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RXR Partial Agonist Produced by Side Chain Repositioning of Alkoxy RXR Full Agonist Retains Antitype 2 Diabetes Activity without the Adverse Effects

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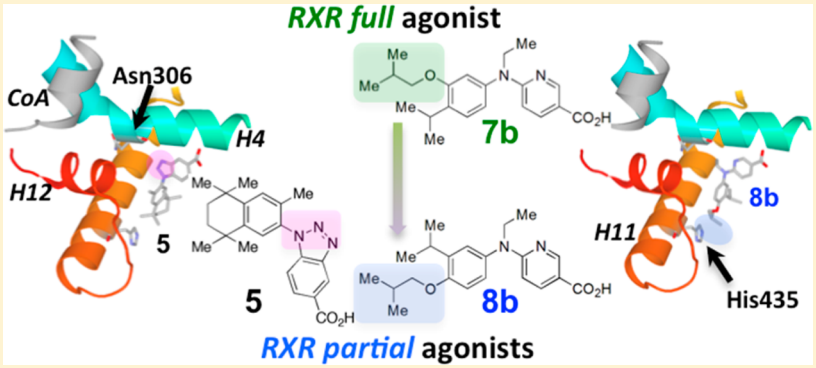
Supporting Information



ABSTRACT: We previously reported RXR partial agonist CBt-PMN (1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-1*H*-benzotriazole-5-carboxylic acid: **5**, EC_{50} = 143 nM, E_{max} = 75%), which showed a potent glucose-lowering effect without causing serious adverse effects. However, it remains important to elucidate the structural requirements for RXR efficacy and the glucose-lowering effect because RXR-permissive heterodimers such as PPAR/RXR or LXR/RXR are reported to be activated differently depending upon the chemical structure of RXR agonists. In this work, we show that an RXR partial agonist, NEt-4IB (6-[ethyl-(4-isobutoxy-3-isopropylphenyl)amino]pyridine-3-carboxylic acid: **8b**, EC_{50} = 169 nM, E_{max} = 55%), can be obtained simply by repositioning the side chains (interchanging the isobutoxy and isopropoxy groups) at the hydrophobic moiety of the RXR full agonist NEt-3IB (6-[ethyl-(3-isobutoxy-4-isopropylphenyl)amino]pyridine-3-carboxylic acid: **7b**, EC_{50} = 19 nM). NEt-4IB (**8b**) showed antitype 2 diabetes activity without the above side effects upon repeated oral administration to mice at 10 mg/kg/day, similarly to **5**.

INTRODUCTION

The pathogenesis of many diseases associated with a modern lifestyle, such as cancer, circulatory disease, type 2 diabetes, and Alzheimer's disease, often appears to involve impaired homeostasis of major body systems. Western medicine generally aims to treat such diseases with drugs targeting specific receptors or enzymes associated with each individual disease. In contrast,

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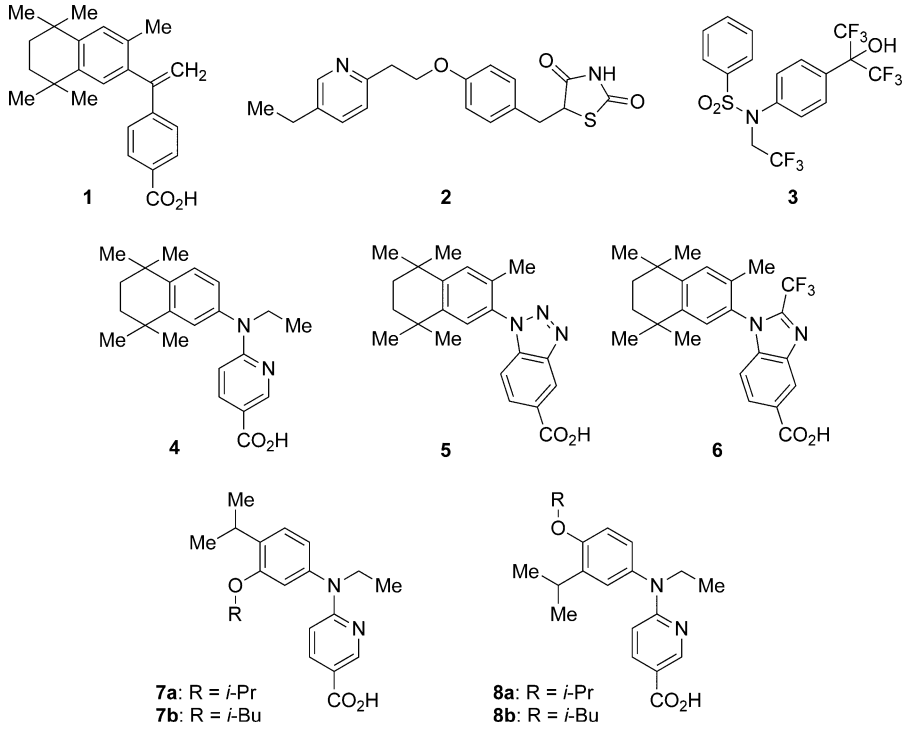

Figure 1. Chemical structures of RXR ligands **1**, **4**–**8**, PPARγ agonist **2**, and LXR agonist **3**.

traditional Chinese medicine or Japanese Kampo medicine, which is a Japanese development of traditional Chinese medicine, aims to treat such diseases by normalizing or improving patients' overall physical condition. Our aim is to develop a westernized Kampo medicine[1] by synthesizing small molecules that target receptors or enzymes but focusing on targets that play critical roles in whole homeostatic systems and regulatory networks. With this goal in mind, we have focused on retinoid X receptors (RXRs), which are key regulators of sugar/lipid metabolism.

RXRs are nuclear receptors that control gene transcription dependently upon ligand binding.[2] RXRs act as homodimers or as heterodimers with peroxisome proliferator-activated receptors (PPARs), liver X receptors (LXRs), and other receptors.[3−5] PPARs are involved in glucose/lipid metabolism, and the PPARγ agonist pioglitazone (**2**) (Figure 1) is used for the treatment of type 2 diabetes.[6] LXRs control glucose/cholesterol metabolism,[7,8] and the LXR agonist T0901317 (**3**) (Figure 1) is reported to elevate HDL cholesterol and to decrease very low density lipoprotein (VLDL), low density lipoprotein (LDL) cholesterol, and arteriosclerosis lesion area in LDL receptor knockout (LDLR KO) mice fed a high-fat diet.[9] Heterodimers of RXRs with PPARs and LXRs are known to be activated synergistically by RXR agonists in the presence of agonists of the heterodimer partners.[10,11] Moreover, interestingly, these heterodimers can be activated by RXR agonists alone even in the absence of any heterodimer partner agonist (so-called permissive effect).[12,13] Thus, targeting RXRs is a reasonable strategy to modulate glucose/lipid metabolism, which is deeply related to diseases associated with a modern lifestyle, via the synergistic or permissive effect of RXRs.

RXR agonists are well-known to be effective in the treatment of various diseases.[14] For example, RXR full agonist bexarotene (LGD1069: **1**) (Figure 1) is used for the treatment of cutaneous T cell lymphoma (CTCL) in the United States[15] and is also effective against type 2 diabetes, metabolic syndrome,[16] Alzheimer's disease,[17] and Parkinson's disease.[18] However, RXR agonists, including bexarotene (**1**) and NEt-TMN (**4**),[19,20] cause various side effects including elevation of blood triglyceride,[21,22] weight gain,[23] hepatomegaly,[23,24] and hypothyroidism.[25] Thus, we are interested in finding ways to eliminate the side effects of RXR agonists, by analogy with the idea of reviving abandoned drugs by eliminating or avoiding their side effects.[1]

The adverse effects mentioned above are all associated with RXR full agonists, which maximally activate RXRs. We considered that moderate activation of RXR might be enough to normalize the body systems, and so we focused on RXR partial agonists whose maximum activation of RXR is lower than that of RXR full agonists or whose transcriptional efficacy is restricted to a particular subset of genes. Although several RXR partial agonists have been reported,[26−28] only CBt-PMN (**5**) (Figure 1) has been evaluated in vivo.[29] This compound showed a potent glucose-lowering effect, together with improvements of insulin secretion and glucose tolerance in type 2 diabetes model mice, without inducing the serious adverse effects caused by RXR full agonists. We also discussed the mechanism of the RXR partial agonistic activity of **5** and reported that **6** is an RXR partial agonist having similar E_{max} (67 ± 2%) and lower EC_{50} (15 ± 0 nM) to those of **5** (E_{max} = 75 ± 4%, EC_{50} = 143 ± 2 nM).[30] However, the level of RXR activating efficacy required to achieve a glucose-lowering effect remains unclear. In addition, the beneficial or adverse effects of RXR agonists may be related to not only RXR activation, but also RXR heterodimer activation, because RXR agonists may differentially activate RXR heterodimers.[31,32] Thus, in this work, we created a novel RXR partial agonist having a distinct chemical structure from **5** and whose activating efficacy toward RXR is weaker than that of **5** and we evaluated its beneficial effects and side effects in mice and rats.

RESULTS AND DISCUSSION

To create new RXR partial agonists, we focused on NEt-4IP (**8a**), which was previously found during the process of developing

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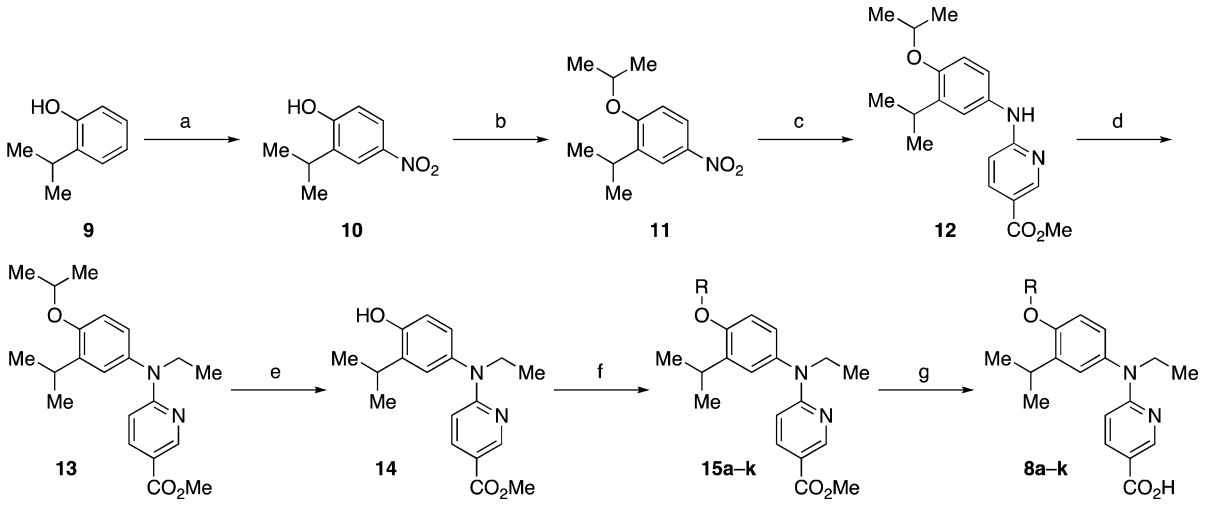
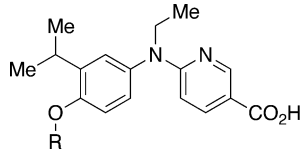
Scheme 1[a]



[a](a) HNO_3, $ZnCl_2$, EtOAc, ultrasonication, 48%; (b) *i*-PrBr, K_2CO_3, KI, DMF, 80%; (c) (1) H_2, Pd/C, MeOH, (2) 6-chloronicotinic acid, $MeSO_3H$, dioxane, (3) H_2SO_4, MeOH, 73%; (d) Et-I, NaH, DMF, 75%; (e) $AlCl_3$, CH_2Cl_2, 97%; (f) R-X, K_2CO_3, KI, DMF, 16–94%; (g) (1) 2 N NaOH, MeOH, (2) 2 N HCl, 12%–93%.

Table 1. Transactivation Activity of Compounds 8a–k toward RXRα in COS-1 Cells



		RXRα[a,b]				
		efficacy (%)				
compd	R	1 μM	10 μM	EC_{50} (nM)[c]	E_{max} (%)[c]	lowest conc for E_{max} (μM)[c]
1		100	85 ± 6	22 ± 2	100	3.8 ± 0.9
8a	*i*-Pr	72 ± 1	83 ± 3	293 ± 29	83 ± 2	4.6 ± 1.5
8b	*i*-Bu	53 ± 0	54 ± 3	169 ± 3	55 ± 2	4.0 ± 1.5
8c	*c*-Pr-CH_2	52 ± 6	64 ± 0	324 ± 62	62 ± 2	14.7 ± 7.7
8d	CH_2=CMe-CH_2	78 ± 6	83 ± 6	218 ± 55	83 ± 3	12.9 ± 10.1
8e	Me_2C=CH-CH_2	77 ± 2	84 ± 2	268 ± 32	84 ± 3	8.4 ± 5.9
8f	*n*-Bu	53 ± 3	75 ± 3	567 ± 57	73 ± 3	20.0 ± 0.0
8g	*n*-Pen	37 ± 2	58 ± 3	772 ± 80	57 ± 4	12.7 ± 8.7
8h	*n*-Hex	28 ± 1	51 ± 1	918 ± 53	52 ± 1	28.7 ± 4.3
8i	Ph-CH_2	52 ± 1	75 ± 1	540 ± 4	73 ± 1	24.3 ± 4.3
8j	Ph-$(CH_2)_2$	15 ± 3	49 ± 2	1130 ± 60	45 ± 1	4.0 ± 5.8
8k	Ph-$(CH_2)_3$	20 ± 2	44 ± 2	1030 ± 80	42 ± 2	10.0 ± 5.0

[a]COS-1 cells were transfected with three kinds of vectors consisting of RXRα, 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. [b]The relative transactivation activity is based on the luciferase activity of 1 μM **1** (RXR full agonist) taken as 100%. All values represent the mean value of at least three separate experiments with triplicate determinations. [c]Each value was determined from full dose–response curves.

