Ascochlorin Derivatives as Ligands for Nuclear Hormone Receptors

Marie Togashi,[†,‡,§] Satoshi Ozawa,[†,‡] Shoko Abe,[∥] Tomoyuki Nishimura,[∥] Mie Tsuruga,[†] Kunio Ando,[⊥] Gakuzo Tamura,[⊥] Shigefumi Kuwahara,[∥] Makoto Ubukata,[#] and Junji Magae*[,†]

Department of Biotechnology, Institute of Research and Innovation, 1201 Takada, Kashiwa 277-0861, Japan, Graduate School of Agricultural Science, Tohoku University, Aoba-ku, Sendai 981-8555, Japan, Nuclear Research Institute, KSP, 3-2-1 Sakada, Takatsu-ku, Kawasaki 213-0012, Japan, and Biotechnology Research Center, Toyama Prefectural University, Kosugi, Toyama 939 0398, Japan

Received December 17, 2002

Nuclear receptor family proteins are structurally related transcription factors activated by specific lipophilic compounds. Because they are activated by a variety of hormonal molecules, including retinoic acid, vitamin D, and steroid hormones, they are assumed to be promising targets for clinical drugs. We previously found that one ascochlorin (**1**) derivative, 4-*O*-carboxymethyl-ascochlorin (**2**), is a potent agonist of peroxisome proliferator activated receptor γ (PPARγ). Here, we synthesized derivatives of **1**, designated as a lead compound, to create new modulators of nuclear hormone receptors. Two derivatives, 4-*O*-carboxymethyl-2-*O*-methylascochlorin (**9**) and 4-*O*-isonicotinoyl-2-*O*-methylascochlorin (**10**), showed improved agonistic activity for PPARγ and induced differentiation of a progenitor cell line, C3H10T1/2. We also found that **1**, dehydroascofuranon (**29**), and a 2,4-*O*-diacetyl-1-carboxylic acid derivative of **1** (**5**) specifically activated estrogen receptors, PPARα, and an androgen receptor. All of the derivatives (**1**−**29**) activated the pregnane X receptor. These results suggest that the chemical structure of **1** is useful in designing novel modulators of nuclear receptors.

Introduction

Ascochlorin (**1**) and ascofuranone (**20**) that were originally isolated as antiviral and antifungal compounds are antibiotics possessing a unique prenyl−phenol moiety.[1] In addition to their antimicrobial activities, these molecules modulate various physiological events in animals, including hypolipidemic activity,[2−6] improvement of type I and type II diabetes,[7−11] suppression of hypertension,[12] tumor supression,[13,14] and immunological modulation.[15,16] They also induce differentiation of human and mouse myeloid cell lines.[17] Recently, we found that 4-*O*-carboxymethyl ascochlorin (**2**) transcriptionally activated and directly interacted with peroxisome proliferator activated receptor γ (PPARγ),[18] a nuclear hormone receptor involved in the development of diabetes.[19,20]

Nuclear hormone receptors are a family of transcription factors that induce the transcription of target genes when activated by their specific lipophilic ligand, including steroid hormones, retinoic acid, and lipid metabolites. These proteins exhibit strong structural similarities, and all contain separable domains for DNA binding and ligand binding.[21−25] Currently, 70 nuclear receptors are known, but specific endogenous ligands have been identified for only half of them. Because nuclear hormone receptors have been implicated in many biochemical pathways, any number of human diseases can result from defects in these proteins. Therefore, drugs that specifically modulate the transcription activity of nuclear receptors are expected to have a promising therapeutic potential to treat disease.

In the present study, we synthesized new derivatives of **1** and **20** in order to improve the agonistic activity of **2** on PPARγ and succeeded in obtaining new, more potent agonists for PPARγ. We also found several agonists for other nuclear hormone receptors. The results suggest that the chemical structure of **1** is useful in designing nuclear hormone receptor modulators (Figure 1).

Results

Prediction of Three-Dimensional Structure of 2 in PPARγ. The rationale for designing new derivatives was based on the hypothesis that the molecular structure of **2** mimics the three-dimensional structure of the antidiabetic ligand rosiglitazone (Figure 2). X-ray crystallography has revealed that rosiglitazone forms a ternary complex containing the PPARγ ligand-binding domain (LBD) and amino acids of human steroid receptor coactivating factor-1.[26,27] We searched the pharmacophore model of **2** using CONFLEX calculations and discovered a resemblance among stable derivatives of **2** and rosiglitazone with respect to molecular size and shape. This analysis led us to predict that the aromatic portion of **2** can act as a bioisosteric replacement for the thiazolidine part of rosiglitazone, maintaining high affinity binding and receptor activation. We predict that the carboxylic acid of **2** could interact with H449 and the oxygen atom of the C-1 aldehyde interacts with H323 of PPARγ LBD. The other hydrophobic parts of **2** would be free to interact in a relatively nonspecific manner with the large ligand-binding pocket as mentioned by Nolte et al.[26] Therefore, we synthesized derivatives of **1** and **20**, focusing mainly on the aromatic ring.

* To whom correspondence should be addressed. Tel: +81-4-7144-9142. Fax: +81-4-7144-9007. E-mail: jmagae@sannet.ne.jp.
† Institute of Research and Innovation.
‡ These authors contributed equally to this work.
§ Present address: Diabetes Center, University of California at San Francisco, San Francisco, CA 94143.
∥ Tohoku University.
⊥ Nuclear Research Institute.
Toyama Prefectural University.

10.1021/jm0205649 CCC: $25.00
Published on Web 08/19/2003

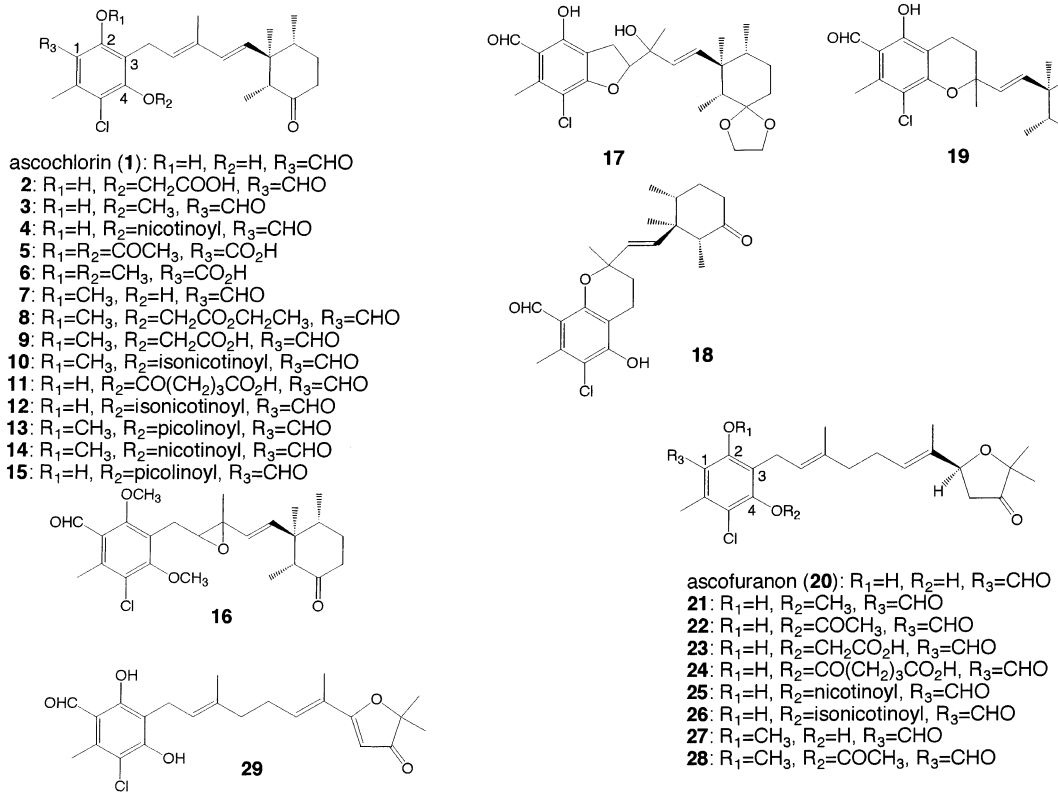

Figure 1. Chemical structures of derivatives.

