analysis was performed by analysis of variance (ANOVA).

Significant differences: * $p < 0.05$ vs. vehicle. ** $p < 0.01$ vs. vehicle.

Table S2. Plasma parameters of male and female SD rats after oral administration of vehicle or **6c** at 30 mg/kg/day for 28 consecutive days (n = 2–6)

	Male			Female		
	Vehicle	**6c**	Reference[a]	Vehicle	**6c**	Reference[a]
AST (U/I)	65.7 ± 6.3	91.7 ± 3.7**	87.0–114.0	69.7 ± 4.8	67.8 ± 2.1	85.0–123.0
ALT (U/I)	30.0 ± 1.7	50.2 ± 2.3**	28.0–40.0	21.7 ± 1.2	37.0 ± 1.2**	25.0–36.0
γ-GTP (U/I)	6.7 ± 0.9	7.0 ± 0.0	0.0–1.0	7.0 ± 0.6	5.8 ± 0.2*	0.0–0.4
ALP (U/I)	703.7 ± 58.6	864.0 ± 94.2	–	382.5 ± 46.5	387.7 ± 31.4	–
CRE (mg/dL)	0.2 ± 0.0	0.2 ± 0.0	0.5–0.6	0.2 ± 0.0	0.2 ± 0.0	0.5–0.6
BUN (mg/dL)	15.5 ± 0.4	14.4 ± 1.4	13.0–16.0	14.5 ± 0.6	15.5 ± 2.0	11.0–16.0
TG (mg/dL)	61.3 ± 9.9	49.0 ± 4.6	61.0–99.0	18.7 ± 4.1	45.2 ± 4.6**	42.0–74.0
TCHO (mg/dL)	50.0 ± 2.5	60.3 ± 4.0	54.0–74.0	57.7 ± 6.9	99.7 ± 6.4**	67.0–87.0

a. These data are taken from the Clinical Laboratory Parameters for Crl:CD(SD) Rats (CRL_Mar, 2006) by Charles River®.

Data are mean ± SEM. Statistical analysis was performed by t-test. Significant differences: * $p < 0.05$ vs. vehicle. ** $p < 0.01$ vs. vehicle.

Table S3. Organ weights of male or female SD rats after oral administration of vehicle or **6c** at 30 mg/kg/day for 28 consecutive days (n = 3–6)

	Male		Female	
	Vehicle	**6c**	Vehicle	**6c**
Weight (g)	339.0 ± 19.1	352.1 ± 7.0	210.2 ± 13.7	206.1 ± 5.4
Brain (g)	1.9 ± 0.1	1.9 ± 0.1	1.7 ± 0.0	1.8 ± 0.0
Liver (g)	10.2 ± 0.8	12.7 ± 0.6*	5.7 ± 0.4	7.6 ± 0.1**
Kidney (g)	2.7 ± 0.1	2.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.1
Spleen (g)	0.6 ± 0.0	0.7 ± 0.0*	0.5 ± 0.0	0.5 ± 0.1
Testis (g)	6.1 ± 0.2	6.4 ± 0.2		

Data are mean ± SEM; Statistical analysis was performed by t-test. Significant differences: * $p < 0.05$ vs. vehicle. ** $p < 0.01$ vs. vehicle.