RXR full agonists NEt-3IP (**7a**) and NEt-3IB (**7b**), because **8a** showed a lower RXR activation efficacy than either **7a** or **7b**.[33] Additionally, because changing the isopropoxy group of **7a** to various other alkoxy groups afforded ligands with distinct RXR heterodimer activation properties,[30] we applied this approach to the isopropoxy group of **8a**.

Various alkoxy derivatives were synthesized according to Scheme 1. 2-Isopropylphenol (**12**) was nitrated in the presence of zinc chloride under ultrasonication to give **13**, and the hydroxyl group was converted to an isopropoxy group to afford **14**. Reduction of the nitro group was performed, and the coupling reaction of the resulting amine and 6-chloronicotinic acid in the presence of mesyl acid as a catalyst gave **15**. Next, **16** was obtained by *N*-ethylation of the linking nitrogen, and deprotection of the isopropoxy group of **16** with aluminum chloride afforded common intermediate **17**. Reaction of **17** with various alkyl halides and hydrolysis of the methyl ester gave the target molecules **8a–j**.

RXR-activating efficacy of the compounds obtained was evaluated using a luciferase transcription assay in COS-1 cells. The data are shown in Table 1 as relative values with respect to 100% activation by RXR full agonist **1** at 1 μM. All the compounds synthesized showed all reduced E_{max} values compared to **1**. Because we wished to know whether compounds whose RXR activation efficacy is lower than that of **5** show pharmacological

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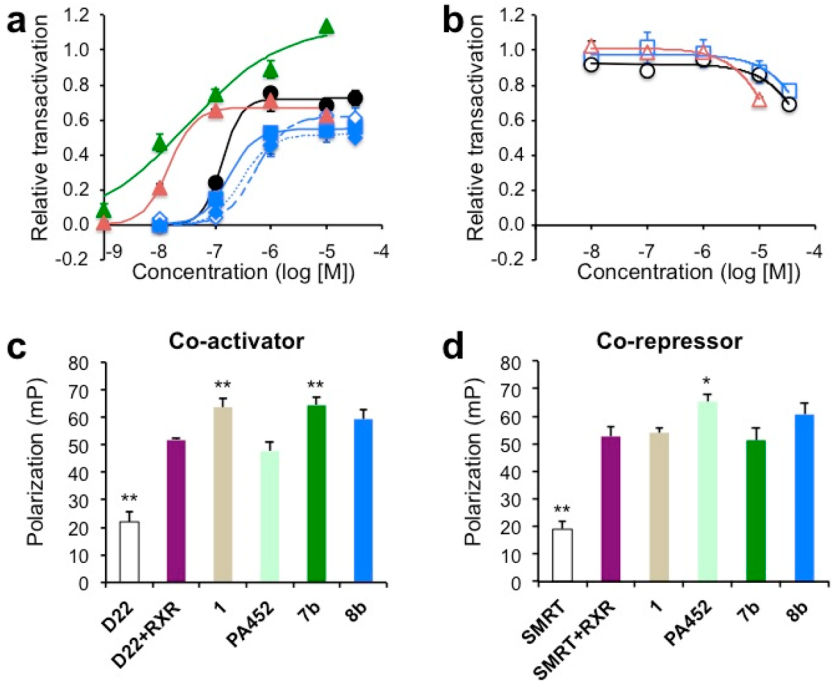

Figure 2. Evaluation of transactivation activity toward RXRα and cofactor recruitment. (a) Relative transactivation activity of CBt-PMN (**5**: closed black circles), CBTF-PMN (**6**: closed purplish-red triangles), NEt-3IB (**7b**: closed green triangles), and **8b** (closed blue squares) toward RXRα. Blue broken lines and dotted lines show the activity of **8b** toward RXRβ and RXRγ, respectively. COS-1 cells were transfected with three kinds of vectors consisting of an RXR 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 results for **5** and **6** are taken from ref 30. (b) Relative transactivation activity of **5** with 1 μM **1** (open black circles), **6** with 1 μM **1** (open purplish-red triangles), or **8b** with 1 μM **1** (open blue squares) toward RXRα. Luciferase activity of **1** at 1 μM was defined as 1. Data shown are the average (n = 3) ± SEM. The results for **5** and **6** were taken from ref 30. (c,d) Changes in fluorescence polarization of each fluorescein-labeled cofactor with RXR in the presence or absence of each test compound. (c) 5 nM fluorescein-labeled coactivator peptide D22. (d) Fluorescein-labeled corepressor peptide SMRT-ID2 (5 nM). Fluorescence polarization values, expressed in mP, are the average (n = 3) ± SEM. The data were analyzed by one-way ANOVA followed by Bonferroni test. *, $p < 0.05$; **, $p < 0.01$ vs cofactor peptide with RXR. Results for D22, D22 + RXR, **1**, and PA452 were taken from ref 30 because these experiments were performed at the same time.

effects or not, we focused on compound **8b** (NEt-4IB), whose E_{max} value was lowest of all and EC_{50} value was lower than **8h** (Table 1).

Evaluation of dose-dependency toward RXR revealed that compound **8b** showed 50−60% efficacy toward not only RXRα but also RXRβ and RXRγ (Figure 2a). To confirm the RXR partial antagonist character of **5**, **6**, and **8b**, they were each examined in combination with RXR full agonist **1** at 1 μM (Figure 2b). Like **5** and **6**, **8b** showed partial inhibition of the activity of **1** in proportion to the concentration of **8b**, confirming that **8b** is an RXR partial agonist.

RXRs bind to a corepressor (CoR), which inhibits DNA transcription, in the absence of agonists. Binding of RXRs to agonists induces conformational change of helix 12 of RXRs and promotes dissociation of CoR and association of a coactivator (CoA), leading to induction of DNA transcription of target genes.[34] That is, RXR ligands that favor a stable RXR−CoA complex are RXR full agonists, while RXR ligands that favor a stable RXR−CoR complex are RXR full antagonists. RXR partial agonists are thought to induce an equilibrium of the two complexes. These phenomena can be detected by fluorescence polarization (FP) measurements using fluorescence-labeled cofactor peptides.[30] Thus, cofactor behavior was examined by FP assay in the presence of an RXR full agonist, an RXR full antagonist, an RXR partial agonist, or **8b** (Figure 2c,d). The FP value of **8b** for CoA was much higher than that of RXR antagonist PA452,[35] although lower than that of RXR full agonist **1** or **7b** (Figure 2c). On the other hand, the FP value of PA452 for CoR was clearly increased, while that of RXR full agonist **1** or **7b** was not increased and that of **8b** showed a nonsignificant increase. Taken together, these results indicate that compound **8b** recruited both CoA and CoR and support the conclusion that **8b** is an RXR partial agonist.

We found that **8b** at 10 μM did not activate any RAR subtype (Figure 3a). We also examined heterodimer activation by **8b**. Although **8b** did not activate PPARγ or LXRα in the absence of RXRα (Figure 3c,e), it induced activation of LXRα/RXRα or PPARγ/RXRα heterodimers generated by cotransfection of RXRα with PPARγ or LXRα, i.e., permissive activation occurred (Figure 3d,f). In addition, **8b** also showed RXRα partial agonistic activity with HepG2 instead of COS-1 as host cells (Supporting Information, Figure S1a). On the other hand, PPARγ/RXRα activation by **8b** was similar to that by **5**, and LXRα/RXRα activation by **8b** was much lower than that by **5** or **7b** (Supporting Information, Figure S1b,c).

Next, to understand the mechanism of the RXR partial agonistic activity of **8b**, we performed docking simulation using AutoDock4.2 (Figure 4).[40] Compounds **5** and **6** are proposed to induce RXR partial activation not by direct interference with helix 12 but via electrostatic repulsion with Asn306 in helix 4.[30] Our simulation predicted that the isobutoxy group at the 4′ position on the hydrophilic phenyl group of **8b** is located close to His435 in helix 11 of RXR. Steric interference between the isobutoxy group of **8b** and His435 is thought to influence helix 12 indirectly, which might produce a conformation that allows RXRs to bind either CoA or CoR to produce RXR partial activation.

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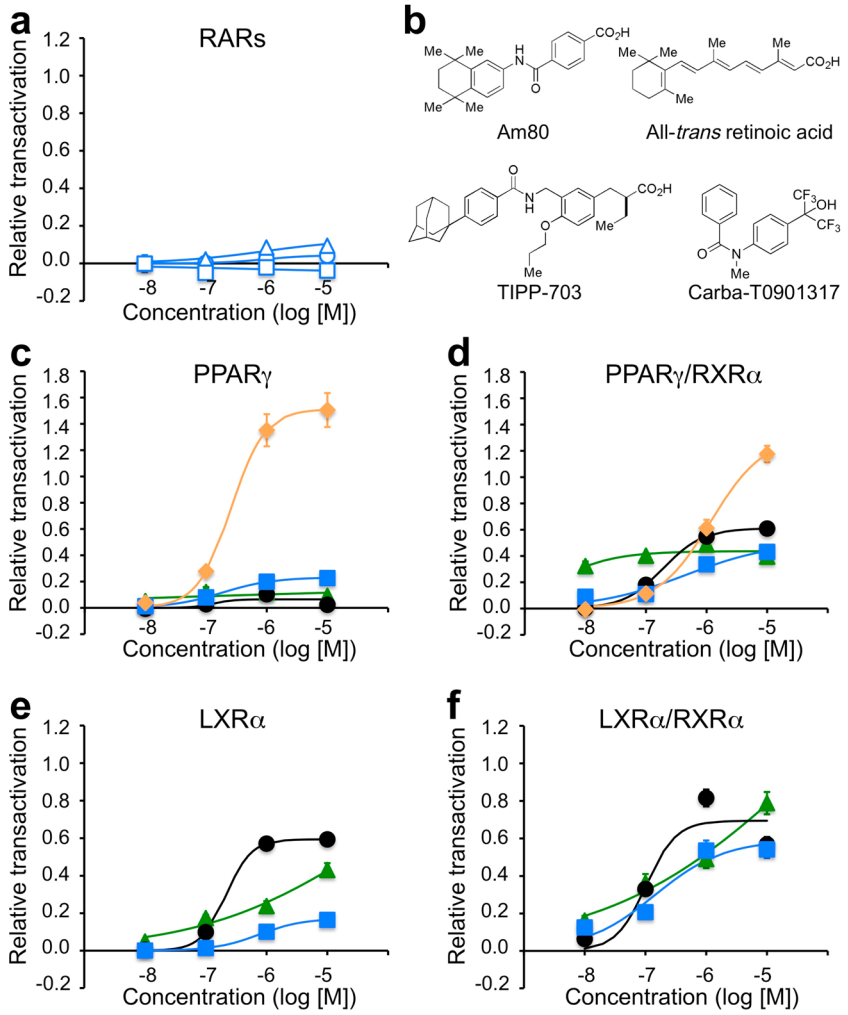

Figure 3. Relative transactivation activity of **5**, **7b**, and **8b** toward RARα, RARβ, RARγ, PPARγ, PPARγ/RXRα, LXRα, and LXRα/RXRα using COS-1 cells. (a) Relative transactivation activity of **8b** toward RARα (open blue circles), RARβ (open blue triangles), and RARγ (open blue squares). COS-1 cells were transfected with three kinds of vectors: each RAR receptor subtype, a luciferase reporter gene under the control of the appropriate RAR response element (tk-TREPII-Luc), and secreted alkaline phosphatase (SEAP) gene as a background. Luciferase activity of Am80 (RARα/β dual agonist) or ATRA (RAR pan agonist) at 1 μM was defined as 1 for RARα and RARβ or for RARγ, respectively. Data shown are the average (n = 3) ± SEM. (b) Chemical structures of Am80 (references36,37), all-*trans* retinoic acid, TIPP-703 (ref 38) (PPAR pan agonist), and carba-T0901317 (ref 39) (LXR pan agonist). (c–f) Relative transactivation activity of **5** (closed black circles), **7b** (closed green triangles), and **8b** (closed blue squares) toward LXRα, LXRα/RXRα, PPARγ, and PPARγ/RXRα. (c,d) Relative transactivation activity of **2** (closed orange diamonds) toward PPARγ and PPARγ/RXRα. COS-1 cells were transfected with four kinds of vectors, consisting of RXRα, a partner receptor (PPARγ or LXRα), the partner response element (tk-PPREx3-Luc for PPARγ or tk-rBARx3-Luc for LXRα), and secreted alkaline phosphatase (SEAP) gene as a background. Luciferase activity of TIPP-703 (PPAR pan agonist) or carba-T0901317 (LXR pan agonist) at 1 μM was defined as 1 for LXRα or LXRα/RXRα, PPARγ, or PPARγ/RXRα, respectively. Data shown are the average (n = 3) ± SEM. The data for **5** was taken from ref 29.

The results suggest that **5** and **8b** induce partial activation through different mechanisms.

In a preliminary in vivo study of **8b**, the blood concentration after oral administration was evaluated and compared with that of RXR full agonist NEt-3IB (**7b**), a regioisomer of **8b**. Single oral administration of NEt-4IB (**8b**) at 30 mg/kg gave a blood concentration of about 13 μM **8b** at 1 h, which is higher than the concentration producing E_{max} (Figure 5a). The blood concentration after oral administration of **7b** at 30 mg/kg reached about 0.75 μM at 1 h, which again is higher than the concentration producing E_{max}. The reason why NEt-3IB (**7b**) and NEt-4IB (**8b**) gave significantly different blood concentrations was examined by using positron emission tomography (PET) tracers, and the results will be reported elsewhere.[42] Briefly, although many factors are known to affect absorption, we found that higher intestinal absorbability and lower biliary excretion contribute to the higher blood concentration of **8b** after oral administration compared with **7b**. However, because oral administration of each compound at 30 mg/kg gave a sufficient blood concentration to obtain E_{max} in each case, adverse effects were evaluated at this dosage.