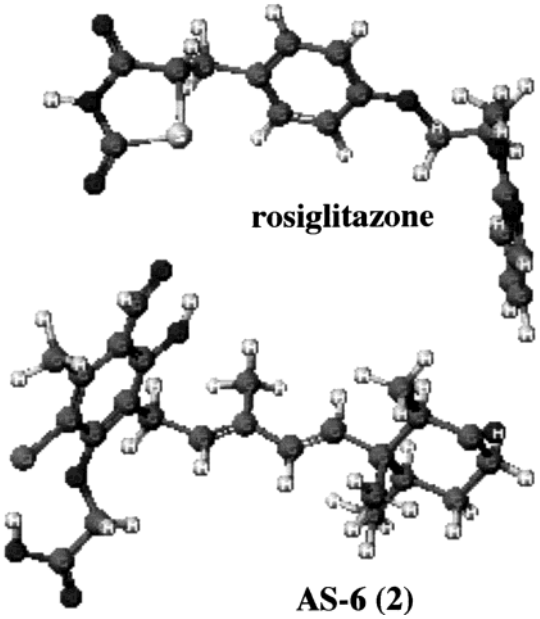

Figure 2. Stable conformations of compound **2** and rosiglitazone.

Synthesis of Ascochlorin-Related Compounds. The synthetic routes for the derivatives of **1** are summarized in Figure 3, and the structures of the derivatives are shown in Figure 1. Regioselective modification to the C-4 phenolic alcohol of **1** was achieved by taking advantage of the difference in reactivities between it and the C-2 phenolic alcohol, which forms an intramolecular hydrogen bond with the C-1 aldehyde. Acetylation of **1** with acetic anhydride in pyridine gave 4-*O*-acetylascochlorin (**30**) in an 89% yield. Methylation of the C-2 phenolic alcohol of **30** afforded the methyl ether product, which was hydrolyzed to produce a key compound, 2-*O*-methylascochlorin (**7**). 4-*O*-Isonicotinoyl-2-*O*-methylascochlorin (**10**) and 4-*O*-carboxymethyl-2-*O*-methylascochlorin (**9**) were prepared by isonicotynoylation of **7** and introduction of a carboxymethyl group into **7** via the intermediate **8** (4-*O*-(ethoxycarbonyl)methyl-2-*O*-methylascochlorin), respectively. Methylation of **1** with excess methyl iodide afforded the dimethyl derivative **3**, which was treated with *m*-CPBA to give the monoepoxide (2,4-di-*O*-methylascochlorin oxide, **16**). The aldehyde of **3** was converted to the acid derivative (2,4-di-*O*-methylascochlorinic acid, **6**) by oxidation with sodium chlorite.

Treatment of **1** with concentrated sulfuric acid gave a mixture of cyclic products, **18** and **19**, in yields of 20 and 30%, respectively. 4-*O*-Nicotinoylascochlorin (**4**) and 4-*O*-isonicotinoylascochlorin (**12**) were prepared from **1** by selective acylation with nicotinoyl chloride and isonicotinoyl chloride in pyridine, respectively. The 4-*O*-glutarate derivative (4-*O*-(4-carboxybutanoyl)ascochlorin, **11**) was obtained by acylation of **1** with glutaric anhydride in pyridine in the presence of a catalytic amount of 4-(dimethylamino)pyridine.

Although acetylation of the C-2 alcohol in **1** with acetic anhydride hardly proceeded, 2,4-di-*O*-acetylascochlorin (**31**) was obtained by treatment with acetyl chloride in pyridine. The 1-carboxylic acid derivative (2,4-di-*O*-acetylascochlorinic acid, **5**) was prepared from **31** in the same manner as **6**. A cyclic derivative (**17**) was unintentionally obtained from **31**.

Synthesis of Ascofuranone-Related Compounds. Procedures similar to those used to synthesize ascochlorin derivatives were applicable to preparation of the ascofuranone derivatives, 4-*O*-methylascofuranone (**21**), 4-*O*-acetylascofuranone (**22**), 4-*O*-carboxylmethylasco-

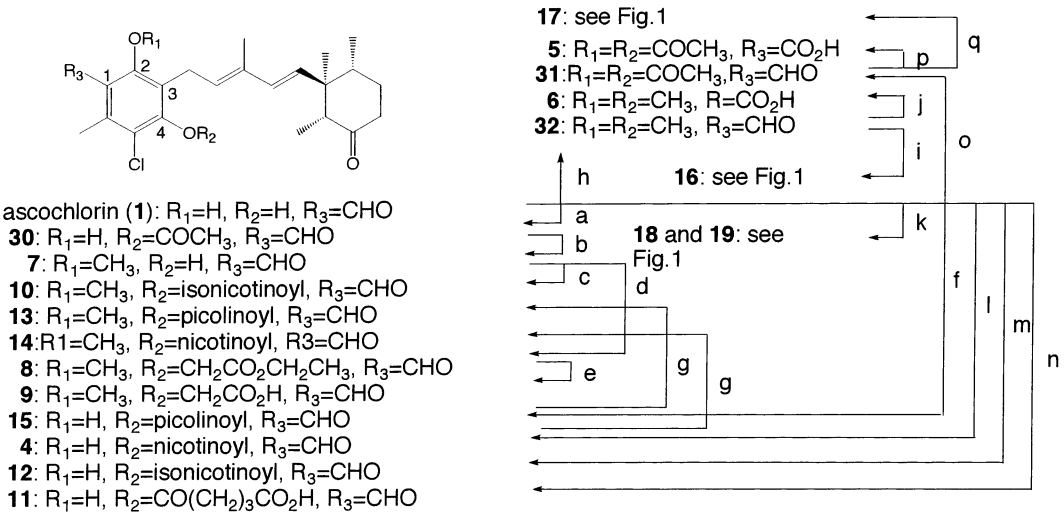

Figure 3. Synthetic schemes for derivatives. Key: (a) Ac_2O, Py, 89%. (b) (i) MeI, K_2CO_3, acetone, 87%; (ii) 1% aqueous NaOH, MeOH, 55%. (c) Isonicotinoyl chloride hydrochloride, Py, 90%. (d) Ethyl bromoacetate, NaH, DMF, 77%. (e) K_2CO_3 (20%), MeOH, 63%. (f) Picolinoyl chloride hydrochloride, Et_2N, Py, 50%. (g) MeI, K_2CO_3, acetone, 97% for **13**, 95% for **14**. (h) Excess MeI, K_2CO_3, acetone, 89%. (i) MCPBA, $NaHCO_3$, CH_2Cl_2, 16%. (j) $NaClO_2$, *t*-BuOH, 2-methyl-2-butene, NaH_2PO_4/H_2O, 87%. (k) Concentrated H_2SO_4, 20% for **18** and 33% for **19**. (l) Nicotinoyl chloride hydrochloride, Py, 39%. (m) Isonicotinoyl chloride hydrochloride, Py, 79%. (n) Glutaric anhydride DMAP, Py, 42%. (o) AcCl, Py, 88%. (p) $NaClO_2$, *t*-BuOH, 2-methyl-2-butene, NaH_2PO_4/H_2O, 64%. (q) (i) $(TMSOCH_2)_2$, TMSOTf, CH_2Cl_2, 33%; (ii) MCPBA, $NaHCO_3$, CH_2Cl_2; (iii) MeOK, 18-crown-6, hexamethyldisilane, THF, 56%.

furanone (**23**), 4-*O*-(4-carboxybutanoyl)ascofuranone (**24**), 4-*O*-nicotinoylascofuranone (**25**), 4-*O*-isonicotinoyl-2-*O*-methylascofuranone (**26**), 2-*O*-methylascofuranone (**27**), and 4-*O*-acetyl-2-methylascofuranone (**28**). The structures of these derivatives appear in Figure 1. Dehydroascofuranone (**29**) was obtained by oxidative treatment of **20** with silver oxide in the presence of sodium hydroxide.

