Identification of a Naturally Occurring Rexinoid, Honokiol, That Activates the Retinoid X Receptor

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Screening of a total of 86 crude drugs for retinoid X receptor (RXR) ligands demonstrated that the methanol extract of the bark of *Magnolia obovata* markedly activated the transcriptional activity of RXRα in luciferase reporter assays. Thereafter, honokiol (**1**) was isolated as a constituent able to activate RXR selectively as a natural rexinoid, but not RARα. The activity of **1** was more potent than those of phytanic acid and docosahexaenoic acid, both of which are known to be natural RXR agonists. Honokiol (**1**) is capable of activating a RXR/LXR heterodimer, resulting in the induction of ATP-binding cassette transporter A1 mRNA and protein expression in RAW264.7 cells, as well as an increase in [^{3}H]cholesterol efflux from peritoneal macrophages. These effects of **1** were enhanced synergistically in the presence of an LXR agonist, 22(*R*)-hydroxycholesterol. The results obtained demonstrate that **1**, a newly identified natural rexinoid, regulates the functions of RXR/LXR heterodimer and abrogates foam cell formation by the induction of ABCA1 via activation of the RXR/LXR heterodimer.

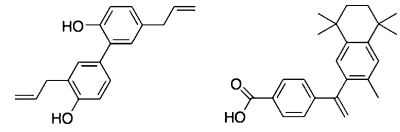
The retinoid X receptor (RXR) is a member of the nuclear receptor superfamily of ligand-activated transcription factors and an obligatory heterodimer partner for a large number of class II nuclear receptors, including the peroxisome proliferator-activated receptor (PPAR), the liver X receptor (LXR), the retinoic acid receptor (RAR), and the farnesoid X receptor.[1,2] RXRs, which consist of three subtypes (α, β, and γ), are unique among nuclear receptors because they form not only various heterodimers but also homodimers.[3]

RXR heterodimers are classified as either permissive or nonpermissive heterodimers.[4,5] Permissive heterodimers can be activated by the ligands of either RXR or its partner receptors and are activated synergistically in the presence of both ligands.[4] Nonpermissive heterodimers are transcriptionally activated by the ligands of RXR partner receptors, but not by RXR ligands, regardless of whether or not the ligands of RXR partner receptors are present.[5] However, recent accumulating evidence has indicated that nonpermissive RXR heterodimers can be activated by RXR ligands depending on factors such as tissue specificity, the cellular environment, or the ability of RXR ligands to recruit various coactivators or co-repressors, regardless of the presence or absence of RXR partner-receptor ligands.[6−9] These studies indicate that RXR plays more than a subordinate role in those heterodimers thought to be nonpermissive.

Several synthetic agonists selective for RXRs relative to RARs, referred to as rexinoids, have been developed and found to exert beneficial effects in various animal models of insulin resistance, diabetes, obesity, and atherosclerosis.[10,11] Rexinoids have potent glucose-lowering, insulin-sensitizing, antiobesity, and antiatherosclerotic effects, which possibly reflect their thiazolidinedione (a PPARγ agonist)-like activity via RXR/PPARγ activation. However, rexinoid activity appears to differ from that induced by thiazolidinediones.[12,13] Rexinoids reduce the progression of atherosclerosis in apoE-knockout or -knockin mice by enhancing the capacity of macrophages to efflux excess cholesterol via ATP-binding cassette transporter A1 (ABCA1) induced by the RXR/LXR heterodimer.[14,15] Rexinoids also increase the uptake and β-oxidation of saturated fatty acids in cultured skeletal muscle cells from diabetic humans and Zucker diabetic rats,[13,16] which indicates that increased fatty acid oxidation in diabetic skeletal muscle is a potential mechanism for insulin sensitization by rexinoids. Furthermore, rexinoids protect cardiomyocytes and endothelial cells from apoptosis by H_2O_2-induced and high-glucose-induced oxidative stress, respectively.[17,18]