Compounds **7b** and **8b** were repeatedly administered orally to mice or rats at 30 mg/kg/day, and weight change and other parameters were evaluated. Figure 5b,c,d and Table 2 show weight change, liver weight, blood triglyceride, and other blood parameters before and after consecutive administration for 7 days in mice. The changes of liver weight and blood triglyceride in the group treated with **8b** were similar to those in the case of the vehicle control, whereas those in the group treated with **7b** were significantly increased (Figure 5c,d and Table 2); however, there was no significant difference in weight change between the groups given **7b** and **8b** (Figure 5b). We previously found that

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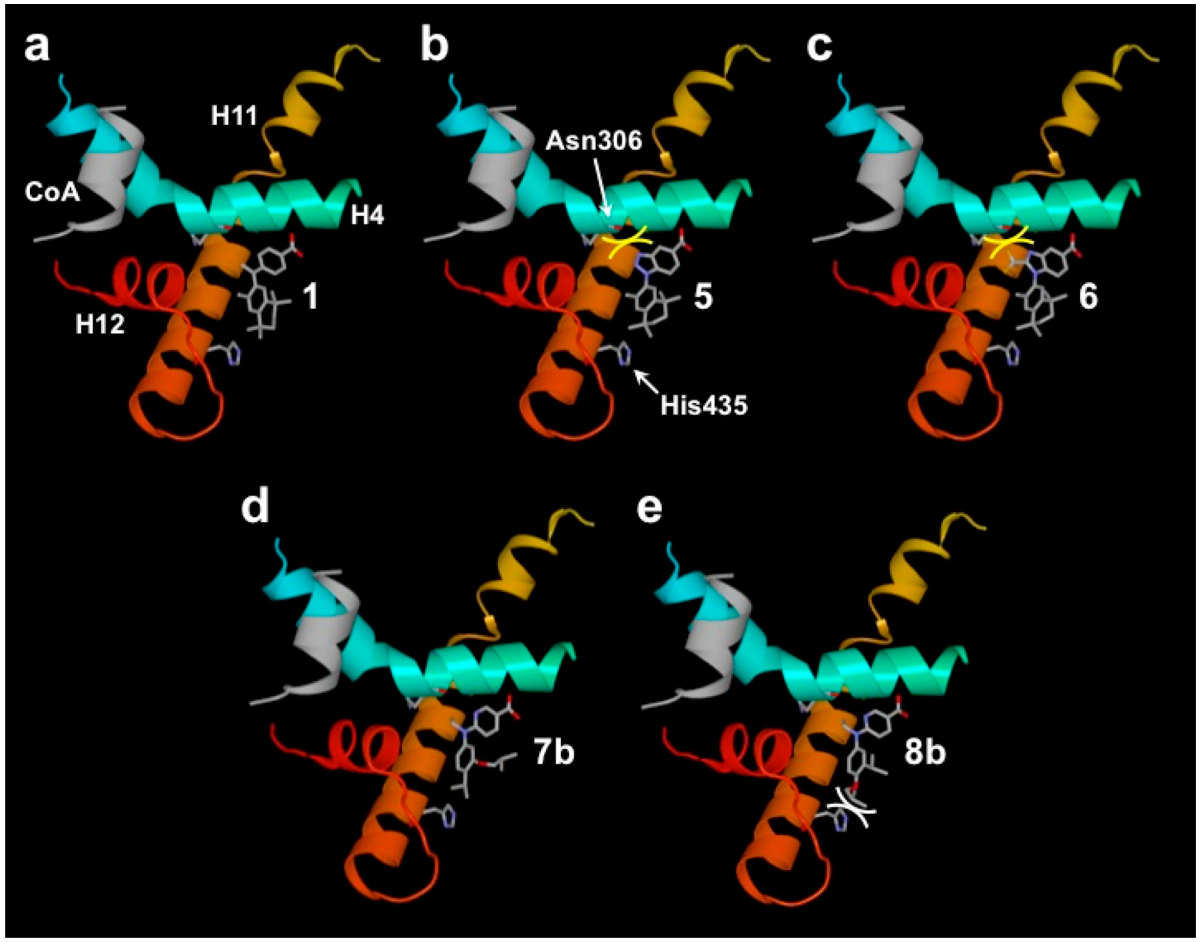

Figure 4. Proposed docking structures of each RXR agonist in the RXRα LBP obtained with AutoDock4.2 (ref 40). (a) Crystal structure of RXRα LBD in complex with **1** (pdb code: 4K6I) (ref 41). (b–e) Docking models obtained with AutoDock4.2. a, c, d, and e were modeled with **5**, **6**, **7b**, and **8b**, respectively. Electronic and steric repulsions were indicated by yellow and white curves, respectively.

RXR full agonists **4**, **7a**, and **7b** similarly activate RXR but differently activate PPAR/RXR and LXR/RXR,[30] and **7b** induced lower degrees of hepatomegaly and blood triglyceride elevation than **4** and **7a**, even though all of them showed similar blood glucose-lowering action on repeated administration.[20] Because the liver weight and blood triglyceride increases induced by **7b** after consecutive administration for 7 days in mice were smaller than those caused by RXR full agonists **4** and **7a**,[20] **8b** clearly shows reduced side effects compared to the full agonists. Among other blood parameters examined, only blood urea nitrogen (BUN) was elevated by **8b** (Table 2). The drug exposures in the single-dose pharmacokinetic studies of **7b** or **8b** would not necessarily reflect those in the multiday accumulated dosing studies, but nevertheless, these results at least indicate that **8b** shows reduced side effects compared to **7b**. Further studies, including evaluation of drug exposures and dose-dependency studies, would be needed to evaluate the efficacy in detail, because many factors, such as cascade effects, total exposure, and peak-to-trough ratio can influence agonist effects. And, although further investigation is required, a PET tracer experiment in mice revealed that excretion of **8b** is not renal, but biliary,[42] so that renal function should not be an issue. In rats administered **8b** orally at 30 mg/kg/day for consecutive 28 days, food intake, water intake, and body weight change were similar to those of the vehicle control group (Supporting Information, Figure S2). The RXR full agonist **7b** produced hepatomegaly, whereas RXR partial agonist **8b** did not (Supporting Information, Table S1). Although blood triglyceride was elevated in females by **8b**, the increase was less than that caused by **7b**. Other blood parameters were not impaired by **8b** but were by **7b** (Supporting Information, Table S2). In short, **8b** did not induce significant weight gain, hepatomegaly, or TG elevation even after prolonged administration. These results strongly suggest that the partial agonist character of **8b** is the reason for the reduced incidence of adverse effects. Nevertheless, we cannot completely rule out the possibilities that the adverse effects of **7b** are due in part to off-target activities or are caused in part by its metabolites, and further studies are desirable to confirm the critical role of partial antagonism.

Because **8b** had no significant adverse effect upon repeated oral administration at 30 mg/kg/day, we next examined its antitype 2 diabetes effect in an animal model because not only RXR partial agonists **5** and **6** but also RXR full agonist **7b** show a glucose-lowering effect in KK-A^{y} type 2 diabetes model mice.[29,43] The insulin sensitizer pioglitazone (**2**) was used as a positive control. Repeated oral administration of **2** at 10 mg/kg/day reduced water intake similarly to **8b** (Figure 6b) and caused significant weight gain (Figure 6c) and hepatomegaly (Table 3), but did not reduce glucose, in accordance with the findings of Arakawa et al.[44] On the other hand, **8b** at the same dosage showed a marked glucose-lowering effect (Figure 6a–d, Table 3) and a tendency for improvement of OGTT (Figure 6e) without

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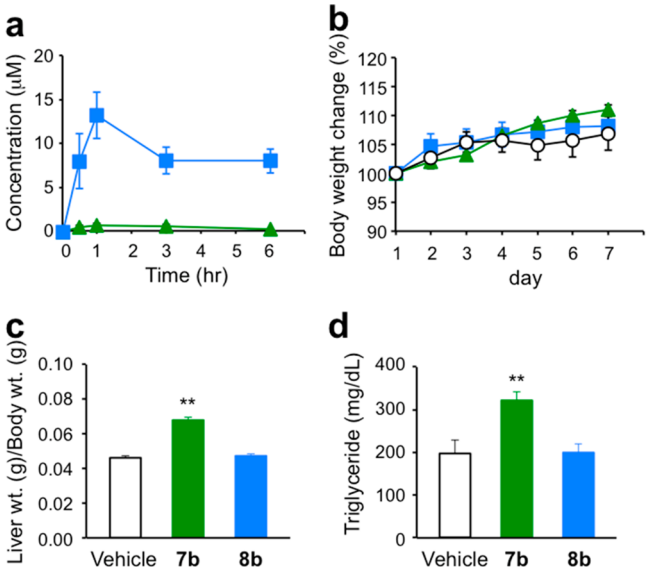

Figure 5. Comparison between compounds **7b** and **8b** in male ICR mice. (a) Plasma concentrations of **7b** (closed green triangles) and **8b** (closed blue squares) (single oral dose of 30 mg/kg). The horizontal scale is elapsed time in hours, and the vertical scale is normalized by the micromolar concentration. Data shown are the average (n = 4–7) ± SEM. (b–d) Evaluation of adverse effects of repeated oral administration of vehicle (open), **7b** (green), and **8b** (blue) at 30 mg/kg/day to male ICR mice for 7 consecutive days. (b) Time course of body weight change for 7 days. Effects of compounds on (c) liver weight/body weight and (d) serum triglyceride. Data shown are the average (n = 8) ± SEM. The data were analyzed by one-way ANOVA followed by Bonferroni test. **: $p < 0.01$ vs vehicle.

significant weight gain or hepatomegaly. Because this dosage is lower than that used to evaluate adverse effects, it appears that this compound produces antitype 2 diabetes effects without the adverse effects produced by RXR full agonists. RT-PCR examination of changes in the expression levels of genes associated with glucose/lipid metabolism in the liver of KK-A^y mice (Figure 6f) showed that **8b** increased *Gck* similarly to RXR partial agonist **5**,[29] although the difference from the vehicle control was not significant. Thus, it is possible that one of the mechanisms of the glucose-lowering effect of **5** is induction of glycolysis via *Gck*. In Ames tests using five strains, **8b** did not exhibit mutagenic properties (Supporting Information, Table S3). These results indicate that **8b** is a promising candidate drug for the treatment of type 2 diabetes.

CONCLUSION

Compound **8b** is an RXR partial agonist with a maximum RXR efficacy of 55% in luciferase reporter gene assay and shows an antitype 2 diabetes effect without the serious side effects caused by full agonists in our animal models. Because RXR full agonists are reported to show therapeutic effects against not only type 2 diabetes but also Alzheimer's disease and Parkinson's disease in animal models, our findings also suggest that RXR partial agonists may be candidate therapeutic agents for those diseases too. The mechanism of the activity of **8b** appears to be distinct from those of other partial agonists involving steric interaction with His435 in helix 11 of RXR. This mechanism may open up a new approach to the design of RXR partial agonists as candidate drugs.

EXPERIMENTAL SECTION

Chemistry. *General.* Flash column chromatography was performed using Kanto Chemical Silica Gel 60 (spherical) 40–50 μm. Melting points were determined with a Yanagimoto hot-stage melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a JEOL JMN-AL-300 (300 MHz), Varian VXR-300 (300 MHz), or Varian VXR-500 (500 MHz) spectrometer. ^{13}C NMR spectra were recorded on a Varian VXR-400 (100 MHz) spectrometer. The purity of all tested compounds was >95%, as confirmed by combustion analysis. Combustion analysis was carried out with a Yanagimoto MT-5 CHN recorder elemental analyzer, and results were within ±0.4% of the theoretical values. FAB-MS was carried out with a VG70-SE.

*4-[1-(3,5,5,8,8-Pentamethyl-5,6,7,8-tetrahydronaphthalen-2-yl)-ethenyl]benzoic Acid (**1**).* This compound was prepared according to ref 45.

*NEt-3IB (**7b**) and NEt-4IP (**8a**).* These compounds were prepared according to ref 33.

*2-Isopropyl-4-nitrophenol (**10**).* Conc HNO_3 (3.4 mL, 45 mmol) was added to a solution of 2-isopropylphenol (6.1 mL, 45 mmol) in EtOAc (200 mL) in an ice bath. The reaction mixture was placed in an ultrasonic reactor, $ZnCl_2$ (6.1 g, 45 mmol) was added, and the whole was stirred for 0.8 h. The reaction mixture was washed with H_2O (2 × 100 mL) and brine (100 mL) and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:5) to yield **10** (4.9 g, 60%) as a brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.13 (d, J = 2.8 Hz, 1H), 8.00 (dd, J = 9.0, 2.8 Hz, 1H), 6.83 (d, J = 9.0 Hz, 1H), 6.04 (s, 1H), 3.26 (sep, J = 7.0 Hz, 1H), 1.30 (d, J = 7.0 Hz, 6H).

*Isopropoxy-2-isopropyl-4-nitrobenzene (**11**).* 2-Bromopropane (990 μL, 11 mmol), K_2CO_3 (2.6 g, 19 mmol), and KI (800 mg, 4.8 mmol) were added to a solution of **10** (1.7 g, 9.6 mmol) in dry DMF (4.8 mL). The reaction mixture was stirred at 70 °C under an Ar atmosphere for 3.0 h, then poured into 2 N HCl (70 mL) and extracted

Table 2. Serum Parameters of Male ICR Mice after Oral Administration of Vehicle, 7b, or 8b at 30 mg/kg/day for 7 Consecutive Days[a]

	vehicle	7b	8b	reference value[c]
AST (U/I)	47.6 ± 4.9	77.5 ± 22	48.4 ± 4.0	80.5 ± 67
ALT (U/I)	16.6 ± 0.87	30.9 ± 4.7**	23.4 ± 1.2	45 ± 16
γ-GTP (U/I)	5.38 ± 0.38	5.88 ± 0.23	6.00 ± 0.33	
ALP (U/I)	327 ± 28	594 ± 43**	367 ± 32	
CRE (mg/dL)	ND[b]	ND	ND	
BUN (mg/dL)	23.6 ± 1.6	25.7 ± 1.3	30.7 ± 2.9**	14 ± 3
TG (mg/dL)	197 ± 33	321 ± 22**	199 ± 22	192 ± 66
TCHO (mg/dL)	126 ± 9.3	182 ± 23**	160 ± 8.1	164 ± 27
GLU (mg/dL)	91.1 ± 8.2	216 ± 25**	129 ± 8.7	164 ± 27

[a]Data shown are the average (n = 8) ± SEM. The data were analyzed by one-way ANOVA followed by Bonferroni test. Significant differences: **$p < 0.01$ vs vehicle. [b]ND means not detected. [c]Taken from the Clinical Laboratory Parameters for Crl:CD1(ICR) Mice (50–70 day old) by Charles River. Analyzing equipment: Alfa Wassermann Ace Alera.