Modulation of PPARγ Activity. Twelve derivatives, **4**, **5**, **7**−**15**, and **27**, activated the transcriptional activity of PPARγ, as indicated in Table 1 and Figure 4. The presence of an oxidized aldehyde group (compounds **5** and **6**) failed to induce agonistic activity for PPARγ. On the contrary, a methylated C-2 alcohol in **1** (**7**) resulted in PPARγ activation to the same degree as for **2**. Introduction of both 2-*O*-methyl and 4-*O*-carboxymethyl groups (**9**) resulted in additive PPARγ activation. The ethyl ester derivative of **9** (compound **8**) showed a decreased additive effect. Modification of the side chain (compound **16**) or bonding between a phenolic alcohol and a double bond of the side chain (compounds **17**−**19**) produced compounds that did not induce PPARγ activation. Acylation of the C-4 alcohol with a longer carboxylic acid unit (compound **11**) failed to improve the agonistic activity of **2**. Isomers of **4** (**12** and 4-*O*-picolinoylascochlorin, **15**) comparably activated PPARγ. Methylation of the C-2 hydroxyl group in **12** and **15**, as in the case of **9**, improved agonistic activity of the corresponding derivatives, **10** and 2-*O*-methyl-4-*O*-picolinoylascochlorin (**13**), while methylation of **4** (2-*O*-methyl-4-*O*-nicotinoylascochlorin, **14**) did not. For derivatives of **20**, methylation of the C-2 hydroxyl group (compound **27**) induced slight activation of PPARγ. None of the derivatives (**1**−**29**) synthesized showed antagonistic activity against PPARγ activated by 1 μM of the agonist pioglitazone. Agonistic activity of **2**, **12**, **13**, and **15** decreased at the highest concentration, because of the nonspecific toxicity of the compound as shown in proliferation inhibition by these compounds (Table 1).

Table 1. Summary of Biological Activity of Ascochlorin and Ascofuranone Derivatives[a]

	growth inhibition	minimum effective concentration (μM)					
compds	IC_{50} (μM)	PPARγ	PPARα	PXR	ERα	ERβ	AR
1	1.0	ND	ND	1	1	1	ND
2	56	30	ND	30	ND	ND	ND
3	1.8	ND	ND	1	ND	ND	ND
4	4.7	10	ND	3	ND	ND	ND
5	32	ND	ND	1	ND	ND	1
6	56	ND	ND	1	ND	ND	ND
7	5.6	10	ND	1	ND	ND	ND
8	3.2	3	ND	1	ND	ND	ND
9	56	3	ND	3	ND	ND	ND
10	5.6	0.3	ND	3	ND	ND	ND
11	0.6	10	ND	1	ND	ND	ND
12	10	10	ND	1	ND	ND	ND
13	38	30	ND	1	ND	ND	ND
14	37	100	ND	10	ND	ND	ND
15	5.6	3	ND	1	ND	ND	ND
16	10	ND	ND	1	ND	ND	ND
17	18	ND	ND	3	ND	ND	ND
18	56	ND	ND	3	ND	ND	ND
19	18	ND	ND	1	ND	ND	ND
20	3.2	ND	ND	3	ND	ND	ND
21	5.6	ND	ND	1	ND	ND	ND
22	10	ND	ND	1	ND	ND	ND
23	32	ND	ND	30	ND	ND	ND
24	3.2	ND	ND	10	ND	ND	ND
25	10	ND	ND	10	ND	ND	ND
26	10	ND	ND	10	ND	ND	ND
27	18	30	ND	1	ND	ND	ND
28	10	ND	ND	1	ND	ND	ND
29	56	ND	10	1	ND	ND	ND

[a] ND indicates not detected.

Activation of Other Nuclear Receptors by Ascochlorin-Related Compounds. Through a vast screening for ligands of nuclear hormone receptors based on reporter assays, we found that all of the derivatives activated pregnane X receptor (PXR) (Table 1). One of the most potent derivatives, **12**, activated PXR as effectively as clotrimazole. In contrast, only **1** activated estrogen receptors (ER) α and β (Figure 5A,B). Compounds **29** and **5** specifically activated PPARα and

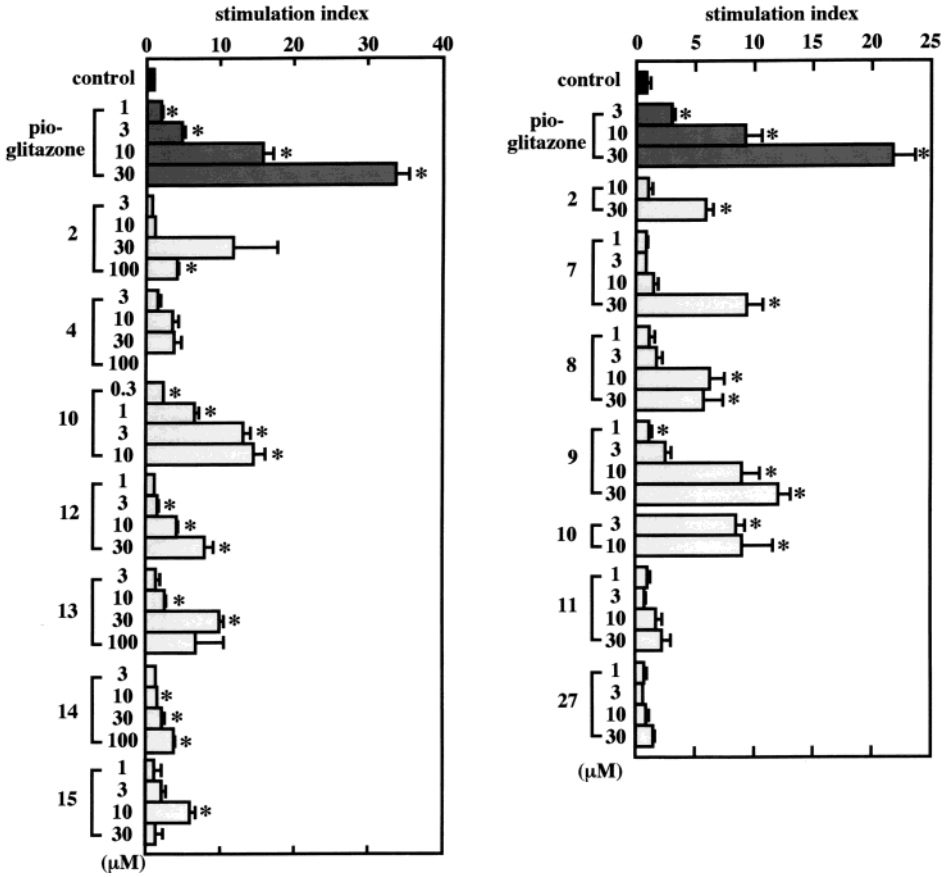

Figure 4. Agonistic activity for PPARγ of derivatives. U2OS cells transfected with expression plasmids for nuclear receptor, together with the corresponding reporter plasmids and pCMV-β-galactosidase as an internal control. The cells were incubated with each ascochlorin derivative for 24 h, as described in the Experimental Section. Agonistic activity of the reagents is expressed as stimulation index. Values are the means of triplicate cultures. Bars represent standard deviation. *, Statistically significant as compared to control ($P < 0.05$, *t*-test).

an androgen receptor (AR), respectively (Figure 5C,D). None of the derivatives (**1**−**29**) activated other nuclear hormone receptors, which included retinoic acid receptor, retinoid X receptor (RXR), glucocorticoid receptor, mineralocorticoid receptor, PPARδ, thyroid hormone receptor, liver X receptor, and farnesoid X receptor (data not shown).