Regardless of their numerous beneficial effects, rexinoids often raise triglyceride levels, suppress the thyroid hormone axis, and induce hepatomegaly, although the effects are not common among all rexinoids. These adverse effects have limited the development and the clinical application of these synthetic compounds as therapeutic agents for the treatment of type 2 diabetes and insulin resistance. In this context, bexarotene is the only synthetic rexinoid approved for clinical use, i.e., the treatment of cutaneous T-cell lymphomas.[19]

As the literature contains only a few reports of natural RXR ligands, it is likely that further exploration of natural rexinoids will lead to the development of pharmacological profiles that have potential therapeutic value for the treatment of diabetes, atherosclerosis, hepatic diseases, and cancer. The present study was therefore undertaken to identify novel naturally occurring RXR-specific rexinoids and to examine the ability of honokiol to activate RXR/LXR heterodimer by measuring LXR target gene expression.



Honokiol (**1**) Bexarotene (**2**)

Results and Discussion

Identification of Honokiol (1) as a Novel Natural Product Rexinoid. A total of 86 crude herbal drugs frequently used in Kampo prescriptions (traditional Japanese medicine) were selected and screened for RXRα agonist activity using a RXRα luciferase reporter assay. Among them, only the methanol extract of *Magnolia obovata* Thunb. (Magnoliaceae) showed strong transcriptional

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activation in this assay (Figure S1, Supporting Information). Therefore, the isolation of active constituents from the methanol extract was undertaken using conventional phytochemical methods. Compound **1** was isolated as a RXRα agonist from *M. obovata*. When the agonistic activity was assessed precisely in the RXRα luciferase reporter assay, **1** was capable of activating reporter gene transcription in a dose-dependent manner with an EC_{50} of 11.8 μM (Figure 1A). The transcriptional activation by **1** was completely abolished by a RXR-specific antagonist, PA452, as also shown with the synthetic agonist bexarotene (**2**) (Figure 1B). As the maximal activation ranged from one-third to one-half of that of **2**, **1** appears to be a partial agonist for RXR. When the activation of RARα by **1** was examined, this compound failed to activate RARα (Figure 1A) as well as LXRα, PPARγ, and PPARδ at the concentrations used in this study (Figure S2, Supporting Information). Honokiol (**1**), the major constituent of the bark of *M. ovobata* and *M. officinalis*, has been thus far demonstrated to show many pharmacological activities, including anti-inflammatory,[21,22] antiarrhythmic,[23] anxiolytic,[24] antitumor,[25,26] antithrombotic,[27] antiarthritic,[28] and antiallergic effects.[29] In the present study, **1** was identified clearly as a novel, naturally occurring rexinoid that can selectively activate RXRα compared with RARα. Although the data are not shown, magnolol, another major constituent of the bark of *M. ovobata*, also activated RXRα, but less potently than honokiol.

As natural RXR ligands, the vitamin A derivative 9-*cis*-retinoic acid (RA), a chlorophyll metabolite, phytanic acid, and various unsaturated fatty acids [particularly linoleic, linolenic, and docosahexaenoic acids (DHA)] are known to bind to RXRs.[30−34] We therefore compared their binding activity to RXRα in the yeast two-hybrid assay (Figure 1C). Honokiol (**1**) exhibited much higher binding activity to RXRα than phytanic acid and DHA, although it was less potent than 9-*cis*-RA. 9-*cis*-RA is a well-known RXR ligand, which also binds to RAR, indicating that it is not a rexinoid. Further, the search for 9-*cis*-RA in tissue with the aid of radioligand precursors (i.e., tracers) has been largely unsuccessful. Thus, the physiological importance of 9-*cis*-RA as an endogenous RXR ligand remains highly controversial.[35] DHA has been reported to bind to and activate RXR in addition to PPARs, although with low affinify (ca. 60−100 μM).[32,33] In the present study, we were unable to clearly confirm RXR activation in the yeast two-hybrid assay at concentrations up to 50 μM. Phytanic acid has also been identified as a RXR ligand as well as a PPARα ligand,[36] but its physiological importance remains obscure. When compared with **1** in the yeast two-hybrid assay, phytanic acid showed much less activity. These results demonstrate that honokiol (**1**) is the first natural potent rexinoid demonstrated to activate RXR selectively thus far.