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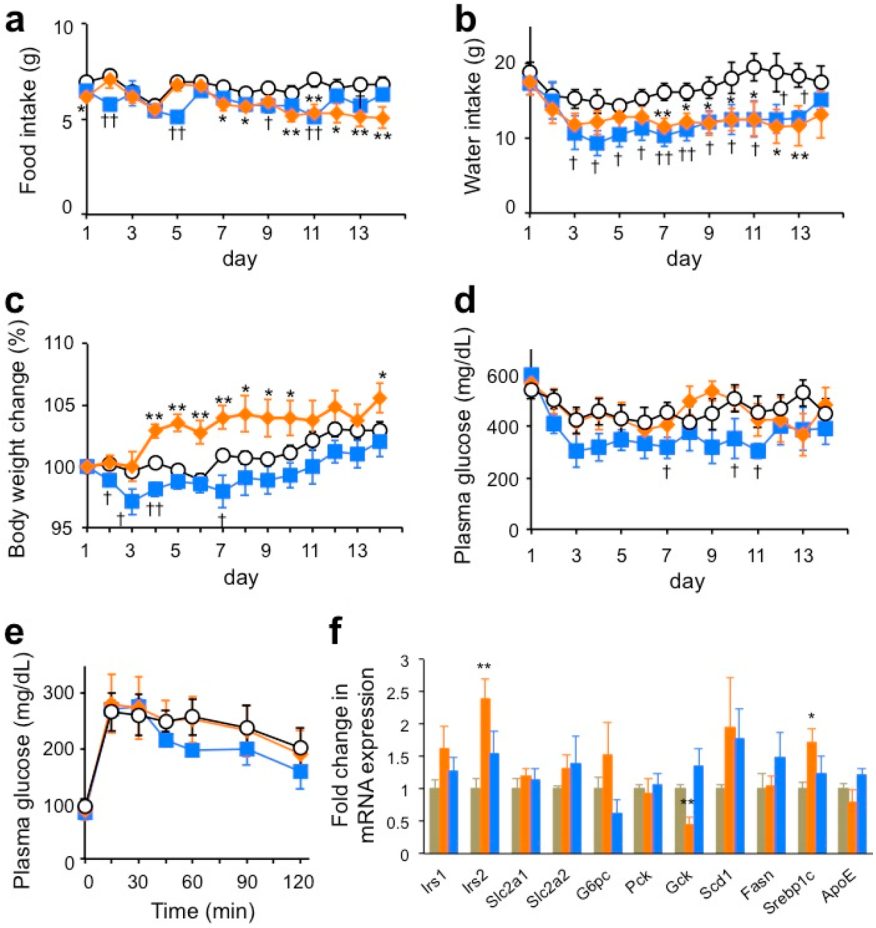

Figure 6. Evaluation of antitype 2 diabetes effects of repeated oral administration of pioglitazone (**2**: closed orange diamonds) or **8b** (closed blue squares) at 10 mg/kg/day to male KK-A^y mice for 14 consecutive days. Open black circles indicate vehicle treatment. Time course of (a) food intake, (b) water intake, (c) body weight change, and (d) blood glucose levels. Data shown are the average (n = 4−6) ± SEM. The data were analyzed by one-way ANOVA followed by Bonferroni test. Significant differences: * (for **2**), † (for **8b**) $p < 0.05$; ** (for **2**), †† (for **8b**) $p < 0.01$, vs vehicle, respectively. (e) Oral glucose tolerance test (OGTT) results of male KK-A^y mice treated with vehicle (open black circles), pioglitazone (**2**) (closed orange diamonds), or **8b** (closed blue squares) at 10 mg/kg/day for 14 consecutive days (n = 4−5). (f) Fold changes in mRNA expression of *Irs1* (i), *Irs2* (ii), *Slc2a1* (iii), *Slc2a2* (iv), *G6pc* (v), *Pck* (vi), *Gck* (vii), *Scd1* (viii), *Fasn* (ix), *Srebp1c* (x), and *ApoE* (xi) in liver tissue of male KK-A^y mice treated with vehicle, **2**, or **8b** at 10 mg/kg/day for 14 consecutive days (n = 3−4). The gray, orange, and blue bars indicate vehicle, **2**, and **8b** treatment, respectively. Data shown are the average ± SEM. The data were analyzed by one-way ANOVA followed by Bonferroni test. Significant differences: *$p < 0.05$ or **$p < 0.01$ vs vehicle. Data for vehicle and pioglitazone (**2**) were taken from ref 30 because these experiments were performed at the same time.

Table 3. Liver Weight and Serum Parameters of Male KK-A^y Mice after Oral Administration of Vehicle, Pioglitazone (2), or 8b at 10 mg/kg/day for 14 Consecutive Days[a]

	vehicle	2	8b
liver weight/body weight (%)	3.78 ± 0.096	4.95 ± 0.33**	3.68 ± 0.18
AST (U/I)	49.6 ± 2.3	45.3 ± 8.4	41.8 ± 1.9
ALT (U/I)	17.6 ± 0.93	21.3 ± 1.9*	17.0 ± 0.58
γ-GTP (U/I)	5.80 ± 0.58	7.33 ± 2.3	5.25 ± 0.63
ALP (U/I)	358 ± 43	396 ± 44	397 ± 40
CRE (mg/dL)	ND[b]	ND	ND
BUN (mg/dL)	21.9 ± 1.1	22.2 ± 1.3	21.6 ± 1.8
TG (mg/dL)	122 ± 11	124 ± 28	101 ± 12
TCHO (mg/dL)	153 ± 11	147 ± 3.8	146 ± 6.5

[a]Data shown are the average (n = 3−5) ± SEM. The data were analyzed by one-way ANOVA followed by Bonferroni test. Significant differences: **$p < 0.01$ vs vehicle. [b]ND means "not detected".

with EtOAc (3 × 80 mL). The organic layer was collected, washed with H_2O (2 × 80 mL) and brine (80 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:15) to yield **11** (1.7 g, 80%) as an orange oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.13 (d, J = 2.5 Hz, 1H), 8.07 (dd, J = 9.0, 3.0 Hz, 1H), 6.86 (d, J = 9.0 Hz, 1H), 4.70 (sep, J = 6.0 Hz, 1H), 3.31 (sep, J = 7.0 Hz, 1H), 1.40 (d, J = 6.0 Hz, 6H), 1.24 (d, J = 7.0 Hz, 6H).

*Methyl 6-[(4-Isopropoxy-3-isopropylphenyl)amino]pyridine-3-carboxylate (**12**).* Activated 10% Pd−C (catalytic amount) was added to a solution of **11** (4.7 g, 21 mmol) in MeOH (30 mL). The reaction mixture was stirred under an H_2 atmosphere at room temperature for 5.5 h, then filtered through Celite, and the Celite cake was washed with EtOAc. The combined filtrate and washing were evaporated under reduced pressure. 6-Chloronicotinic acid (3.7 g, 23 mmol) and $MeSO_3H$ (1.5 mL, 23 mmol) were added to a solution of the residue in dry dioxane (30 mL). This mixture was stirred at 120 °C under an Ar atmosphere for 17 h and then evaporated under reduced pressure. Conc H_2SO_4 (2.0 mL) was added to a solution of the residue in dry MeOH (20 mL). The reaction mixture was stirred at 80 °C under an Ar atmosphere for 5.7 h, evaporated under reduced pressure, neutralized with 2 N NaOH, and then extracted with EtOAc (3 × 80 mL). The organic layer was collected, washed with H_2O (2 × 100 mL) and brine (100 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:8 to 1:5) to yield **12** (5.6 g, 73%) as a

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brown solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.79 (d, J = 2.0 Hz, 1H), 8.00 (dd, J = 9.0, 2.0 Hz, 1H), 7.12−7.09 (m, 2H), 6.90 (s, 1H), 6.85 (d, J = 9.0 Hz, 1H), 6.64 (d, J = 9.0 Hz, 1H), 4.54 (sep, J = 6.0 Hz, 1H), 3.88 (s, 3H), 3.33 (sep, J = 7.0 Hz, 1H), 1.36 (d, J = 6.0 Hz, 6H), 1.20 (d, J = 7.0 Hz, 6H).

*Methyl 6-[Ethyl-(4-isopropoxy-3-isopropylphenyl)amino]-pyridine-3-carboxylate (**13**).* Compound **12** (1.2 g, 3.6 mmol) was added to a suspension of NaH (200 mg, 5.0 mmol, 60% dispersion in oil) in dry DMF (6.0 mL) at rt under an Ar atmosphere. The mixture was stirred for 5 min, then iodoethane (400 μL, 5.0 mmol) was added, and stirring was continued for 0.3 h. The reaction mixture was poured onto ice and extracted with EtOAc (3 × 40 mL). The organic layer was collected, washed with H_2O (2 × 50 mL) and brine (30 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:5 to 1:1) to yield **13** (960 mg, 75%) as a brown oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.83 (d, J = 2.5 Hz, 1H), 7.77 (dd, J = 9.0, 2.5 Hz, 1H), 7.01 (d, J = 2.5 Hz, 1H), 6.95 (dd, J = 8.5, 2.5 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.15 (d, J = 9.0 Hz, 1H), 4.58 (sep, J = 6.0 Hz, 1H), 4.00 (q, J = 7.0 Hz, 2H), 3.85 (s, 3H), 3.32 (sep, J = 7.0 Hz, 1H), 1.38 (d, J = 6.0 Hz, 6H), 1.22 (t, J = 7.0 Hz, 3H), 1.19 (d, J = 7.0 Hz, 6H).

*Methyl 6-[Ethyl-(4-hydroxy-3-isopropylphenyl)amino]pyridine-3-carboxylate (**14**).* $AlCl_3$ (1.2 g, 9.0 mmol) was added to a solution of **13** (960 mg, 2.7 mmol) in dry CH_2Cl_2 (14 mL). The reaction mixture was stirred at rt under an Ar atmosphere for 3.5 h, then poured into H_2O (100 mL) and extracted with EtOAc (3 × 50 mL). The organic layer was collected, washed with H_2O (2 × 50 mL) and brine (50 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:4) to yield **14** (820 mg, 97%) as a pale-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.83 (d, J = 2.5 Hz, 1H), 7.78 (dd, J = 9.0, 2.5 Hz, 1H), 7.02 (d, J = 2.5 Hz, 1H), 6.90 (dd, J = 8.5, 2.5 Hz, 1H), 6.82 (d, J = 8.5 Hz, 1H), 6.15 (d, J = 9.0 Hz, 1H), 5.06 (s, 1H), 4.00 (q, J = 7.0 Hz, 2H), 3.86 (s, 3H), 3.22 (sep, J = 7.0 Hz, 1H), 1.25 (d, J = 7.0 Hz, 6H), 1.24 (t, J = 7.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 166.89, 160.79, 152.44, 151.05, 137.44, 136.78, 136.15, 126.34, 116.45, 113.96, 107.35, 51.68, 45.48, 27.10, 22.46, 12.94. FAB-MS m/z: 315 $[M + H]^+$.

*Methyl 6-[Ethyl-(4-isobutoxy-3-isopropylphenyl)amino]pyridine-3-carboxylate (**15b**).* 1-Bromo-2-methylpropane (370 μL, 3.4 mmol), K_2CO_3 (240 mg, 1.7 mmol), and KI (catalytic amount) were added to a solution of **14** (310 mg, 1.0 mmol) in dry DMF (10 mL). The reaction mixture was stirred at 60 °C under an Ar atmosphere for 18 h, then poured into H_2O (100 mL) and extracted with EtOAc (3 × 30 mL). The organic layer was collected, washed with H_2O (2 × 50 mL) and brine (30 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:6) to yield **15b** (300 mg, 81%) as a pale-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.83 (d, J = 2.5 Hz, 1H), 7.77 (dd, J = 9.0, 2.5 Hz, 1H), 7.62 (d, J = 2.5 Hz, 1H), 6.97 (dd, J = 8.5, 2.5 Hz, 1H), 6.86 (d, J = 8.5 Hz, 1H), 6.14 (d, J = 9.0 Hz, 1H), 4.00 (q, J = 7.0 Hz, 2H), 3.85 (s, 3H), 3.77 (sep, J = 6.0 Hz,1H), 3.36 (sep, J = 7.0 Hz, 1H), 2.22−2.08 (m, 1H), 1.22 (d, J = 7.0 Hz, 6H), 1.22 (t, J = 7.0 Hz, 3H), 1.08 (d, J = 6.5 Hz, 6H).

*Methyl 6-[Ethyl-(4-cyclopropylmethoxy-3-isopropylphenyl)-amino]pyridine-3-carboxylate (**15c**).* (Bromomethyl)cyclopropane (100 μL, 1.1 mmol), K_2CO_3 (190 mg, 1.4 mmol), and KI (60 mg, 0.35 mmol) were added to a solution of **14** (220 mg, 0.69 mmol) in dry DMF (3.0 mL). The reaction mixture was stirred at 60 °C under an Ar atmosphere for 4.0 h, then poured into H_2O (20 mL), acidified with 2 N HCl, and extracted with EtOAc (3 × 30 mL). The organic layer was collected, washed with H_2O (2 × 30 mL) and brine (30 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:6) to yield **15c** (240 mg, 94%) as a pale-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.82 (dd, J = 2.5, 0.5 Hz, 1H), 7.76 (dd, J = 9.0, 2.5 Hz, 1H), 7.02 (d, J = 2.5 Hz, 1H), 6.95 (dd, J = 8.5, 2.5 Hz, 1H), 6.84 (d, J = 8.5 Hz, 1H), 6.14 (d, J = 9.0, 0.5 Hz, 1H), 4.00 (q, J = 7.0 Hz, 2H), 3.86 (d, J = 7.5 Hz, 2H), 3.85 (s, 3H), 3.37 (sep, J = 7.0 Hz, 1H), 1.23−1.20 (m, 5H), 0.67−0.62 (m, 2H), 0.40−0.38 (m, 2H).