Effects on Cell Function of Ascochlorin-Related Compounds. It is known that activators of PPARγ induce differentiation of adipocytes.[19,20] As expected, derivatives that were potent activators of PPARγ also induced differentiation of C3H10T1/2 progenitor cells into adipocytes, as judged by induction of glycerol phosphate dehydrogenase (GPDH), a marker enzyme of mature adipocytes. Correlated to agonistic activity on PPARγ, compound **9** more effectively induced differentiation than did **2** or **7**, while compound **10**, which has comparable PPARγ agonistic activity, was less effective than **9**, probably because of its cytotoxicity. Compounds **14** and **15** did not induce adipocyte differentiation, whereas **13**, which had a more potent PPARγ agonistic activity, did (Figure 6). Inhibition of cell proliferation was decreased in most derivatives except **11** (Table 1). Toxicities of **6**, **9**, **18**, and **29** were decreased to the same level as **2**.

Discussion

Because the calculated partial negative charge on the C-1 carbonyl oxygen atom of **2** is lower than that of **1** (−0.322 vs −0.334 au), we were interested in synthesizing derivative **7**, which has a much lower partial negative charge at the C-1 oxygen atom (−0.280 au). As expected, **7** activated PPARγ; we therefore synthesized **9**, a hybrid compound of **2** and **7**. Compound **9**, which has the lowest partial negative charge (−0.272 au) on the C-1 oxygen atom among these three compounds, demonstrated improved agonistic activity on PPARγ. Methylation at the C-2 alcohol of **12** (compound **10**) improved agonistic activity in the same manner as **9**.

Therefore, we next synthesized **13** (−0.265 au) and **14** (−0.274 au), hybrid compounds of **15** (−0.323 au) and **4** (−0.323 au), respectively, with **7**. Although we observed improved maximum agonistic activity for compound **13**, the activity of **14** was not as obvious (Figure 4A). These data suggest that the moderate partial charge on the oxygen atom of the C-1 aldehyde is one of the important parameters in establishing the agonistic activity on PPARγ of these derivatives. Molecular shape and size are also important as evidenced by the behavior of the compound **7** type cyclic derivatives, **17**−**19**, which did not show any PPARγ agonistic activity.

Among the derivatives synthesized in this study, two compounds, **9** and **10**, had the most potent agonistic activity, and **9** effectively induced adipocyte differentiation. The inhibitory concentration for cell proliferation by **9** decreased as it did for **2**. Compound **10** activated PPARγ and induced adipocyte differentiation at lower

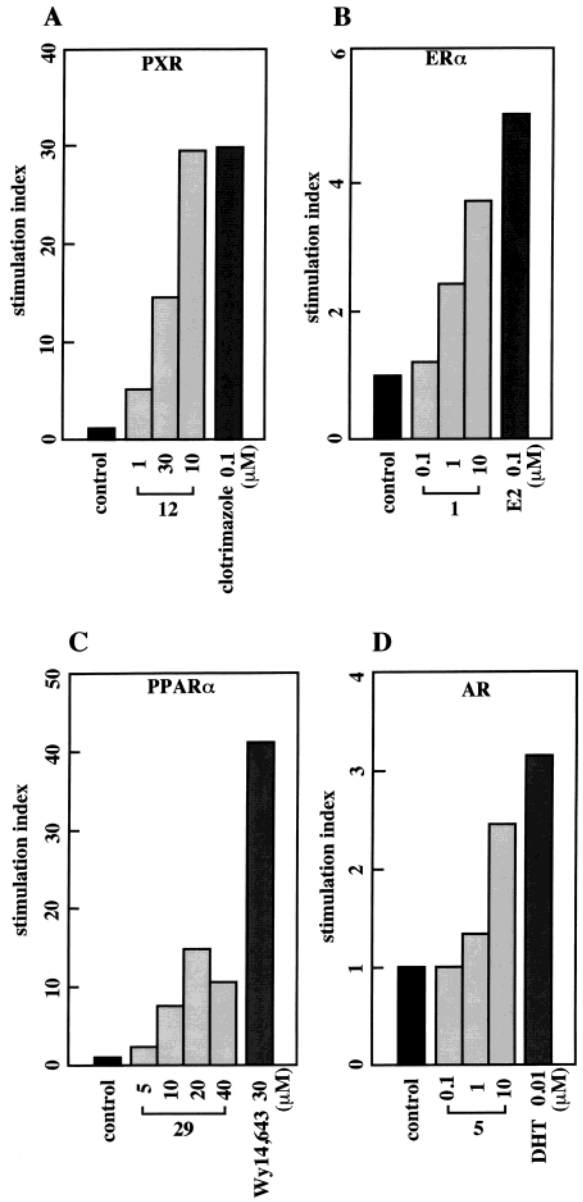

Figure 5. Activation of nuclear receptors by derivatives. U2OS cells were transfected with expression vectors for Gal4-PXR (A), ER (B), Gal4-PPARα (C), or AR (D), together with the corresponding reporter plasmids and pCMV-β-galactosidase as an internal control. The cells were incubated with ascochlorin derivatives for 24 h, as described in the Experimental Section. Agonistic activity of the reagents is expressed as stimulation index. The representative result in more than three experiments is shown. Values are the means of triplicate cultures. Bars represent standard deviation.

concentrations than did **2** and **9**; this compound activated the PPARγ at 1 μM. The toxicity of **10** was also improved relative to the original compound, **1**. Although compounds with the thiazolidinedione structure, including rosiglitazone, troglitazone, and pioglitazone, activate PPARγ and induce adipocyte differentiation more effectively than do **9** and **10**, they sometimes exhibit severe hepatotoxicity. For this reason, troglitazone has been withdrawn from clinical use. Compounds **9** and **10** are promising candidates for clinical study as nonthiazolidinedione antidiabetic drugs.

Although PPARγ activation and adipocyte differentiation induced by the ascochlorin derivatives were mostly correlated, several exceptions were observed. For example, **10** did not show improved activity to induce adipocyte differentiation, although it did activate PPARγ more potently than did **4**. Moreover, **20** and **29** significantly induced differentiation of adipocytes (data not shown), whereas they had little or no activity for activation of PPARγ. Troglitazone acts as a partial agonist of PPARγ in muscle and kidney cells, while it behaves as its full agonist in 3T3L1 adipocytes.[28] FMOC-L-leucine, a chemically distinct PPARγ ligand, induces a particular allosteric configuration of PPARγ, resulting in differential cofactor recruitment.[29] This compound improves insulin sensitivity; yet, it has a lower adipogenic activity. It is also reported that coactivator interactions induced by a natural ligand, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, could not be observed with troglitazone.[30] Differences in chemical structure among the ascochlorin derivatives might affect the set of cofactors recruited to PPARγ and the set of target genes induced in different cell types, resulting in distinct pharmacological properties. Alternatively, it is also possible that these derivatives induce adipocyte differentiation through cellular factors other than PPARγ.

We found that all of the derivatives of **1** and **20** activated PXR as strongly as clotrimazole. PXR is a nuclear receptor involving detoxification of hydrophobic substances including xenobiotics and bile acids.[31−35] PXR binds as a heterodimer with RXR to DNA binding elements in the regulatory regions of cytochrome P450 3A monooxygenase and a number of other genes involved in the metabolism and elimination of these toxins from the body. The PXR LBD has a large spherical ligand-binding cavity that allows it to interact with a wide range of hydrophobic chemicals,[36] explaining why all of the derivatives of **1** and **20** activated PXR. Although PXR protects the body by facilitating the rapid clearance of the drug, it may potentially give rise to the harmful drug−drug interactions. Thus, modulation of PXR activity will be important for the clinical use of derivatives in future.