Honokiol (1) Increases mRNA Levels of ABCA1 and G1 by Activating the RXR/LXR Heterodimer. Rexinoids are known to be active and permissive for heterodimers such as RXR/LXR and PPARs/RXR.[10] Therefore, the properties of **1** as a rexinoid were examined in terms of activation of RXR/LXR heterodimer. The ATP-binding cassette transporter A1 (ABCA1) and ABCG1 are induced in macrophages following the activation of RXR/LXR heterodimer by oxidized cholesterol, which is intimately implicated in the control of cholesterol homeostasis in macrophages. To confirm that **1** acts as a rexinoid in cells, the ability of this compound to induce the expression of ABCA1 and ABCG1 in RAW264.7 cells was evaluated. As shown in Figure 2A, an LXR agonist, 22(*R*)-HC, and bexarotene (**2**) increased mRNA levels of ABCA1 significantly as the respective agonists for the permissive RXR/LXR heterodimer. Compound **1** was capable of enhancing mRNA levels of ABCA1 in a dose-dependent manner, and these effects were completely abolished by a RXR antagonist, HX531. Additionally, **1** was found to be capable of increasing mRNA levels of ABCG1 as well as ABCA1, and this effect was completely abrogated by HX531 (Figure 2B). Next, when the protein levels of ABCA1 were determined by western blot analysis, either **1** or **2** elevated the protein levels of ABCA1 (Figure 2C). Further, **1** increased synergistically the protein levels of ABCA1 in the presence of 22(*R*)-HC, as also shown with **2**. These results demonstrate that **1** can activate RXR/LXR heterodimer as a rexinoid.