*Methyl 6-{Ethyl-[3-isopropyl-4-(2-methylallyloxy)phenyl]amino}-pyridine-3-carboxylate (**15d**).* 3-Bromo-2-methylpropene (100 μL, 1.0 mmol), K_2CO_3 (69 mg, 0.50 mmol), and KI (catalytic amount) were added to a solution of **14** (80 mg, 0.25 mmol) in dry DMF (3.0 mL). The reaction mixture was stirred at 60 °C under an Ar atmosphere for 4.0 h, poured into H_2O (60 mL), acidified with 2 N HCl, and then extracted with EtOAc (3 × 20 mL). The organic layer was collected, washed with H_2O (40 mL) and brine (40 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:6) to yield **15d** (73 mg, 78%) as a pale-yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.83 (dd, J = 2.5, 0.5 Hz, 1H), 7.77 (dd, J = 9.0, 2.5 Hz, 1H), 7.03 (d, J = 2.5 Hz, 1H), 6.97 (dd, J = 9.0, 2.5 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.14 (dd, J = 9.0, 0.5 Hz, 1H), 5.15 (s, 1H), 5.02 (s, 1H), 4.47 (s, 2H), 4.01 (q, J = 7.0 Hz, 2H), 3.85 (s, 3H), 3.39 (sep, J = 7.0 Hz, 1H), 1.88 (s, 3H), 1.22 (t, J = 7.0 Hz, 3H), 1.22 (d, J = 7.0 Hz, 6H).

*Methyl 6-{Ethyl-[3-isopropyl-4-(3-methyl-but-2-enyloxy)phenyl]-amino}pyridine-3-carboxylate (**15e**).* 1-Bromo-3-methyl-2-butene (210 μL, 1.8 mmol), K_2CO_3 (330 mg, 2.4 mmol), and KI (catalytic amount) were added to a solution of **14** (190 mg, 0.60 mmol) in dry DMF (20 mL). The reaction mixture was stirred at 120 °C under an Ar atmosphere for 21 h, then poured into H_2O (120 mL) and extracted with EtOAc (3 × 50 mL). The organic layer was collected, washed with H_2O (2 × 50 mL) and brine (50 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:12) to yield **15e** (62 mg, 27%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.83 (dd, J = 2.5, 0.5 Hz, 1H), 7.78 (dd, J = 9.0, 2.5 Hz, 1H), 7.02 (d, J = 2.5 Hz, 1H), 6.97 (dd, J = 8.5, 2.5 Hz, 1H), 6.89 (d, J = 8.5 Hz, 1H), 6.16 (dd, J = 9.0, 0.5 Hz, 1H), 5.52 (t, J = 6.5 Hz, 1H), 4.55 (d, J = 6.5 Hz, 2H), 4.01 (q, J = 7.0 Hz, 2H), 3.85 (s, 3H), 3.35 (sep, J = 7.0 Hz, 1H), 1.82 (s, 3H), 1.76 (s, 3H), 1.26 (t, J = 7.0 Hz, 3H), 1.20 (d, J = 7.0 Hz, 6H).

*Methyl 6-[(4-Butoxy-3-isopropylphenyl)ethylamino]pyridine-3-carboxylate (**15f**).* 1-Bromobutane (69 μL, 0.64 mmol), K_2CO_3 (88 mg, 0.64 mmol), and KI (catalytic amount) were added to a solution of **14** (100 mg, 0.32 mmol) in dry DMF (3.0 mL). The reaction mixture was stirred at 60 °C under an Ar atmosphere for 3.0 h, poured into H_2O (60 mL), acidified with 2 N HCl, and then extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (40 mL) and brine (40 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:6) to yield **15f** (69 mg, 59%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.84 (d, 1H, J = 2.0 Hz), 7.78 (dd, J = 9.0, 2.5 Hz, 1H), 7.03 (d, J = 2.5 Hz, 1H), 6.98 (dd, J = 8.5, 2.5 Hz, 1H), 6.89 (d, J = 9.0 Hz, 1H), 6.15 (d, J = 9.0 Hz, 1H), 4.01 (q, J = 7.0 Hz, 2H), 4.01 (t, J = 6.0 Hz, 2H), 3.85 (s, 3H), 3.34 (sep, J = 7.0 Hz, 1H), 1.87−1.78 (m, 2H), 1.62−1.51 (m, 2H), 1.22 (t, J = 7.0 Hz, 3H), 1.21 (d, J = 7.0 Hz, 6H), 1.01 (t, J = 7.5 Hz, 3H).

*Methyl 6-[Ethyl-(3-isopropyl-4-pentyloxyphenyl)amino]pyridine-3-carboxylate (**15g**).* *n*-Amyl bromide (82 μL, 0.66 mmol), K_2CO_3 (91 mg, 0.66 mmol), and KI (catalytic amount) were added to a solution of **14** (104 mg, 0.33 mmol) in dry DMF (3.0 mL). The reaction mixture was stirred at 60 °C under an Ar atmosphere for 6.5 h, poured into H_2O (60 mL), acidified with 2 N HCl, and then extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (40 mL) and brine (40 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:6) to yield **15g** (76 mg, 60%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.84 (d, J = 2.5 Hz, 1H), 7.78 (dd, J = 9.0, 2.5 Hz, 1H), 7.06 (d, J = 2.5 Hz, 1H), 6.98 (dd, J = 8.5, 2.5 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.15 (d, J = 9.0 Hz, 1H), 4.01 (q, J = 7.0 Hz, 2H), 4.00 (t, J = 7.0 Hz, 2H), 3.86 (s, 3H), 3.34 (sep, J = 7.0 Hz, 1H), 1.89−1.80 (m, 2H), 1.55−1.41 (m, 4H), 1.22 (t, J = 6.5 Hz, 3H), 1.21 (d, J = 6.5 Hz, 6H), 1.01 (t, J = 7.0 Hz, 3H).

*Methyl 6-[Ethyl-(4-hexyloxy-3-isopropylphenyl)amino]pyridine-3-carboxylate (**15h**).* 1-Iodohexane (97 μL, 0.66 mmol), K_2CO_3 (91 mg, 0.66 mmol), and KI (catalytic amount) were added to a solution of **14** (105 mg, 0.33 mmol) in dry DMF (3.0 mL). The reaction mixture was stirred at 60 °C under an Ar atmosphere for 3.7 h, poured into H_2O

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(60 mL), acidified with 2 N HCl, and then extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (40 mL) and brine (40 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:6) to yield **15h** (110 mg, 82%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.84 (d, J = 2.0 Hz, 1H), 7.78 (dd, J = 9.0, 2.5 Hz, 1H), 7.02 (d, J = 2.5 Hz, 1H), 6.98 (dd, J = 8.5, 2.5 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.15 (d, J = 9.0 Hz, 1H), 4.01 (q, J = 6.5 Hz, 2H), 4.00 (t, J = 6.5 Hz, 2H), 3.85 (s, 3H), 3.34 (sep, J = 7.0 Hz, 1H), 1.88−1.79 (m, 2H), 1.57−1.52 (m, 2H), 1.40−1.35 (m, 4H), 1.22 (t, J = 7.0 Hz, 3H), 1.22 (d, J = 6.5 Hz, 6H), 0.92 (t, J = 7.0 Hz, 3H).

*Methyl 6-[(4-Benzyloxy-3-isopropylphenyl)ethylamino]pyridine-3-carboxylate (**15i**).* Benzyl bromide (48 μL, 0.40 mmol), K_2CO_3 (69 mg, 0.40 mmol), and KI (catalytic amount) were added to a solution of **14** (106 mg, 0.34 mmol) in dry DMF (3.0 mL). The reaction mixture was stirred at 60 °C under an Ar atmosphere for 2.5 h, then poured into H_2O (60 mL) and extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (2 × 40 mL) and brine (30 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:6) to yield **15i** (76 mg, 55%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.83 (d, J = 2.5 Hz, 1H), 7.78 (dd, J = 9.0, 2.5 Hz, 1H), 7.48−7.35 (m, 5H), 7.06−6.97 (m, 3H), 6.16 (d, J = 9.0 Hz, 1H), 5.12 (s, 2H), 4.01 (q, J = 7.0 Hz, 2H), 3.85 (s, 3H), 3.42 (sep, J = 7.0 Hz, 1H), 1.23 (d, J = 7.0 Hz, 6H), 1.22 (t, J = 7.0 Hz, 3H).

*Methyl 6-[Ethyl-(3-isopropyl-4-phenethyloxyphenyl)lamino]-pyridine-3-carboxylate (**15j**).* (2-Bromoethyl)benzene (190 μL, 1.4 mmol), K_2CO_3 (170 mg, 1.2 mmol), and KI (catalytic amount) were added to a solution of **14** (220 mg, 0.70 mmol) in dry DMF (4.0 mL). The reaction mixture was stirred at 80 °C under an Ar atmosphere for 16 h, then poured into H_2O (100 mL) and extracted with EtOAc (3 × 30 mL). The organic layer was washed with water (2 × 40 mL) and brine (30 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:9 to 1:3) to yield **15j** (46 mg, 16%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.82 (d, J = 2.0 Hz, 1H), 7.76 (dd, J = 9.0, 2.0 Hz, 1H), 7.33−7.32 (m, 5H), 7.00 (d, J = 2.5 Hz, 1H), 6.95 (dd, J = 8.5, 2.5 Hz, 1H), 6.86 (d, J = 8.5 Hz, 1H), 6.13 (d, J = 9.0 Hz, 1H), 4.21 (t, J = 6.5 Hz, 2H), 4.00 (q, J = 7.0 Hz, 2H), 3.85 (s, 3H), 3.30 (sep, J = 7.0 Hz, 1H), 3.15 (t, J = 6.5 Hz, 2H), 1.21 (t, J = 7.0 Hz, 3H), 1.16 (d, J = 7.0 Hz, 6H).

*Methyl 6-{Ethyl-[(3-isopropyl-4-phenylpropoxy)phenyl]amino}-pyridine-3-carboxylate (**15k**).* 3-Phenylpropyl bromide (54 μL, 0.36 mmol), K_2CO_3 (83 mg, 0.60 mmol), and KI (17 mg, 0.10 mmol) were added to a solution of **14** (75 mg, 0.24 mmol) in dry DMF (3.0 mL). The reaction mixture was stirred at 90 °C under an Ar atmosphere for 14 h, then poured into H_2O (100 mL) and extracted with EtOAc (3 × 30 mL). The organic layer was washed with water (2 × 40 mL) and brine (30 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure. The residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:6) to yield **15k** (76 mg, 73%) as a white oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.83 (d, J = 2.5 Hz, 1H), 7.78 (d, J = 9.0, 2.5 Hz, 1H), 7.35−7.22 (m, 5H), 7.03 (d, J = 2.5 Hz, 1H), 6.96 (dd, J = 8.5, 2.5 Hz, 1H), 6.84 (d, J = 8.5 Hz, 1H), 6.15 (d, J = 9.0 Hz, 1H), 4.01 (t, J = 6.0 Hz, 2H), 4.01 (q, J = 7.5 Hz, 2H), 3.85 (s, 3H), 3.37 (sep, J = 7.0 Hz, 1H), 2.87 (t, J = 7.0 Hz, 2H), 2.22−2.12 (m, 2H), 1.24 (d, J = 7.0 Hz, 6H), 1.22 (t, J = 7.5 Hz, 3H).

*6-[Ethyl-(4-isobutoxy-3-isopropylphenyl)amino]pyridine-3-carboxylic Acid (**8b**).* To a solution of **15b** (300 mg, 0.81 mmol) in MeOH (10 mL) were added 2 N NaOH (4.0 mL) and THF (3.0 mL). The reaction mixture was stirred at 60 °C for 2.0 h and then evaporated under reduced pressure. The residue was neutralized with 2 N HCl and extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (2 × 40 mL) and brine (30 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure, and the residue was recrystallized from MeOH to yield **8b** (140 mg, 49%) as white needles; mp 162.0−164.0 °C. IR (KBr) cm^{-1}: 1677 (CO). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.90 (d, J = 2.5 Hz, 1H), 7.81 (dd, J = 9.0, 2.5 Hz, 1H), 7.03 (d, J = 2.5 Hz, 1H), 6.98 (dd, J = 8.5, 2.5 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.16 (d, J = 9.0 Hz, 1H), 4.00 (q, J = 7.0 Hz, 2H), 3.77 (d, J = 6.0 Hz, 2H), 3.49 (s, 1H), 3.36 (sep, J = 7.0 Hz, 1H), 1.23 (t, J = 7.0 Hz, 3H), 1.22 (d, J = 7.0 Hz, 6H), 1.08 (d, J = 6.5 Hz, 6H). FAB-MS m/z: 357 $[M + H]^+$. Anal. Calcd for $C_{21}H_{28}N_2O_3$: C, 70.76; H, 7.92; N, 7.86. Found: C, 70.49; H, 7.64; N, 7.73.