In addition, activation of PXR might contribute to modulation of cholesterol metabolism of ascochlorin derivatives, because **3** and **20** have hypocholesterolemic activity despite the lack of PPARγ activation.[2−6] We also found that **1**, **6**, and **29** activated ERα and β, AR, and PPARα, respectively. Sex steroids slow the rate of bone remodeling, protect against bone loss, and elongate the life span of mature bone cells.[37] They also have neuromodulatory and neuroprotective roles.[38] The inhibitory effect of estrogens on atherogenesis is well-documented in numerous animal models.[39] Dietary consumption of weak agonists of ER improves insulin resistance[40] and is associated with a decreased breast cancer risk.[41] PPARα is considered a major regulator of intra- and extracellular lipid metabolism.[42] AR and PPARα also modulate immunological responses.[43,44] These observations suggest the therapeutic potential of the present derivatives in the treatment of osteoporosis, neural injuries, metabolic diseases, cardiovascular diseases, immune diseases such as inflammation and autoimmune diseases, and cancers.

Conclusions

We synthesized new derivatives of **1** and **20** in order to improve the agonistic activity of **2** on PPARγ and

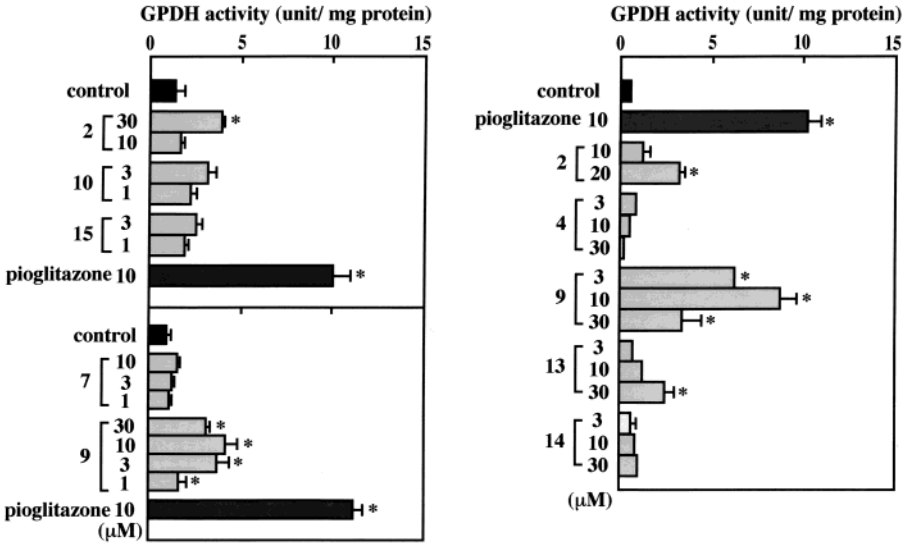

Figure 6. Adipocyte differentiation of C3H10T1/2 cells induced by derivatives. C3H10T1/2 cells were induced to differentiate into mature adipocytes in the presence of ascochlorin derivatives, as described in the Experimental Section. Adipocyte differentiation was evaluated through the induction of GPDH activity. Values are the means of triplicate cultures. Bars represent standard deviation. *, Statistically significant as compared to control ($p < 0.05$, *t*-test).

succeeded to obtain more new potent agonists for PPARγ through the modification of the aromatic ring. We also found that all of the derivatives activated PXR, **29** activated PPARα, and **1** and **5** were specific ligands for ERs and AR. These results demonstrate that the chemical structures of **1** and **20** are useful for discovering new ligands of nuclear receptors, which could be used in the treatment of human diseases including diabetes, arteriosclerosis, and chronic inflammation.

Experimental Section

Prediction of Three-Dimensional Structure. CONFLEX and MOPAC calculations as well as quantitative structure−activity relationship analyses were executed using the CAChe Work System on a Power Macintosh G3. Calculations of partial charge for **1**, **2**, **7**, **9**, and others were done with 3-methyl model compounds instead of real side chain compounds. The three-dimensional structure of PPARγ from the Protein Data Bank was displayed using MolScript on an SGI Indy computer.

Synthesis of Derivatives. Compound 30. 2-Chloro-4-formyl-5-hydroxy-3-methyl-6-[(2*E*,4*E*)-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-2,4-pentadienyl]phenyl Acetate. Acetic anhydride (2 mL, 21.2 mmol) was added to a solution of **1** (400 mg, 0.989 mmol) in pyridine (2 mL). The mixture was stirred overnight at room temperature and then poured into ice water and extracted with ethyl ether. The extract was washed with brine, dried ($MgSO_4$), and evaporated. The residue was purified by silica gel column chromatography (hexane−acetone, 95:5) to give an oil, which was crystallized to a pure compound **30** (350 mg, 89%); mp 144 °C. IR (film): 1775, 1715, 1645 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 0.70 (3H, s), 0.81 (3H, d, $J = 7.0$ Hz), 0.84 (3H, d, $J = 7.0$ Hz), 1.57−1.67 (1H, m), 1.89 (3H, s), 1.90−1.97 (2H, m), 2.36−2.44 (3H, m), 2.37 (3H, s), 2.65 (3H, s), 3.44 (2H, d, $J = 7.0$ Hz), 5.37 (1H, t, $J = 7.0$ Hz), 5.39 (1H, d, $J = 16.0$ Hz), 5.87 (1H, d, $J = 16.0$ Hz), 10.30 (1H, s), 12.55 (1H, s). Anal. ($C_{25}H_{31}ClO_5$) C, H.

4-*O*-Acetyl-2-*O*-methylascochlorin (33). 2-Chloro-4-formyl-5-methoxy-3-methyl-6-[(2*E*,4*E*)-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-2,4-pentadienyl]phenyl Acetate. A solution of **30** (500 mg, 1.12 mmol) in acetone (30 mL) was heated at reflux for 2 h with methyl iodide (2 mL, 32.1 mmol) and potassium carbonate (190 mg, 1.37 mmol). The resultant mixture was filtered, and evaporation of the filtrate gave **33** (450 mg, 87%) after crystallization from hexane−acetone (95:5); mp 114 °C. IR (film): 1780, 1715 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 0.71 (3H, s), 0.81 (3H, d, $J = 7.0$ Hz), 0.84 (3H, d, $J = 7.0$ Hz), 1.58−1.68 (1H, m), 1.90 (3H, br s), 1.91−1.97 (2H, m), 2.32 (3H, s), 2.35−2.44 (2H, m), 2.63 (3H, s), 3.46 (2H, d, $J = 7.0$ Hz), 3.83 (3H, s), 5.35 (1H, t, $J = 7.0$ Hz), 5.42 (1H, d, $J = 16.0$ Hz), 5.89 (1H, d, $J = 16.0$ Hz), 10.44 (1H, s). Anal. ($C_{26}H_{33}ClO_5$) C, H.

Compound 7. 3-Chloro-4-hydroxy-6-methoxy-2-methyl-5-[(2*E*,4*E*)-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-2,4-pentadienyl]benzaldehyde. A 1% aqueous NaOH solution (20 mL) was added to a stirred solution of **33** (300 mg, 0.717 mmol) in methanol, and the mixture was heated at reflux for 2 h. The resultant mixture was acidified with dilute hydrochloric acid, and MeOH was then removed in vacuo. The aqueous residue was extracted with EtOAc. The organic solution was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane−acetone, 95:5) to give **7** (150 mg, 55%); mp 126 °C. IR (film): 1715, 1665 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 0.70 (3H, s), 0.81 (3H, d, $J = 7.0$ Hz), 0.84 (3H, d, $J = 7.0$ Hz), 1.57−1.67 (1H, m), 1.89−1.96 (2H, m), 1.93 (3H, br s), 2.34−2.46 (3H, m), 2.66 (3H, s), 3.56 (2H, d, $J = 7.0$ Hz), 3.84 (3H, s), 5.40 (1H, d, $J = 16.0$ Hz), 5.50 (1H, t, $J = 7.0$ Hz), 5.90 (1H, d, $J = 16.0$ Hz), 6.29 (1H, s), 10.37 (1H, s). Anal. ($C_{24}H_{31}ClO_4$) C, H; C: calcd, 68.81; found, 69.25.