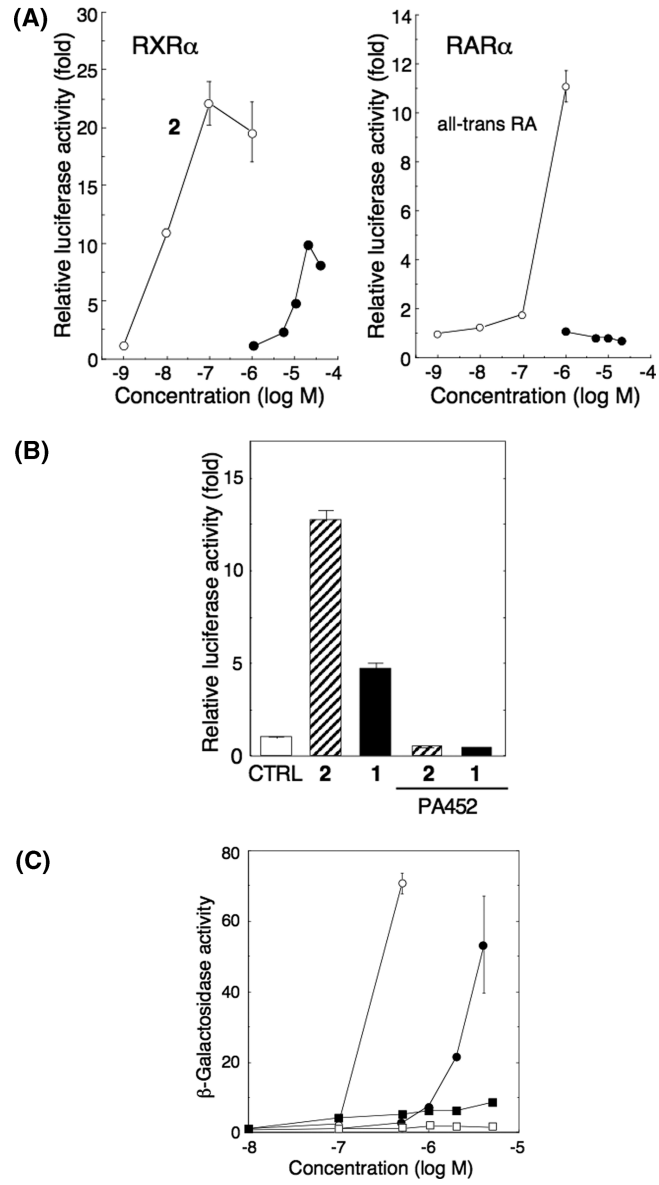

Figure 1. Activation of RXRα by honokiol (**1**). (A) HEK293 cells were co-transfected with the respective nuclear receptor expression vectors and luciferase reporter plasmids together with pCMX-β-gal, as described in the Experimental Section. Six hours after transfection, the cells were treated with increasing concentrations of honokiol (**1**) (solid circles), bexarotene (**2**) (RXR agonist), and all-*trans*-retinoic acid (RAR agonist) for 48 h, or (B) the cells were treated with **1** and **2** at concentrations of 10 μM and 10^{-8} M, respectively, in the absence or presence of RXR pan-antagonist PA452 (10 μM) for 48 h. The activity was normalized using β-gal and expressed as fold induction relative to that of vehicle-treated cells. Data are represented as means ± SD of three determinants from a representative of three independent experiments, which showed similar results. (C) RXRα binding activities of natural RXR agonists were compared using a yeast two-hybrid assay as described in the Experimental Section. 9-*cis*-RA (open circles), phytanic acid (solid squares), DHA (open squares), and **1** (solid circles) were incubated with yeast suspension at increasing concentrations at 30 °C for 18 h. β-Galactosidase activity is presented as the means ± SD of three determinants.