*6-[Ethyl-(4-cyclopropylmethoxy-3-isopropylphenyl)amino]-pyridine-3-carboxylic Acid (**8c**).* To a solution of **15c** (130 mg, 0.35 mmol) in MeOH (8.0 mL) were added 2 N NaOH (5.0 mL) and THF (2.0 mL). The reaction mixture was stirred at 60 °C for 1.5 h and then evaporated under reduced pressure. The residue was poured into satd NH_4Cl and extracted with EtOAc (3 × 30 mL). The organic layer was washed with water (2 × 40 mL) and brine (40 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure, and the residue was recrystallized from MeOH to yield **8c** (120 mg, 93%) as white needles; mp 194.0−196.0 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.65 (dd, J = 2.5, 0.8 Hz, 1H), 7.76 (dd, J = 9.0, 2.5 Hz, 1H), 7.05−7.01 (m, 3H), 6.13 (d, J = 9.0, 0.8 Hz, 1H), 3.94 (q, J = 7.0 Hz, 2H), 3.88 (q, J = 7.0 Hz, 2H), 1.14 (d, J = 7.0 Hz, 6H), 1.12 (t, J = 7.0 Hz, 3H), 0.62−0.56 (m, 2H), 0.38−0.33 (m, 2H). FAB-MS m/z: 355 $[M + H]^+$. Anal. Calcd for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90. Found: C, 70.99; H, 7.25; N, 7.81.

*6-{Ethyl-[3-isopropyl-4-(2-methylallyloxy)phenyl]amino}pyridine-3-carboxylic Acid (**8d**).* To a solution of **15d** (73 mg, 0.20 mmol) in MeOH (3.0 mL) was added 2 N NaOH (3.0 mL). The reaction mixture was stirred at 60 °C for 2.0 h and then evaporated under reduced pressure. The residue was neutralized with 2 N HCl and extracted with EtOAc (3 × 30 mL). The organic layer was washed with water (40 mL) and brine (40 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure, and the residue was recrystallized from MeOH to yield **8d** (26 mg, 37%) as white needles; mp 162.0−163.5 °C. IR (KBr) cm^{-1}: 1677 (CO). ^{1}H NMR (300 MHz, $CDCl_3$) δ: 8.91 (d, J = 2.0 Hz, 1H), 7.82 (dd, J = 9.0, 2.5 Hz, 1H), 7.05 (d, J = 2.5 Hz, 1H), 6.99 (dd, J = 8.5, 2.5 Hz, 1H), 6.90 (d, J = 9.0 Hz, 1H), 6.17 (d, J = 9.0 Hz, 1H), 5.15 (s, 1H), 5.02 (s, 1H), 4.47 (s, 2H), 4.03 (q, J = 7.0 Hz, 2H), 3.39 (sep, J = 7.0 Hz, 1H), 1.88 (s, 3H), 1.23 (t, J = 7.0 Hz, 3H), 1.23 (d, J = 7.0 Hz, 6H). FAB-MS m/z: 355 $[M + H]^+$. Anal. Calcd for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90. Found: C, 71.54; H, 7.52; N, 7.95.

*6-{Ethyl-[3-isopropyl-4-(3-methyl-but-2-enyloxy)phenyl]amino}-pyridine-3-carboxylic Acid (**8e**).* To a solution of **15e** (62 mg, 0.16 mmol) in MeOH (3.0 mL) were added 2 N NaOH (3.0 mL) and THF (2.0 mL). The reaction mixture was stirred at 60 °C for 0.75 h and then evaporated under reduced pressure. The residue was poured into satd NH_4Cl (40 mL) and extracted with EtOAc (3 × 30 mL). The organic layer was washed with water (2 × 30 mL) and brine (30 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure, and the residue was recrystallized from MeOH to yield **8e** (7.2 mg, 12%) as white needles; mp 139.5−141.0 °C. IR (KBr) cm^{-1}: 1671 (CO). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.66 (d, J = 2.5 Hz, 1H), 7.76 (dd, J = 9.0, 2.5 Hz, 1H), 7.06−7.05 (m, 3H), 6.14 (d, J = 9.0 Hz, 1H), 5.48 (t, J = 6.5 Hz, 1H), 4.58 (d, J = 6.5 Hz, 2H), 3.94 (q, J = 7.0 Hz, 2H), 1.78 (s, 3H), 1.73 (s, 3H), 1.15 (d, J = 7.0 Hz, 6H), 1.12 (t, J = 7.0 Hz, 3H). FAB-MS m/z: 369 $[M + H]^+$. Anal. Calcd for $C_{22}H_{28}N_2O_3$: C, 71.71; H, 7.66; N, 7.60. Found: C, 71.77; H, 7.62; N, 7.57.

*6-[(4-Butoxy-3-isopropylphenyl)ethylamino]pyridine-3-carboxylic Acid (**8f**).* To a solution of **15f** (69 mg, 0.19 mmol) in MeOH (3.0 mL) was added 2 N NaOH (3.0 mL). The reaction mixture was stirred at 60 °C for 4.0 h and then evaporated under reduced pressure. The residue was neutralized with 2 N HCl and extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (40 mL) and brine (40 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure, and the residue was recrystallized from MeOH to yield **8f** (29 mg, 44%) as white needles; mp 161.0−163.5 °C. IR (KBr) cm^{-1}: 1613 (CO). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 8.66 (d, J = 2.0 Hz, 1H), 7.76 (dd, J = 9.0, 2.5 Hz, 1H), 7.09−7.00 (m, 3H), 6.13 (d, J = 9.0 Hz, 1H), 4.01 (t, J = 6.0 Hz, 2H), 3.93 (q, J = 7.0 Hz, 2H), 3.24 (sep, J = 7.0 Hz, 1H), 1.79−1.70 (m, 2H), 1.55−1.43 (m, 2H), 1.16 (d, J = 7.0 Hz, 6H), 1.11 (t, J = 7.0 Hz, 3H), 0.95 (t, J = 7.5 Hz, 3H). FAB-MS m/z: 357 $[M + H]^+$. Anal. Calcd for $C_{21}H_{28}N_2O_3$: C, 70.76; H, 7.92; N, 7.86. Found: C, 70.70; H, 7.73; N, 7.86.

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*6-[Ethyl-(3-isopropyl-4-pentyloxyphenyl)amino]pyridine-3-carboxylic Acid (**8g**).* To a solution of **15g** (76 mg, 0.20 mmol) in MeOH (6.0 mL) were added 2 N NaOH (3.0 mL) and THF (1.5 mL). The reaction mixture was stirred at 60 °C for 3.0 h and then evaporated under reduced pressure. The residue was neutralized with 2 N HCl and extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (40 mL) and brine (40 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure and the residue was recrystallized from MeOH to yield **8g** (47 mg, 64%) as pale-yellow needles; mp 154.5−156.0 °C. IR (KBr) cm^{-1}: 1691 (CO). ^1H NMR (300 MHz, DMSO-d_6) δ: 8.67 (d, J = 2.5 Hz, 1H), 7.77 (dd, J = 9.0, 2.5 Hz, 1H), 7.10−7.01 (m, 3H), 6.14 (d, J = 9.0 Hz, 1H), 4.02 (t, J = 6.0 Hz, 2H), 3.94 (q, J = 7.0 Hz, 2H), 3.26 (sep, J = 7.0 Hz, 1H), 1.82−1.73 (m, 2H), 1.49−1.37 (m, 4H), 1.17 (d, J = 6.5 Hz, 6H), 1.12 (t, J = 7.0 Hz, 3H), 0.92 (t, J = 7.0 Hz, 3H). FAB-MS m/z: 371 $[M + H]^+$. Anal. Calcd for $C_{22}H_{30}N_2O_3$: C, 71.32; H, 8.16; N, 7.56. Found: C, 71.16; H, 7.98; N, 7.29.

*6-[Ethyl-(4-hexyloxy-3-isopropylphenyl)amino]pyridine-3-carboxylic Acid (**8h**).* To a solution of **15h** (109 mg, 0.27 mmol) in MeOH (6.0 mL) were added 2 N NaOH (3.0 mL) and THF (1.5 mL). The reaction mixture was stirred at 60 °C for 2.3 h and then evaporated under reduced pressure. The residue was neutralized with 2 N HCl and extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (40 mL) and brine (40 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure, and the residue was recrystallized from MeOH to yield **8h** (64 mg, 61%) as white needles; mp 167.0−169.0 °C. IR (KBr) cm^{-1}: 1654 (CO). ^1H NMR (300 MHz, DMSO-d_6) δ: 8.67 (d, J = 2.5 Hz, 1H), 7.77 (dd, J = 9.0, 2.5 Hz, 1H), 7.10−7.01 (m, 3H), 6.14 (d, J = 9.0 Hz, 1H), 4.02 (t, J = 6.0 Hz, 2H), 3.94 (q, J = 7.0 Hz, 2H), 3.26 (sep, J = 7.0 Hz, 1H), 1.81−1.72 (m, 2H), 1.50−1.44 (m, 2H), 1.37−1.30 (m, 4H), 1.17 (d, J = 7.0 Hz, 6H), 1.12 (t, J = 7.0 Hz, 3H), 0.89 (t, J = 7.0 Hz, 3H). FAB-MS m/z: 385 $[M + H]^+$. Anal. Calcd for $C_{23}H_{32}N_2O_3$: C, 71.84; H, 8.39; N, 7.29. Found: C, 71.63; H, 8.38; N, 7.37.

*6-[(4-Benzyloxy-3-isopropylphenyl)ethylamino]pyridine-3-carboxylic Acid (**8i**).* To a solution of **15i** (76 mg, 0.19 mmol) in MeOH (8.0 mL) were added 2 N NaOH (3.0 mL) and THF (2.0 mL). The reaction mixture was stirred at 60 °C for 2.0 h and then evaporated under reduced pressure. The residue was neutralized with 2 N HCl and extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (2 × 40 mL) and brine (30 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure and the residue was recrystallized from MeOH to yield **8i** (38 mg, 52%) as colorless cubes; mp 183.0−184.5 °C. IR (KBr) cm^{-1}: 1674 (CO). ^1H NMR (300 MHz, DMSO-d_6) δ: 12.36 (br s, 1H), 8.65 (d, J = 2.5 Hz, 1H), 7.76 (dd, J = 9.0, 2.5 Hz, 1H), 7.51−7.35 (m, 5H), 7.16−7.04 (m, 3H), 6.14 (d, J = 9.0 Hz, 1H), 5.15(s, 2H), 3.95 (q, J = 7.0 Hz, 2H), 1.19 (d, J = 7.0 Hz, 6H), 1.13 (t, J = 7.0 Hz, 3H). FAB-MS m/z: 391 $[M + H]^+$. Anal. Calcd for $C_{24}H_{26}N_2O_3$: C, 73.82; H, 6.71; N, 7.17. Found: C, 73.58; H, 6.93; N, 7.14.

*6-[Ethyl-(3-isopropyl-4-phenethyloxyphenyl)lamino]pyridine-3-carboxylic Acid (**8j**).* To a solution of **15j** (46 mg, 0.11 mmol) in MeOH (6.0 mL) were added 2 N NaOH (2.0 mL) and THF (2.0 mL). The reaction mixture was stirred at 60 °C for 4.3 h and then evaporated under reduced pressure. The residue was neutralized with 2 N HCl and extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (2 × 30 mL) and brine (30 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure, and the residue was purified by flash column chromatography (EtOAc:*n*-hexane = 1:4), then was recrystallized from MeOH to yield **8j** (25 mg, 58%) as colorless cubes; mp 167.5−169.0 °C. IR (KBr) cm^{-1}: 1693 (CO). ^1H NMR (300 MHz, DMSO-d_6) δ: 8.63 (d, J = 2.5 Hz, 1H), 7.72 (dd, J = 9.0, 2.5 Hz, 1H), 7.36−7.22 (m, 5H), 7.03−6.99 (m, 3H) 6.10 (d, J = 9.0, 2.5 Hz, 1Hx), 4.23 (t, J = 6.5 Hz, 2H), 3.93 (q, J = 7.0 Hz, 2H), 3.19 (sep, J = 7.0 Hz, 1H), 3.08 (t, J = 6.5 Hz, 2H), 1.12 (t, J = 7.0 Hz, 3H), 1.09 (d, J = 7.0 Hz, 6H). FAB-MS m/z: 405 $[M + H]^+$. Anal. Calcd for $C_{25}H_{28}N_2O_3$: C, 74.23; H, 6.98; N, 6.93. Found: C, 74.33; H, 6.88; N, 7.06.

*6-{Ethyl-[(3-isopropyl-4-phenylpropoxy)phenyl]amino}pyridine-3-carboxylic Acid (**8k**).* To a solution of **15k** (76 mg, 0.18 mmol) in MeOH (7.0 mL) were added 2 N NaOH (2.0 mL) and THF (3.0 mL). The reaction mixture was stirred at 60 °C for 2.0 h and then evaporated under reduced pressure. The residue was poured into satd NH_4Cl and extracted with EtOAc (3 × 30 mL). The organic layer was washed with water (2 × 30 mL) and brine (30 mL) and dried over $MgSO_4$. The solution was evaporated under reduced pressure and recrystallized from CH_2Cl_2/*n*-hexane to yield **8k** (56 mg, 75%) as colorless plates; mp 171.5−176.0 °C. IR (KBr) cm^{-1}: 1660 (CO). ^1H NMR (300 MHz, DMSO-d_6) δ: 8.65 (d, J = 2.0 Hz, 1H), 7.75 (d, J = 9.0, 2.5 Hz, 1H), 7.33−7.17 (m, 5H), 7.05−7.01 (m, 3H), 6.13 (d, J = 9.0 Hz, 1H), 4.01 (t, J = 6.0 Hz, 2H), 3.94 (q, J = 7.0 Hz, 2H), 2.81 (t, J = 8.0 Hz, 2H), 2.13−2.04 (m, 2H), 1.20 (d, J = 7.0 Hz, 6H), 1.13 (t, J = 7.0 Hz, 3H). FAB-MS m/z: 419 $[M + H]^+$. Anal. Calcd for $C_{26}H_{30}N_2O_3$: C, 74.61; H, 7.22; N, 6.69. Found: C, 74.70; H, 7.23; N, 6.69.