Compound 8. Ethyl {2-Chloro-4-formyl-5-methoxy-3-methyl-6-[(2*E*,4*E*)-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-2,4-pentadienyl]phenoxy}acetate. Sodium hydride (60%, dispersion in mineral oil, 23.1 mg, 0.578 mmol) was washed with dry pentane and suspended in dimethylformamide (DMF; 1 mL). To the above suspension was dropped **7** (0.22 g, 0.525 mmol) in DMF (2 mL). The mixture was stirred at room temperature for 30 min, and ethyl bromoacetate (0.0641 mL, 0.578 mmol) was added. The resulting solution was stirred for 4 h at 50 °C, poured into a saturated NH_4Cl solution, and extracted with ether. The extract was washed with water and brine successively, dried, and concentrated in vacuo. The residue was subjected to silica gel column chromatography (hexanes−EtOAc) to give **8** as a colorless gum (0.204 g, 77%). IR (film): 1765, 1713, 1694 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 0.70 (3H, s), 0.80 (3H, d, $J = 6.7$ Hz), 0.83 (3H, d, $J = 6.9$ Hz), 1.31 (3H, t, $J = 7.0$ Hz), 1.58−1.67 (1H, m), 1.90 (3H, br s), 1.90−1.96 (2H, m), 2.34−2.44 (3H, m), 2.63 (3H, s), 3.65 (2H, d, $J = 7.0$ Hz), 3.88 (3H, s), 4.29 (2H, q, $J = 7.0$ Hz), 4.61 (2H, s), 5.39 (1H, d, $J = 16.0$

Hz), 5.46 (1H, t, J = 7.0 Hz), 5.89 (1H, d, J = 16.0 Hz), 10.42 (1H, s). Anal. ($C_{28}H_{37}Cl\ O_6$) C, H.

Compound 9. {**2-Chloro-4-formyl-5-methoxy-3-methyl-6-[(2*E*,4*E*)-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-2,4-pentadienyl]phenoxy**}**acetic Acid.** A 20% aqueous potassium carbonate solution (1 mL, 1.45 mmol) was added to a solution of **8** (0.207 g, 0.409 mmol) in methanol, and the mixture was stirred for 2 h at room temperature. The reaction mixture was acidified to pH 2 with a 3 N aqueous HCl solution and extracted with ether. The extract was washed with water and brine, dried ($MgSO_4$), and evaporated in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH) to give **9** as a colorless gum (0.122 g, 63%). IR (film): 1715, 1696 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 0.70 (3H, s), 0.81 (3H, d, J = 6.8 Hz), 0.83 (3H, d, J = 6.8 Hz), 1.62 (1H, qd, J = 13.5, 6.0 Hz), 1.91 (3H, br s), 1.91−1.97 (2H, m), 2.34−2.45 (3H, m), 2.63 (3H, s), 3.62 (2H, d, J = 6.9 Hz), 3.84 (3H, s), 4.65 (2H, s), 5.42 (1H, d, J = 16.0 Hz), 5.43 (1H, t, J = 6.9 Hz), 5.89 (1H, d, J = 16.0 Hz), 10.42 (1H, s). Anal. ($C_{26}H_{33}ClO_6$) C, H.

Compound 10. 2-Chloro-4-formyl-5-methoxy-3-methyl-6-[(2*E*,4*E*)-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-2,4-pentadienyl]phenyl Pyridine-4-carboxylate. Isonicotinoyl chloride hydrochloride (71.7 mg, 0.403 mmol) was added to a solution of **7** (61.3 mg, 0.146 mmol) in dry pyridine (1 mL). The reaction mixture was stirred for 3 h at room temperature, water was added, and the solution was stirred for an additional 30 min. The resulting mixture was extracted with EtOAc, and the extract was washed with aqueous $CuSO_4$ solution, water, saturated aqueous $NaHCO_3$ solution, and brine successively, dried ($MgSO_4$), and concentrated in vacuo. The residue was subjected to silica gel column chromatography (hexanes−EtOAc) to produce **10** (69.3 mg, 90%) as a colorless gum. IR (film): 1760, 1710, 1700 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 0.69 (3H, s), 0.76−0.83 (6H, br), 1.59−1.64 (1H, m), 1.65 (3H, br s), 1.85−1.96 (2H, m), 2.32−2.45 (3H, m), 2.67 (3H, s), 3.39−3.50 (1H, br), 3.50−3.63 (1H, br), 3.88 (3H, s), 5.29 (1H, d, J = 16.0 Hz), 5.34 (1H, t, J = 7.0 Hz), 5.82 (1H, d, J = 16.0 Hz), 7.79−7.99 (2H, m), 8.87 (2H, m), 10.49 (1H, s). Anal. ($C_{30}H_{34}ClNO_5$) C, H, N.

2,4-Di-*O*-methylascochlorin (32). 3-Chloro-4,6-dimethoxy-2-methyl-5-[(2*E*,4*E*)-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-2,4-pentadienyl]benzaldehyde. MeI (2 mL, 32.1 mmol) and potassium carbonate (2 g, 14.5 mmol) were added to a solution of **1** (400 mg, 0.989 mmol) in acetone (20 mL). The mixture was heated at reflux for 3 h and filtered. The filtrate was concentrated in vacuo and crystallized from hexane to afford **32** (380 mg, 89%); mp 101 °C. IR (film): 1732, 1690 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 0.70 (3H, s), 0.81 (3H, d, J = 7.0 Hz), 0.83 (3H, d, J = 7.0 Hz), 1.57−1.67 (1H, m), 1.93 (3H, br s), 1.91−1.97 (2H, m), 2.34−2.46 (3H, m), 2.64 (3H, s), 3.55 (2H, d, J = 7.0 Hz), 3.83 (3H, s), 3.88 (3H, s), 5.41 (1H, d, J = 16.0 Hz), 5.47 (1H, t, J = 7.0 Hz), 5.89 (1H, d, J = 16.0 Hz), 10.42 (1H, s). Anal. ($C_{25}H_{33}ClO_4$) C, H.

Compound 16. 3-Chloro-4,6-dimethoxy-2-methyl-5-[(*E*)-2,3-epoxy-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-4-pentenyl]benzaldehyde. $NaHCO_3$ (0.111 mg) and *m*-chloroperbenzoic acid (70%, 157 mg, 0.637 mmol) were added to a solution of **32** (229 mg, 0.530 mmol) in CH_2Cl_2 (7 mL). The mixture was stirred for 3 h in an ice bath, diluted with water, and extracted with ether. The organic layer was washed with 10% sodium thiosulfate aqueous solution, saturated $NaHCO_3$ aqueous solution, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was subjected to silica gel column chromatography (hexanes−EtOAc, 5:1) to give **16** (39 mg, 16%). IR (film): 1715, 1696, 1553, 1458, 1379, 1309, 1228, 1100 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 0.67 (3H, s), 0.78 (1.5H, d, J = 7.0 Hz), 0.804 (1.5H, d, J = 7.0 Hz), 0.806 (1.5H, d, J = 7.0 Hz), 0.83 (1.5 H, d, J = 7.0 Hz), 1.557 (1.5H, s), 1.562 (1.5H, s), 1.56−1.65 (1H, m), 1.87−1.95 (2H, m), 2.32−2.44 (3H, m), 2.65 (3H, s), 2.97−3.00 (2H, m), 3.00−3.05 (1H, m), 3.87 (3H, s), 3.93 (3H, s), 5.24 (0.5H, d, J = 16.0 Hz), 5.25 (0.5H, d, J = 16.0 Hz), 5.53 (0.5H, d, J = 16.0 Hz), 5.5.54 (0.5H, d, J = 16.0 Hz), 10.42 (1H, s). Anal. ($C_{25}H_{33}ClO_5$) C, H.