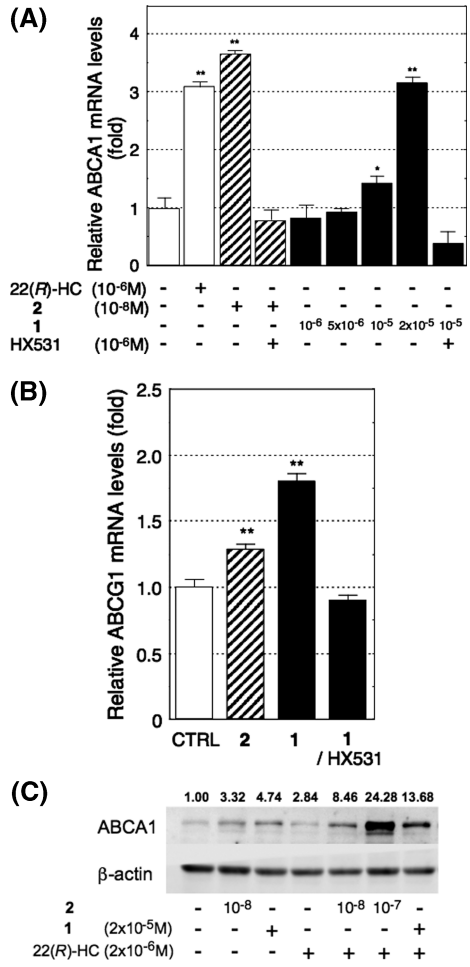

Figure 2. Induction of ABCA1 and G1 in RAW264.7 cells by honokiol (**1**). (A) RAW264.7 cells were treated with **1**, 22(*R*)-HC, or bexarotene (**2**) at the indicated concentrations for 8 h in the absence or presence of RXR antagonist HX531. mRNA levels of ABCA1 were measured with quantitative real-time RT-PCR analysis, normalized using the β-actin levels, and expressed as fold induction relative to that of vehicle-treated cells. Data are represented as means ± SD of three wells from a representative of three independent experiments, which showed similar results. $*p < 0.05$, $**p < 0.01$ vs vehicle control (CTRL). (B) RAW264.7 cells were treated with **1** at a concentration of 20 μM or **2** at 10^{-8} M in the absence or presence of HX531 (1 μM) for 8 h. mRNA levels of ABCG1 were measured as described above. Data are represented as described for part A. (C) RAW264.7 cells were treated with **1**, **2**, and 22(*R*)-HC at the indicated concentrations for 24 h. Protein levels of ABCA1 were determined by western blot analysis, as described in the Experimental Section. Data were a representative of three independent experiments, which showed similar results. The numbers above the bands represent the ratio of band density of ABCA1 to β-actin.

Honokiol (1) Activates ApoAI-Mediated Cholesterol Efflux from Macrophages. The effects of honokiol (**1**) on apoAI-mediated cholesterol efflux from mouse peritoneal macrophages loaded with [^{3}H]-cholesterol in the presence of acetyl-LDL were next examined. Single treatment of macrophages with **1** or bexarotene (**2**) enhanced cholesterol efflux slightly, but significantly (Figure 3). Intriguingly, treatment with **1** or **2** in combination with 22(*R*)-HC significantly increased the amount of cholesterol efflux from cholesterol-loaded macrophages. These results demonstrate that either a single treatment with **1** or treatment with **1** in combination with an LXR agonist is able to induce ABCA1 expression, in turn leading to the prevention of foam cell formation. Taken together, **1** appears to participate efficiently in the regulation

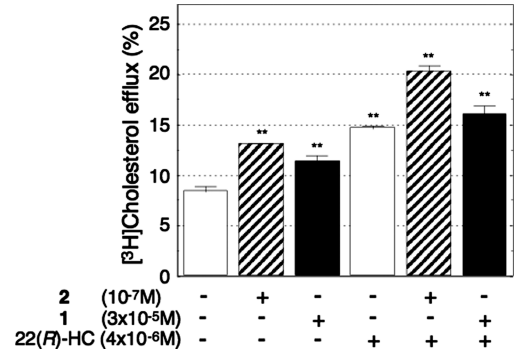

Figure 3. ApoAI-mediated cholesterol efflux from peritoneal macrophages. Thioglycolate-elicited peritoneal macrophages were incubated with [^{3}H]cholesterol (2 μCi/mL) together with acetyl-LDL (50 μg/mL) for 24 h. To determine apoAI-mediated cholesterol efflux, the cells were incubated with honokiol (**1**) or bexarotene (**2**) at the indicated concentrations in the absence or presence of 22(*R*)-HC in medium containing 0.3% bovine serum albumin and 10 μg/mL free apoAI. After 24 h, the amount of [^{3}H]cholesterol excreted into the medium was measured using a liquid scintillation counter. Data are represented as means ± SD of three wells from a representative of two independent experiments, which showed similar results. $**p < 0.01$ vs vehicle control.

of cholesterol homeostasis in macrophages via the activation of RXR/LXR heterodimer as a rexinoid. However, as ABCA1 is known to be induced by PPARs/RXR heterodimers via LXR induction,[37−39] the activation of PPARs/RXR heterodimers by **1** may contribute partially to the prevention of foam cell formation. A study concerning which permissive RXR heterodimers are activated by **1** is now in progress.

Potentially beneficial effects of rexinoids on cancer, diabetes, insulin resistance, and atherosclerosis have been reported to date,[10−16] but clinically available rexinoids other than **2** have not yet been developed. As regards their clinical application, rexinoids that can attain a sufficiently high plasma concentration to activate RXR heterodimers and yet not exert any adverse effects possess potential as therapeutic agents against various lifestyle diseases. Indeed, **1** has been well-tolerated in models of arthritis, heart disease, and cancer[27,40] and has been used without noticeable side effects for many years in traditional Japanese or Chinese medicine,[24] which renders it an attractive candidate therapeutic agent for metabolic disorders such as diabetes, and atherosclerosis.