Luciferase Reporter Gene Assay. *Culture of COS-1 and HepG2 Cells.* COS-1 and HepG2 cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% FBS, 0.2% $NaHCO_3$, 4 mM L-glutamine, penicillin (50000 U/L), and streptomycin (50000 μg/L) 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 or HepG2 cells transfected with three kinds of vectors: each receptor subtype, a luciferase reporter gene under the control of the appropriate nuclear receptor response element, and secreted alkaline phosphatase (SEAP) gene as a background. CRBPII-tk-Luc, tk-TREPII-Luc, tk-PPRE×3-Luc, and tk-rBAR×3-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 Effectene transfection reagent (QIAGEN) according to the supplier's protocol. In the case of heterodimer assay, RXRα (0.5 μg), each partner receptor (PPAR or LXRα, 0.5 μg), and the partner response element (4.0 μg) were transfected into COS-1 or HepG2 cells as described above. Test compound solutions were added to the suspension of transfected cells, which were seeded at about 2.0×10^4 cells/well in 96-well white plates (DMSO concentration 1%). 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. This experiment was performed at the same time as the work reported in ref 30. Production and purification of recombinant RXRα protein were done using the GATEWAY technology.[46,47] Destination vectors were generated by insertion of human RXRα DNA (Ultimate Human ORF Clone, Invitrogen)[48] 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 to 1.0 and then 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 further cultured for 2.0 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, 1.0 mM PMSF) at 4 °C. The RXR protein was purified using a His GraviTrap column (GE Healthcare)[49] to give 2.2 mg/L pure protein in the culture medium.

Fluorescence Polarization Assay. This experiment was performed at the same time as the work reported in ref 30. Fluorescein-labeled cofactor peptides were purchased from Invitrogen. 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, 1.0 mM dithiothreitol, 1.0 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 1.0 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.

Molecular Docking. The crystal structure of human RXRα−ligand binding domain (pdb code: 4K6I)[41] was retrieved from the Brookhaven Protein Data Bank: http://www.rcsb.org/pdb/Welcome.do. The structure was also refined by Refmac5 of the CCP4 suite (R_{factor} = 0.207, R_{free} = 0.284).[50] Hydrogen atoms were automatically added at pH 7.0 by the protonate 3D program in the MOE 2010.10 package (Molecular Operating Environment, Chemical Computing Group Inc., Montreal, QC, Canada). All hydrogen atomic coordinates were optimized by the conjugate gradient method with the AMBER99 force field under the Born solvent model. N- and C-termini were ionized $-NH_3^+$ and $-COO^-$, respectively. The 3D structures of ligands used for the docking study were constructed by using Spartan (Wavefunction, Inc.). After semiempirical PM3 calculations, ab initio calculations at the level of HF/6-31G* were performed to find the lowest energy conformers. The AutoDock4.2 molecular docking program[40] 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. *Measurement of Serum Concentration of Test Compounds after Oral Administration at 30 mg/kg to Mice.* Six-week-old male ICR mice were purchased from Charles River Laboratories Japan, Inc.. This experiment was 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. Groups of six-week-old ICR male mice (n = 5−8 in each) were treated with suspensions of test compound at 30 mg/kg (1% ethanol and 0.5% CMC in distilled water) in a volume of 10 mL/kg of body weight by oral administration. At the indicated times (0, 0.5, 1.0, 3.0, and 6.0 h after compound administration), 0.6−1 mL of blood was taken from the inferior vena cava under diethyl ether anesthesia. Each blood sample was centrifuged at 1900g for 5 min at rt. To 100 μL of the resulting plasma were added 100 μL of ice-cold 5.0 mM ammonium acetate solution (adjusted with acetic acid to pH 5.0) and 1.0 mL of ice-cold EtOAc. The resulting mixture was vortexed for 30 s, kept at room temperature for 10 min, and centrifuged at 1900g for 30 s at room temperature. An 800 μL aliquot of the EtOAc phase was removed and concentrated to dryness in a centrifugal evaporator. To the resulting residue was added 100 μL of HPLC eluent solution (for **7b**, 33.3 mM ammonium acetate (adjusted with acetic acid to pH 5.0) with methanol (85:15, v/v); for **8b**, 25 mM ammonium acetate (adjusted with acetic acid to pH 5.0) with methanol (80:20, v/v), respectively). Each 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 mm i.d. × 250 mm, 5 μm, GL Sciences, Tokyo, Japan) kept at 40 °C, using the appropriate HPLC eluent solution (for **7b**, 33.3 mM ammonium acetate (adjusted with acetic acid to pH 5.0) with methanol (85:15, v/v); for **8b**, 25 mM ammonium acetate (adjusted with acetic acid to pH 5.0) with methanol (80:20, v/v), respectively) as a 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−seven-week-old male ICR mice were purchased from Charles River Laboratories Japan, Inc.. This experiment was 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. After arrival of the animals, all were group-housed and acclimated to the colony for 1 or 2 days 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% CMC in distilled water) in 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 5:00 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. Blood samples were centrifuged at 1900g 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.. This experiment was 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. After arrival of the rats, all were group-housed and acclimated to the colony for 6 (male) or 7 (female) days 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, food intake, and water intake were 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) in 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 5:00 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 2000g for 10 min at 4 °C to obtain a serum sample. Measurements of blood parameters were performed according to the method described below.

Evaluation of Blood Glucose-Lowering Activity in KK-A^y Mice. This experiment was performed at the same time as the work reported in ref 30. 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. This experiment was 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. 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, food intake, and water intake were measured at approximately 10:00 a.m. every day for 14 days before dosing. Mice were administered orally with a solution of test compound

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at a dose of 10 mg/kg or with the vehicle (1% ethanol and 0.5% CMC in distilled water) in 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 5:00 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 room temperature. 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). White blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets (PLT) were measured by using a pocH-100i (Sysmex).

Oral Glucose Tolerance test (OGTT). This experiment was performed at the same time as the work reported in refs 29 and 30. 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 1.0 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.

RNA Preparation and Quantitative Real-Time RT-PCR. This experiment was performed according to ref 29. Fifty mg of liver tissue from KK-A^y mice described above was resected and mechanically homogenized with a Polytron PT 10/35 (Kinematica Inc., Littau-Luzern, Switzerland) in 0.5 mL of Trizol reagent (Invitrogen, Carlsbad, CA). Total RNA was extracted as previously described (*S4*). Quantitative real-time RT-PCR analysis was performed using a LightCycler rapid thermal cycler system (Roche Applied Science, Mannheim, Germany) following the protocol previously reported (*S5*). Two μg of total RNA was reverse-transcribed by random hexamer priming using ReverTra Ace (Toyobo, Osaka, Japan). The PCR mixture consisted of 1× SYBR Green PCR Master Mix (Toyobo), which includes DNA polymerase, SYBR Green I Dye, dNTPs, PCR buffer, and 10 pmol forward and reverse primers and cDNA of samples in a total volume of 20 μL. The amplification of a housekeeping gene, *Rps18*, was used to normalize the efficiency of cDNA synthesis and the amount of RNA applied. PCR was performed with initial denaturation at 94 °C for 30 s, followed by amplification for 40 cycles, each cycle consisting of denaturation at 95 °C for 5.0 s, annealing at 60 °C for 15 s, and polymerization at 72 °C for 15 s. Primers used for this study are listed in Supporting Information, Table 3.

Ames Test. *Salmonella enterica* subspecies I, serovar Typhimurium (*Salmonella typhimurium*) strain TA97 [hisO1242, hisD6610 DuvrB gal bio chl1005 rfa1001/pKM101], TA98 [hisD3052 DuvrB gal bio chl1005 rfa1001/pKM101], TA100 [hisG46 DuvrB gal bio chl1005 rfa1001/pKM101], TA102 [hisD(G)8476 DuvrB gal bio chl1005 rfa1001/pKM101 pAQ1], and TA1535 [hisG46 DuvrB gal bio chl1005 rfa1001] were gifts from Dr. B. N. Ames of the University of California, Berkeley.[51] The supernatant fraction of rat liver homogenate (S9) was prepared from Sprague−Dawley rats (male, 6 weeks old) that had been induced with phenobarbital and 5,6-benzoflavone. Positive controls, 2-aminoanthracene (2AA), 6-chloro-9-[3-(2-chloroethylamino)-propylamino]-2-methoxyacridine 2HCl (ICR191), methylnitrosourea (MNU), 2-nitrofluorene (2NF), *N*-nitrosomorpholine (NMOR), 4-nitroquinoline-1-oxide (4NQO), and other reagents were purchased from commercial sources. Compound **8b** and positive controls except MNU and NMOR were dissolved in dimethyl sulfoxide. MNU and NMOR were dissolved in sterilized distilled water.

Genotoxicity assays were performed in accordance with ICH harmonized tripartite guideline guidance on genotoxicity testing and data interpretation for pharmaceuticals intended for human use S2(R1) Current Step 4 version dated 9 November 2011.[51] The recommendation is to use a fully adequate protocol including all strains with and without metabolic activation, a suitable dose range that fulfills criteria for top dose selection, and appropriate positive and negative controls. The recommended set of bacterial strains includes those that detect base substitution and frameshift mutations were *Salmonella typhimurium* TA98, *Salmonella typhimurium* TA100, *Salmonella typhimurium* TA1535, either *Salmonella typhimurium* TA1537 or TA97 or TA97a, and either *Salmonella typhimurium* TA102 or *Escherichia coli* WP2 uvrA or *Escherichia coli* WP2 uvrA (pKM101). For this experiment, five strains were selected (*Salmonella typhimurium* TA98, *Salmonella typhimurium* TA100, *Salmonella typhimurium* TA1535, *Salmonella typhimurium* TA97, and *Salmonella typhimurium* TA102). As the maximum dose level recommended is 5000 μg/plate (or 5 μL/plate for liquid test substance) when not limited by solubility or cytotoxicity, 5000 μg/plate (0.1 mL of 50 mg/mL solution) was selected as the top dose. All assays were performed with plate-incorporation methods[52] and with and without metabolic activation (hereafter referred as +S9 and −S9, respectively). Assays were performed in duplicate. All experiments were carried out in accordance with the Safety Guidelines of Okayama University and the Japanese Government Management Law for Toxic Chemicals (no. 303).

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

NMR data for compound **14**; in vitro and in vivo data are described (PDF, CSV). This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

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

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS USED

RXR, retinoid X receptor; PPAR, peroxisome proliferator-activated receptor; LXR, liver X receptor; E_{max}, maximum efficacy; EC_{50}, half-maximal (50%) effective concentration; Asn, asparagine; His, histamine; SEM, standard error of the mean; CMC, carboxymethyl cellulose; AST, aspartate aminotransferase; ALT, alanine aminotransferase; γ-GTP, γ-glutamyl transpeptidase; ALP, alkaline phosphatase; CRE, creatinine; BUN, blood urea nitrogen; GLU, glucose; Rps18, ribosomal protein S18; Irs, insulin receptor substrate; Slc2a, solute carrier family 2 (facilitated glucose transporter); G6pc, glucose-6-phosphatase, catalytic subunit; Pck, phosphoenolpyruvate carboxykinase; Gck, glucokinase; Scd1, stearoyl-CoA desaturase-1; Fasn, fatty acid synthase; Srebp1c, sterol regulatory element binding protein-1c; ApoE, apolipoprotein E