Compound 6. 3-Chloro-4,6-dimethoxy-2-methyl-5-[(2*E*,4*E*)-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-2,4-pentadienyl]benzoic Acid. $NaH_2PO_4{\cdot}2H_2O$ (0.155 g, 0.993 mmol) and water (2.6 mL) were added to **32** (0.428 g, 0.989 mmol). *tert*-Butyl alcohol (10.4 mL), 2-methyl-2-butene (0.460 mL, 4.34 mmol), and 90% sodium chlorite (0.305, 3.04 mmol) were added to the mixture, which was then stirred for 15 min at room temperature. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated in vacuo to give 0.396 g of crude product. The product was purified by silica gel column chromatography (CH_2Cl_2−MeOH) to give **6** (0.384 g, 87%) as a colorless gum. IR (KBr disk): 3440, 1712 cm^{-1}. NMR (CD_3OD, 500 MHz): 0.70 (3H, s), 0.79 (3H, d, J = 6.8 Hz), 0.82 (3H, d, J = 6.8 Hz), 1.61 (1H, qd, J = 13.4, 4.7 Hz), 1.92 (3H, s), 1.92−2.10 (2H, m), 2.24−2.29 (1H, m), 2.33 (3H, s), 2.50−2.62 (2H, m), 3.53 (2H, d, J = 7.0 Hz), 3.80 (3H, s), 3.81 (3H, s), 5.46 (1H, t, J = 7.0 Hz), 5.48 (1H, d, J = 15.8 Hz), 5.93 (1H, d, J = 15.8 Hz). Anal. ($C_{25}H_{33}Cl\ O_5$) C, H.

Compounds 18 and 19. 6-Chloro-5-hydroxy-2,7-dimethyl-2-[2-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)vinyl]chroman-8-carbaldehyde and 8-Chloro-5-hydroxy-2,7-dimethyl-2-[2-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)vinyl]chroman-6-carbaldehyde. Compound **1** (400 mg, 0.989 mmol) was dissolved in concentrated H_2SO_4 (16 mL) and was left standing for 30 min at room temperature. The reaction mixture was poured into ice water and extracted with EtOAc. The organic layer was washed with water and brine successively, dried ($MgSO_4$), and concentrated in vacuo. The residue was subjected to silica gel column chromatography (hexane−acetone, 95:5−9:1) to give **18** and **19**, which were crystallized to **18** (80 mg, 20%) from hexane−acetone (9:1) and to **19** (130 mg, 33%) from hexane−acetone (95:5), respectively. Compound **18**: mp 65 °C. IR (film): 1710, 1635 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 0.55 (1.5H, d, J = 7.0 Hz), 0.59 (1.5H, d, J = 7.0 Hz), 0.66 (3H, s), 0.79 (1.5H, d, J = 7.0 Hz), 0.82 (1.5 H, d, J = 7.0 Hz), 1.49 (3H, s), 1.54−1.63 (1H, m), 1.69−1.76 (0.5H, m), 1.82−1.95 (2.5H, m), 1.96−2.02 (1H, m), 2.19 (0.5H, q, J = 7.0 Hz), 2.30−2.41 (2.5H, m), 2.50−2.57 (1H, m), 2.66 (1.5H, s), 2.67 (1.5H, s), 2.76 (0.5H, t, J = 5.0 Hz), 2.79 (0.5H, t, J = 5.0 Hz), 5.316 (0.5H, d, J = 16.0 Hz), 5.323 (0.5H, d, J = 16.0 Hz), 5.364 (0.5H, d, J = 16.0 Hz), 5.370 (0.5H, d, J = 16.0 Hz), 6.183 (0.5H, s), 6.185 (0.5H, s), 10.592 (0.5H, s), 10.595 (0.5H, s). Anal. ($C_{23}H_{29}ClO_4$) C, H. Compound **19**: mp 99 °C. IR (film): 1710, 1635 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 0.52 (1.5H, d, J = 7.0 Hz), 0.57 (1.5H, d, J = 7.0 Hz), 0.66 (3H, s), 0.80 (1.5H, d, J = 7.0 Hz), 0.83 (1.5 H, d, J = 7.0 Hz), 1.53 (3H, s), 1.54−1.63 (1H, m), 1.68−1.76 (0.5H, m), 1.79−1.96 (2.5H, m), 1.97−2.03 (1H, m), 2.20 (0.5H, q, J = 7.0 Hz), 2.30−2.40 (2.5H, m), 2.40−2.50 (1H, m), 2.61 (1.5H, s), 2.63 (1.5H, s), 2.74 (0.5H, t, J = 5.0 Hz), 2.78 (0.5H, t, J = 5.0 Hz), 5.304 (0.5H, d, J = 16.0 Hz), 5.311 (0.5H, d, J = 16.0 Hz), 5.346 (0.5H, d, J = 16.0 Hz), 5.359 (0.5H, d, J = 16.0 Hz), 10.13 (0.5H, s), 10.14 (0.5H, s), 12.75 (1H, s). Anal. ($C_{23}H_{29}ClO_4$) C, H; C: calcd, 68.22; found, 68.70.

Compound 4. 2-Chloro-4-formyl-5-hydroxy-3-methyl-6-[(2*E*,4*E*)-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-2,4-pentadienyl]phenyl Pyridine-3-carboxylate. Nicotinoyl chloride hydrochloride (242 mg, 1.42 mmol) was added to a solution of **1** (0.150 g, 0.358 mmol) in dry pyridine (1.5 mL). The reaction mixture was stirred for 2 h at room temperature, after which time water was added and the solution was stirred for an additional 30 min. The resulting mixture was extracted with ether. The extract was washed with aqueous $CuSO_4$ solution, water, saturated aqueous $NaHCO_3$ solution, and brine successively, dried ($MgSO_4$), and concentrated in vacuo. The residue was subjected to silica gel column chromatography (hexanes−EtOAc, 3:1) to give **4** (242 mg, 39%). ^1H NMR ($CDCl_3$, 500 MHz): 0.68 (3H, s), 0.79 (3H, d, J = 7.0 Hz), 0.81 (3H, d, J = 7.0 Hz), 1.56−1.66 (1H, m), 1.71 (3H, s), 1.86−1.95 (2H, m), 2.30−2.44 (3H, m), 2.69 (3H, s), 3.33−3.48 (1H, br), 3.48−3.63 (1H, br), 5.30 (1H, d, J = 16.0 Hz), 5.39 (1H, t, J = 7.0 Hz), 5.83 (1H, d,

J = 16.0 Hz), 7.48−7.51 (1H, m), 8.44−8.47 (1H, m), 8.89−8.91 (1H, m), 9.41−9.42 (1H, m), 10.35 (1H, s), 12.60 (1H, s). Anal. ($C_{29}H_{32}ClNO_5$) C, H, N.

Compound 14. 2-Chloro-4-formyl-5-methoxy-3-methyl-6-[(2*E*,4*E*)-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-2,4-pentadienyl]phenyl Pyridine-3-carboxylate. MeI (0.2 mL, 3.21 mmol) and potassium carbonate (40 mg, 0.289 mmol) were added to a solution of **4** (0.1 g, 0.196 mmol) in acetone (1.0 mL). The mixture was heated at reflux for 2 h and filtered. The filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes−EtOAc, 2:1) to afford **14** (98 mg, 95%). ^{1}H NMR ($CDCl_3$, 300 MHz): 0.68 (3H, s), 0.74−0.83 (6H, br), 1.56 1.66 (1H, m), 1.68 (3H, s), 1.84−1.98 (2H, m), 2.30−2.44 (3H, m), 2.67 (3H, s), 3.38−3.50 (1H, m), 3.50−3.62 (1H, m), 3.88 (3H, s), 5.29 (1H, d, J = 16.0 Hz), 5.36 (1H, t, J = 7.0 Hz), 5.82 (1H, d, J = 16.0 Hz), 7.46 (1H, m), 8.40−8.46 (1H, m), 9.38 9.42 (1H, m), 10.49 (1H, s). Anal. ($C_{30}H_{34}ClNO_5$) C, H, N.