In addition, one of the expected beneficial effects of rexinoids is a reduction in the respective side effects intrinsic to PPARγ, RAR, and VDR agonists when used in combination with rexinoids. Specifically, the therapeutic effects of PPARγ agonist thiazolidinediones, RAR agonist all-*trans*-retinoic acid, and VDR agonist 1,25-dihydroxyvitamin D3 are hampered by their severe side effects;[41−43] however, honokiol combination therapy with low doses of the respective agonists may overcome such problems.

In the present study, we have identified honokiol (**1**) as a naturally occurring potent rexinoid that may serve as a candidate therapeutic agent in the treatment of atherosclerosis. This finding indicates that it is worthwhile exploring natural products to modulate physiological or pathophysiological function. A further detailed study of **1** might also enable the expansion of clinical applications of this compound to include the treatment of lifestyle diseases other than atherosclerosis.

Experimental Section

Reagents. Bexarotene (**2**) was purchased from Toronto Research Chemicals, Inc. (North York, Canada). 22(*R*)-Hydroxycholesterol [22(*R*)-HC], all-*trans*-retinoic acid, and docosahexaenoic acid (DHA) were from Sigma-Aldrich (St. Louis, MO). [^{3}H]Cholesterol was obtained from PerkinElmer Japan (Yokohama, Japan), apoAI from

Athens Research & Technology (Athens, GA), acetyl-LDL from AbD Serotec (Oxford, UK), honokiol (**1**) (99%) from Kishida (Osaka, Japan), phytanic acid from Cayman (Ann Arbor, MI), and chlorophenol red β-D-galactopyranoside from Wako Pure Chemical Industries (Osaka, Japan). The RXR antagonists HX531 and PA452 were kindly provided by Dr. H. Kagechika of Tokyo Medical and Dental University. Crude drugs were obtained from Tsumura & Co. (Tokyo, Japan).

Preparation of Methanol Extracts of Crude Drugs. Eighty-six crude drugs, used for the preparation of Kampo prescriptions (therapeutic drugs in traditional Japanese medicine), most of which are frequently used in the 210 Kampo prescriptions approved by the Ministry of Health, Labour and Welfare, Japan, were selected to prepare methanol extracts as follows. Crude drugs (10 g) were extracted with 100% methanol (100 mL × 3 times) for 24 h at room temperature, and the extracts were filtered, combined, and concentrated *in vacuo*. The resulting residues were dissolved in dimethylsulfoxide.

Animals. Male *dd*Y mice (5−7 weeks of age) were purchased from Shizuoka Laboratory Center (Shizuoka, Japan). All animals were kept in a temperature-controlled room (24 ± 1 °C) on a 12 h light/dark cycle and were given free access to a commercial diet (MF; Oriental Yeast Co., Tokyo, Japan) and water at the Laboratory Animal Center of Aichi Gakuin University. All animal procedures were approved by the institutional animal care and use committee of the School of Pharmacy at Aichi Gakuin University, Japan.

Cell Culture. Thioglycolate-elicited macrophages were harvested from the abdominal cavity of *dd*Y mice 4 days after intraperitoneal injection of 2 mL of 3% thioglycolate medium (Wako Pure Chemical Industries, Osaka, Japan). Cells were seeded at a concentration of 2×10^6 cells/mL and were maintained in RPMI1640 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum (FBS) (Nichirei Biosciences Inc., Tokyo, Japan), 100 U/mL of penicillin (Sigma-Aldrich), and 100 μg/mL of streptomycin (Sigma-Aldrich) for 2 h. After nonadherent cells were removed, the adherent macrophages were incubated with various concentrations of the test compounds in RPMI1640 medium supplemented with 10% FBS.