■ REFERENCES

(1) Yamada, S.; Kakuta, H. Retinoid X receptor ligands: a patent review (2007−2013). *Expert Opin. Ther. Pat.* **2014**, *24*, 443−452.
(2) Germain, P.; Chambon, P.; Eichele, G.; Evans, R. M.; Lazar, M. A.; Leid, M.; De Lera, A. R.; Lotan, R.; Mangelsdorf, D. J.; Gronemeyer, H. International Union of Pharmacology. LXIII. Retinoid X receptors. *Pharmacol. Rev.* **2006**, *58*, 760−772.
(3) Mangelsdorf, D. J.; Thummel, C.; Beato, M.; Herrlich, P.; Schiitz, G.; Umesono, K.; Blumberg, B.; Kastner, P.; Mark, M.; Chambon, P.; Evans, R. M. The nuclear receptor superfamily: the second decade. *Cell* **1995**, *83*, 835−839.
(4) Shulman, A. I.; Larson, C.; Mangelsdorf, D. J.; Ranganathan, R. Structural determinants of allosteric ligand activation in RXR heterodimers. *Cell* **2004**, *116*, 417−429.
(5) Ronald, M.; Evans, D.; Mangelsdorf, D. J. Nuclear receptors, RXR, and the big bang. *Cell* **2014**, *157*, 255−266.
(6) Bortolini, M.; Wright, M. B.; Bopst, M.; Balas, B. Examining the safety of PPAR agonists—current trends and future prospects. *Expert Opin. Drug Saf.* **2013**, *12*, 65−79.
(7) Schultz, J. R.; Tu, H.; Luk, A.; Repa, J. J.; Medina, J. C.; Li, L.; Schwendner, S.; Wang, S.; Thoolen, M.; Mangelsdorf, D. J.; Lustig, K. D.; Shan, B. Role of LXRs in control of lipogenesis. *Genes Dev.* **2000**, *14*, 2831−2838.
(8) Mitro, N.; Mak, P. A.; Vargas, L.; Godio, C.; Hampton, E.; Molteni, V.; Kreusch, A.; Saez, E. The nuclear receptor LXR is a glucose sensor. *Nature* **2007**, *445*, 219−223.
(9) Terasaka, N.; Hiroshima, A.; Koieyama, T.; Ubukata, N.; Morikawa, Y.; Nakai, D.; Inaba, T. T-0901317, a synthetic liver X receptor ligand, inhibits development of atherosclerosis in LDL receptor-deficient mice. *FEBS Lett.* **2003**, *536*, 6−11.
(10) Schulman, I. G.; Shao, G.; Heyman, R. A. Transactivation by retinoid X receptor-peroxisome proliferator-activated receptor gamma (PPARgamma) heterodimers: intermolecular synergy requires only the PPARgamma hormone-dependent activation function. *Mol. Cell. Biol.* **1998**, *18*, 3483−3494.
(11) Yoshikawa, T.; Shimano, H.; Amemiya-Kudo, M.; Yahagi, N.; Hasty, A. H.; Matsuzaka, T.; Okazaki, H.; Tamura, Y.; Iizuka, Y.; Ohashi, K.; Osuga, J.; Harada, K.; Gotoda, T.; Kimura, S.; Ishibashi, S.; Yamada, N. Identification of liver X receptor-retinoid X receptor as an activator of the sterol regulatory element-binding protein 1c gene promoter. *Mol. Cell. Biol.* **2001**, *21*, 2991−3000.
(12) Forman, B. M.; Umesono, K.; Chen, J.; Evans, R. M. Unique response pathways are established by allosteric interactions among nuclear hormone receptors. *Cell* **1995**, *81*, 541−550.
(13) Shulman, A. I.; Larson, C.; Mangelsdorf, D. J.; Ranganathan, R. Structural determinants of allosteric ligand activation in RXR heterodimers. *Cell* **2004**, *116*, 417−429.
(14) Altucci, L.; Leibowitz, M. D.; Ogilvie, K. M.; de Lera, A. R.; Gronemeyer, H. RAR and RXR modulation in cancer and metabolic disease. *Nature Rev. Drug Discovery* **2007**, *6*, 793−810.
(15) Pileri, A.; Delfino, C.; Grandi, V.; Pimpinelli, N. Role of bexarotene in the treatment of cutaneous T-cell lymphoma: the clinical and immunological sides. *Immunotherapy* **2013**, *5*, 427−433.
(16) Mukherjee, R.; Davies, P. J.; Crombie, D. L.; Bischoff, E. D.; Cesario, R. M.; Jow, L.; Hamann, L. G.; Boehm, M. F.; Mondon, C. E.; Nadzan, A. M.; Paterniti, J. R., Jr.; Heyman, R. A. Sensitization of diabetic and obese mice to insulin by retinoid X receptor agonists. *Nature* **1997**, *386*, 407−410.
(17) Cramer, P. E.; Cirrito, J. R.; Wesson, D. W.; Lee, C. Y.; Karlo, J. C.; Zinn, A. E.; Casali, B. T.; Restivo, J. L.; Goebel, W. D.; James, M. J.; Brunden, K. R.; Wilson, D. A.; Landreth, G. E. ApoE-directed therapeutics rapidly clear β-amyloid and reverse deficits in AD mouse models. *Science* **2012**, *335*, 1503−1506.
(18) McFarland, K.; Spalding, T. A.; Hubbard, D.; Ma, J. N.; Olsson, R.; Burstein, E. S. Low dose bexarotene treatment rescues dopamine neurons and restores behavioral function in models of Parkinson's disease. *ACS Chem. Neurosci.* **2013**, *4*, 1430−1438.
(19) Fujii, S.; Ohsawa, F.; Yamada, S.; Shinozaki, R.; Fukai, R.; Makishima, M.; Enomoto, S.; Tai, A.; Kakuta, H. Modification at the acidic domain of RXR agonists has little effect on permissive RXR-heterodimer activation. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5139−5142.
(20) Kakuta, H.; Ohsawa, F.; Yamada, S.; Makishima, M.; Tai, A.; Yasui, H.; Yoshikawa, Y. Feasibility of structural modification of retinoid X receptor agonists to separate blood glucose-lowering action from adverse effects: studies in KKA^{y} type 2 diabetes model mice. *Biol. Pharm. Bull.* **2012**, *35*, 629−633.
(21) Standeven, A. M.; Thacher, S. M.; Yuan, Y. D.; Escobar, M.; Vuligonda, V.; Beard, R. L.; Chandraratna, R. A. Retinoid X receptor agonist elevation of serum triglycerides in rats by potentiation of retinoic acid receptor agonist induction or by action as single agents. *Biochem. Pharmacol.* **2001**, *62*, 1501−1509.
(22) de Vries-van der Weij, J.; de Haan, W.; Hu, L.; Kuif, M.; Oei, H. L.; van der Hoorn, J. W.; Havekes, L. M.; Princen, H. M.; Romijn, J. A.; Smit, J. W.; Rensen, P. C. Bexarotene induces dyslipidemia by increased very low-density lipoprotein production and cholesteryl ester transfer protein-mediated reduction of high-density lipoprotein. *Endocrinology* **2009**, *150*, 2368−2375.
(23) Lenhard, J. M.; Lancaster, M. E.; Paulik, M. A.; Weiel, J. E.; Binz, J. G.; Sundseth, S. S.; Gaskill, B. A.; Lightfoot, R. M.; Brown, H. R. The RXR agonist LG100268 causes hepatomegaly, improves glycaemic control and decreases cardiovascular risk and cachexia in diabetic mice suffering from pancreatic beta-cell dysfunction. *Diabetologia* **1999**, *42*, 545−554.
(24) Standeven, A. M.; Escobar, M.; Beard, R. L.; Yuan, Y. D.; Chandraratna, R. A. Mitogenic effect of retinoid X receptor agonists in rat liver. *Biochem. Pharmacol.* **1997**, *54*, 517−524.
(25) Sherman, S. I. Etiology, diagnosis, and treatment recommendations for central hypothyroidism associated with bexarotene therapy for cutaneous T-cell lymphoma. *Clin. Lymphoma* **2003**, *3*, 249−252.
(26) Canan Koch, S. S.; Dardashti, L. J.; Hebert, J. J.; White, S. K.; Croston, G. E.; Flatten, K. S.; Heyman, R. A.; Nadzan, A. M. Identification of the first retinoid X, receptor homodimer antagonist. *J. Med. Chem.* **1996**, *39*, 3229−3234.
(27) Beard, R. L.; Colon, D. F.; Song, T. K.; Davies, P. J.; Kochhar, D. M.; Chandraratna, R. A. Synthesis and structure−activity relationships of retinoid X receptor selective diaryl sulfide analogs of retinoic acid. *J. Med. Chem.* **1996**, *39*, 3556−3563.
(28) Nahoum, V.; Pérez, E.; Germain, P.; Rodríguez-Barrios, F.; Manzo, F.; Kammerer, S.; Lemaire, G.; Hirsch, O.; Royer, C. A.; Gronemeyer, H.; de Lera, A. R.; Bourguet, W. Modulators of the structural dynamics of the retinoid X receptor to reveal receptor function. *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 17323−17328.

(29) Kakuta, H.; Yakushiji, N.; Shinozaki, R.; Ohsawa, F.; Yamada, S.; Ohta, Y.; Kawata, K.; Nakayama, M.; Hagaya, M.; Fujiwara, C.; Makishima, M.; Uno, S.; Tai, A.; Maehara, A.; Nakayama, M.; Oohashi, T.; Yasui, H.; Yoshikawa, Y. RXR partial agonist CBt-PMN exerts therapeutic effects on type 2 diabetes without the side effects of RXR full agonists. *ACS Med. Chem. Lett.* **2012**, *3*, 427−432.
(30) Ohsawa, F.; Yamada, S.; Yakushiji, N.; Shinozaki, R.; Nakayama, M.; Kawata, K.; Hagaya, M.; Kobayashi, T.; Kohara, K.; Furusawa, Y.; Fujiwara, C.; Ohta, Y.; Makishima, M.; Naitou, H.; Tai, A.; Yoshikawa, Y.; Yasui, H.; Kakuta, H. Mechanism of retinoid X receptor partial agonistic action of 1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)-1*H*-benzotriazole-5-carboxylic acid and structural development to increase potency. *J. Med. Chem.* **2013**, *56*, 1865−1877.
(31) Nishimaki-Mogami, T.; Tamehiro, N.; Sato, Y.; Okuhira, K.; Sai, K.; Kagechika, H.; Shudo, K.; Abe-Dohmae, S.; Yokoyama, S.; Ohno, Y.; Inoue, K.; Sawada, J. The RXR agonists PA024 and HX630 have different abilities to activate LXR/RXR and to induce ABCA1 expression in macrophage cell lines. *Biochem. Pharmacol.* **2008**, *76*, 1006−1013.
(32) Ohsawa, F.; Morishita, K.; Yamada, S.; Makishima, M.; Kakuta, H. Modification at the lipophilic domain of RXR agonists differentially influences activation of RXR heterodimers. *ACS Med. Chem. Lett.* **2010**, *1*, 521−525.
(33) Takamatsu, K.; Takano, A.; Yakushiji, N.; Morohashi, K.; Morishita, K.; Matsuura, N.; Makishima, M.; Tai, A.; Sasaki, K.; Kakuta, H. The first potent subtype-selective retinoid X receptor (RXR) agonist possessing a 3-isopropoxy-4-isopropylphenylamino moiety, NEt-3IP (RXRalpha/beta-dual agonist). *ChemMedChem* **2008**, *3*, 780−787.
(34) Davies, P. J.; Berry, S. A.; Shipley, G. L.; Eckel, R. H.; Hennuyer, N.; Crombie, D. L.; Ogilvie, K. M.; Peinado-Onsurbe, J.; Fievet, C.; Leibowitz, M. D.; Heyman, R. A.; Auwerx, J. Metabolic effects of rexinoids: tissue-specific regulation of lipoprotein lipase activity. *Mol. Pharmacol.* **2001**, *59*, 170−176.
(35) Takahashi, B.; Ohta, K.; Kawachi, E.; Fukasawa, H.; Hashimoto, Y.; Kagechika, H. Novel retinoid X receptor antagonists: Specific inhibition of retinoid synergism in RXR−RAR heterodimer actions. *J. Med. Chem.* **2002**, *45*, 3327−3330.
(36) Kagechika, H.; Kawachi, E.; Hashimoto, Y.; Himi, T.; Shudo, K. Retinobenzoic acids. 1. Structure−activity relationships of aromatic amides with retinoidal activity. *J. Med. Chem.* **1988**, *31*, 2182−2192.
(37) Miwako, I.; Kagechika, H. Tamibarotene. *Drugs Today* **2007**, *43*, 563−568.
(38) Kasuga, J.; Oyama, T.; Hirakawa, Y.; Makishima, M.; Morikawa, K.; Hashimoto, Y.; Miyachi, H. Improvement of the transactivation activity of phenylpropanoic acid-type peroxisome proliferator-activated receptor pan agonists: effect of introduction of fluorine at the linker part. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 4525−4528.
(39) Aoyama, A.; Aoyama, H.; Dodo, K.; Makishima, M.; Hashimoto, Y.; Miyachi, H. LXR antagonists with a 5-substituted phenanthridin-6-one skeleton: synthesis and LXR transrepression activities of conformationally restricted carba-T0901317 analogs. *Heterocycles* **2008**, *76*, 137−142.
(40) Morris, G. M.; Huey, R.; Lindstrom, W.; Sanner, M. F.; Belew, R. K.; Goodsell, D. S.; Olson, A. J. AutoDock4 and AutoDockTools4: automated docking with selective receptor flexibility. *J. Comput. Chem.* **2009**, *30*, 2785−2791.
(41) Boerma, L. J.; Xia, G.; Qui, C.; Cox, B. D.; Chalmers, M. J.; Smith, C. D.; Lobo-Ruppert, S.; Griffin, P. R.; Muccio, D. D.; Renfrow, M. B. Defining the communication between agonist and coactivator binding in the retinoid X receptor α ligand binding domain. *J. Biol. Chem.* **2014**, *289*, 814−826.
(42) Submitted for publication.
(43) Arakawa, K.; Ishihara, T.; Aoto, M.; Inamasu, M.; Saito, A.; Ikezawa, K. Actions of novel antidiabetic thiazolidinedione, T-174, in animal models of non-insulin-dependent diabetes mellitus (NIDDM) and in cultured muscle cells. *Br. J. Pharmacol.* **1998**, *125*, 429−436.
(44) Wagner, C. E.; Jurutka, P. W.; Marshall, P. A.; Groy, T. L.; van der Vaart, A.; Ziller, J. W.; Furmick, J. K.; Graeber, M. E.; Matro, E.; Miguel, B. V.; Tran, I. T.; Kwon, J.; Tedeschi, J. N.; Moosavi, S.; Danishyar, A.; Philp, J. S.; Khamees, R. O.; Jackson, J. N.; Grupe, D. K.; Badshah, S. L.; Hart, J. W. Modeling, synthesis and biological evaluation of potential retinoid X receptor (RXR) selective agonists: novel analogues of 4-[1-(3,5,5,8,8-pentamethyl-5,6,7,8-tetrahydro-2-naphthyl)ethynyl]benzoic acid (bexarotene). *J. Med. Chem.* **2009**, *52*, 5950−5966.
(45) Kain, S. R. Use of secreted alkaline phosphatase as a reporter of gene expression in mammalian cells. *Methods Mol. Biol.* **1997**, *63*, 49−60.
(46) Landy, A. Dynamic, structural, and regulatory aspects of lambda site-specific recombination. *Annu. Rev. Biochem.* **1989**, *58*, 913−949.
(47) Walhout, A. J.; Temple, G. F.; Brasch, M. A.; Hartley, J. L.; Lorson, M. A.; van den Heuvel, S.; Vidal, M. GATEWAY recombinational cloning: application to the cloning of large numbers of open reading frames or ORFeomes. *Methods Enzymol.* **2000**, *328*, 575−592.
(48) Liang, F.; Matrubutham, U.; Parvizi, B.; Yen, J.; Duan, D.; Mirchandani, J.; Hashima, S.; Nguyen, U.; Ubil, E.; Loewenheim, J.; Yu, X.; Sipes, S.; Williams, W.; Wang, L.; Bennett, R.; Carrino, J. ORFDB: an information resource linking scientific content to a high-quality open reading frame (ORF) collection. *Nucleic Acids Res.* **2004**, *32*, D595−D599.
(49) Hengen, P. Purification of His-Tag fusion proteins from *Escherichia coli*. *Trends Biochem. Sci.* **1995**, *20*, 285−286.
(50) Collaborative Computational Project. Number 4. The CCP4 suite: programs for protein crystallography. *Acta. Crystallogr., Sect. D: Biol. Crystallogr.* **1994**, *50*, 760−763.
(51) Maron, D. M.; Ames, B. N. Revised methods for the Salmonella mutagenicity test. *Mutat. Res.* **1983**, *113*, 173−215.
(52) *Safety Guidelines*; International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH): Geneva, 2014; http://www.ich.org/products/guidelines/safety/article/safety-guidelines.html.