Mouse macrophage-like RAW264.7 cells and human embryonic kidney (HEK) 293 cells were provided by the RIKEN BioResource Center (Tsukuba, Japan) through the National Bio-Resource Project of the Ministry of Education, Culture, Sports, Science, and Technology (MEXT) of Japan. RAW264.7 cells and HEK293 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% FBS and Minimum Essential Medium (MEM) containing 10% FBS and nonessential amino acids (Sigma-Aldrich) at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Luciferase Reporter Gene Assay. HEK293 cells were transfected by calcium phosphate co-precipitation with the following: 60 ng of pCMX-hRXR-α and 150 ng of CRBPII-tk-Luc for the RXR reporter assay and 30 ng of pCMX-hRAR-α and 120 ng of tk-RE-Luc for the RAR reporter assay, with the addition of 30 ng of pCMX-β-gal expression vector and carrier DNA pUC18 to yield 600 ng of total DNA per well. After 6 h of transfection, the cells were thoroughly washed with fresh medium and were left to continue incubation in the presence of the crude methanol extracts at 100 μg/mL concentration or compounds at the indicated concentrations in medium containing 10% FBS for another 40 h. Luciferase and β-galactosidase activities of the cell lysates were analyzed using a luminescence reader and a spectrophotometer, respectively. Luciferase activity was normalized relative to the activity of an internal β-galactosidase control and expressed as the relative luciferase activity, which was determined in triplicate experiments.

Yeast Two-Hybrid Assay. Yeast expressing GAL4DBD-RXRα, GAL4AD-TIF2, and *lac*Z reporter plasmids were kindly provided by Dr. J. Nishikawa of Osaka University.[20] Yeast transformants were grown overnight at 30 °C with vigorous shaking in 5 mL of selective medium without leucine and tryptophan. The yeast was harvested by centrifugation at 3000*g* for 15 min and suspended in fresh medium. The absorbance at 630 nm of the cell suspension was measured with a U-2001 spectrophotometer (Hitachi High-Technologies Co., Tokyo, Japan). All assays were performed using the yeast suspension with the absorbance at 630 nm of 0.1. Aliquots (90 μL) of the yeast suspension were cultured in the presence of 10 μL of test compounds for 18 h at 30 °C. The 100 μL of chemically treated yeasts was incubated with 33 μL of Z buffer (0.1 M sodium phosphate (pH 7.9), 10 mM KCl, 1 mM $MgSO_4$) containing 4 mg/mL ZYMOLYASE-20T (Seikagaku Co., Tokyo, Japan) for 30 min at 30 °C. The enzymatic reaction of β-galactosidase was initiated by the addition of 25 μL of 0.5 mg/mL chlorophenol red β-D-galactopyranoside dissolved in 0.1 M sodium phosphate buffer (pH 7.9). When the red color developed, 50 μL of 2 M Na_2CO_3 was added to stop the reaction. The absorbances at 570 and 630 nm were measured, and the activity was calculated according to the following equation: $U = [(\text{absorbance at } 570 \text{ nm}) - (\text{absorbance at } 630 \text{ nm})]/(\text{absorbance at } 630 \text{ nm at the start of the assay})$. β-Galactosidase activity is presented as the means ± SD of three determinations.

Quantitative RT-PCR. Total RNA was isolated from the cultured cells using RNAiso Plus (Takara Bio Inc., Ohtsu, Japan). Following treatment of the RNA samples with DNase (Invitrogen), first strand cDNA was synthesized from 0.5 μg of total RNA using oligo$(dT)_{20}$ RT primer and ReverTra Ace (Toyobo, Japan) according to the manufacturer's instructions. Quantitative real-time PCR (SYBR green) analysis was performed using the Takara-bio TP800 Thermal Cycler Dice real-time system. Levels of mRNA expression were subsequently normalized relative to β-actin mRNA levels and calculated according to the delta−delta Ct method. The primer sequences were as follows: ABCA1, forward primer 5′-AAT TCT CAA GTG CAA ACA CTT CTG G-3′ and reverse primer 5′-GAG GCA TAT GCT TGC GGT ACA-3′; ABCG1, forward primer 5′-GTC TCA GCC TTC TAA AGT TCC TC-3′ and reverse primer 5′-TCT CTC GAA GTG AAT GAA ATT TAT CG-3′; β-actin, forward primer 5′-CAT CCG TAA AGA CCT CTA TGC CAA C-3′ and reverse primer 5′-ATG GAG CCA CCG ATC CAC A-3′.

Western Blot Analysis. After a 24 h incubation period, the macrophages were harvested and lysed in 40 μL of lysis buffer containing 10 mM Tris-base (pH 8.0), 0.1% Triton X-100, 0.15 mM KCl, 5 mM mercaptoethanol, 1.3 mM EDTA, and protease inhibitor cocktail tablets (Roche Diagnostics, Tokyo, Japan) on ice. The lysates were centrifuged at 12000*g* for 15 min at 4 °C, and the resulting supernatant was assayed to determine the protein concentration (Bradford assay, Bio-Rad Laboratories). The supernatant (20 μg protein) was suspended in 0.9 M urea, 0.2% (v/v) Triton X-100, and 0.1% (w/v) dithiothreitol, supplied with 10% (w/v) lithium dodecylsulfate (LDS), and was subjected to 8% LDS-PAGE. After electrophoresis, the proteins in the gel were transferred to a polyvinylidene fluoride membrane (Millipore, Billerica, MA) using an AE6677 horizon blot system (Atto Corp., Tokyo, Japan). The membrane was blocked overnight at 4 °C in a solution of 5% powdered skim milk in Tris-buffered saline (TBS) and then was incubated with anti-ABCA1 antibody (MABI 98-2) diluted 1:50 in TBS containing 0.05% Tween-20 for 2 h at room temperature. The blot was washed in three changes of wash buffer (0.05% Tween-20 in TBS) and then was incubated with alkaline phosphatase-conjugated anti-rat IgG antibody (Santa Cruz Biotechnology, Santa Cruz, CA) in 1% powdered skim milk and 0.05% Tween-20 in TBS for 1 h at room temperature. The blot was thoroughly washed in three changes of wash buffer, and ABCA1 was detected using CDP-Star (Applied Biosystems) as a substrate of alkaline phosphatase. The protein concentration was determined using the LAS-3000 mini system (Fujifilm, Tokyo, Japan). The anti-ABCA1 antibody was kindly provided by Dr. S. Yokoyama of Nagoya City University, Japan.

Cholesterol Efflux Assays. Thioglycolate-elicited peritoneal macrophages were incubated with [^{3}H]cholesterol (2 μCi/mL) in the presence of acetyl-LDL (12.5 μg/mL) for 24 h. The cells were then thoroughly washed and equilibrated for 3 h in RPMI1640 medium containing 3% bovine serum albumin. For the cholesterol efflux, the cells were incubated with test compounds in medium containing 0.3% bovine serum albumin and 10 μg/mL free apoAI. After 24 h, aliquots of the medium were removed, and the [^{3}H]cholesterol excreted into the medium was measured using a liquid scintillation counter. The [^{3}H]cholesterol present in the cells was also determined by a liquid scintillation counter following the extraction of cellular lipids with 2-propanol/*n*-hexane. The amount of excreted cholesterol was calculated as follows: [^{3}H]cholesterol efflux (%) = total [^{3}H]cholesterol in medium × 100/(total [^{3}H]cholesterol in medium + total [^{3}H]cholesterol in the cells).

Statistical Analysis. Data are represented as the means ± SD and were evaluated for statistical significance by one-way ANOVA, followed by Bonferroni's *t*-test. A value of $p < 0.05$ was considered to be statistically significant.

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Supporting Information Available: Summary of screening of methanol extracts of crude drugs for RXRα agonist activity, and the activation of LXRα, PPARγ, and PPARδ by honokiol (**1**) demonstrated by luciferase reporter gene assay. This material is available free of charge via the Internet at http://pubs.acs.org.

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