Compound 13. 2-Chloro-4-formyl-5-methoxy-3-methyl-6-[(2*E*,4*E*)-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-2,4-pentadienyl]phenyl Pyridine-2-carboxylate. Picolinoyl chloride hydrochloride (69 mg, 0.385 mmol) and triethylamine (0.083 mL, 0.593 mmol) were added to a solution of **1** (0.120 g, 0.296 mmol) in dry pyridine. The reaction mixture was heated under reflux for 5 h; saturated aqueous $NaHCO_3$ solution was added, and the mixture stirred for an additional 30 min. The resulting mixture was extracted with EtOAc. The extract was washed with saturated aqueous $NaHCO_3$ solution and brine successively, dried ($MgSO_4$), and concentrated in vacuo. The residue was subjected to silica gel column chromatography (hexanes−EtOAc, 3:1) to give **15** (75 mg, 50%). ^{1}H NMR ($CDCl_3$, 300 MHz): 0.66 (3H, s), 0.77 (3H, d, J = 6.6 Hz), 0.79 (3H, d, J = 6.6 Hz), 1.54−1.68 (1H, m), 1.70 (3H, s), 1.83−1.96 (2H, m), 2.30 2.42 (3H, m), 2.68 (3H, s), 3.44−3.51 (1H, m), 3.51−3.58 (1H, m), 5.27 (1H, d, J = 15.9 Hz), 5.41 (1H, t, J = 7.2 Hz), 5.83 (1H, d, J = 15.9 Hz), 7.60 (1H, ddd, J = 7.7, 4.7, 1.1 Hz), 7.93 (1H, ddd, J = 7.7, 7.7, 1.9 Hz), 8.28 (1H, d, J = 7.7 Hz), 8.88 (1H, dd, J = 4.7 Hz, 1.9 Hz), 10.34 (1H, s), 12.59 (1H, s). Anal. ($C_{29}H_{32}ClNO_5$) C, H, N.

MeI (0.045 mL, 0.723 mmol) and potassium carbonate (10 mg, 0.072 mmol) were added to a solution of **15** (0.023 g, 0.045 mmol) in acetone (1.0 mL). The mixture was heated for 2 h under reflux and filtered. The filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes−EtOAc, 2:1) to afford **13** (23 mg, 97%). ^{1}H NMR ($CDCl_3$, 300 MHz): 0.67 (3H, s), 0.76 (1H, d, J = 6.0 Hz), 0.78 (1H, d, J = 6.0 Hz), 1.54−1.68 (1H, m), 1.68 (3H, s), 1.82−1.98 (2H, m), 2.28−2.44 (3H, m), 2.67 (3H, s), 3.40−3.55 (1H, m), 3.55−3.70 (1H, m), 3.87 (3H, s), 5.26 (1H, d, J = 16.0 Hz), 5.40 (1H, t, J = 7.3 Hz), 5.83 (1H, d, J = 16.0 Hz), 7.59 (1H, ddd, J = 7.7, 4.7, 1.1 Hz), 7.92 (1H, ddd, J = 7.7, 7.7, 1.9 Hz), 8.26 (1H, d, J = 7.7 Hz), 8.87 (1H, dd, J = 4.7, 1.1 Hz), 10.49 (1H, s). Anal. ($C_{30}H_{34}ClNO_5$) C, H, N.

Compound 11. 4-({2-Chloro-4-formyl-5-methoxy-3-methyl-6-[(2*E*,4*E*)-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-2,4-pentadienyl]phenoxy}carbonyl)butanoic Acid. 4-(Dimethylamino)pyridine (4.4 mg, 0.036 mmol) and glutaric anhydride (0.049 g, 0.430 mmol) were added to a solution of **1** (0.150 g, 0.358 mmol) in dry pyridine (1.5 mL). The reaction mixture was stirred at 50 °C overnight, acidified to pH 2 with 1 N HCl, and extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes−EtOAc) to produce **11** as colorless crystals (0.081 g, 42%); mp 120−123 °C. IR (KBr disk): 3000, 1771, 1711, 1649 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): 0.70 (3H, s), 0.81 (3H, d, J = 7.0 Hz), 0.83 (3H, d, J = 7.0 Hz), 1.59−1.66 (1H, m), 1.88 (3H, s), 1.89 1.96 (2H, m), 2.12 (2H, qui, J = 7.2 Hz), 2.34−2.45 (3H, m), 2.55 (2H, t, J = 7.2 Hz), 2.65 (3H, s), 2.76 (2H, t, J = 7.2 Hz), 3.42 (2H, br d, J = 6.0 Hz), 5.35 (1H, t, J = 7.0 Hz), 5.39 (1H, d, J = 16.0 Hz), 5.87 (1H, d, J = 16.0 Hz), 10.30 (1H, s), 12.54 (1H, s). Anal. ($C_{28}H_{35}ClO_7$) C, H.

Compound 12. 2-Chloro-4-formyl-5-methoxy-3-methyl-6-[(2*E*,4*E*)-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-2,4-pentadienyl]phenyl Pyridine-4-carboxylate. Isonicotinoyl chloride hydrochloride (0.242 g, 1.36 mmol) was added to a solution of **1** (0.200 g, 0.494 mmol) in dry pyridine (1 mL). The reaction mixture was stirred for 3 h at room temperature, quenched with water, and stirred for an additional 30 min. The resulting mixture was extracted with ether. The extract was washed with aqueous $CuSO_4$ solution, water, saturated aqueous $NaHCO_3$ solution, and brine successively, dried ($MgSO_4$), and concentrated in vacuo. The resultant crude crystals were recrystallized from hexanes−ether to give **12** (0.199 g, 79%) as colorless crystals; mp 119−121 °C. IR (film): 1752, 1711, 1642 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): 0.68 (3H, s), 0.79 (3H, d, J = 7.0 Hz), 0.81 (3H, d, J = 7.0 Hz), 1.59−1.65 (1H, m), 1.68 (3H, br s), 1.86−1.96 (2H, m), 2.33−2.44 (3H, m), 2.69 (3H, s), 3.34−3.46 (1H, br), 3.46−3.60 (1H, br), 5.29 (1H, d, J = 16.0 Hz), 5.36 (1H, t, J = 7.0 Hz), 5.82 (1H, d, J = 16.0 Hz), 8.01 (2H, br d, J = 4.6 Hz), 8.85 8.95 (2H, br), 10.35 (1H, s), 12.60 (1H, s). Anal. ($C_{29}H_{32}ClNO_5$) C, H, N.

Compound 31. 3-Acetoxy-4-chloro-6-formyl-5-methyl-2-[(2*E*,4*E*)-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-2,4-pentadienyl]phenyl Acetate. Acetyl chloride (0.158 mL, 2.22 mmol) was added dropwise to a solution of **1** (0.200 g, 0.494 mmol) in dry pyridine (1 mL). The reaction mixture was stirred for 4 h at room temperature, quenched with saturated $NaHCO_3$ solution (2 mL), and stirred for an additional 20 min. The reaction mixture was diluted with water and then extracted with ether. The organic layer was washed with saturated aqueous $CuSO_4$ solution, water, and brine successively, dried (Na_2SO_4), and concentrated in vacuo to afford **31** (0.320 g, 88%) as a colorless gum. ^{1}H NMR ($CDCl_3$, 500 MHz): 0.71 (3H, s), 0.81 (3H, d, J = 6.7 Hz), 0.84 (3H, d, J = 6.7 Hz), 1.63 (1H, qd, J = 13.0, 5.5 Hz), 1.86 (3H, s), 1.90−1.97 (2H, m), 2.34 (3H, s), 2.35 (3H, s), 2.36 2.43 (3H, m), 3.35 (2H, d, J = 7.0 Hz), 5.25 (1H, t, J = 7.0 Hz), 5.41 (1H, d, J = 16.0 Hz), 5.87 (1H, d, J = 16.0 Hz), 10.27 (1H, s).

Compound 5. 3-Chloro-4,6-diacetoxy-2-methyl-5-[(2*E*,4*E*)-3-methyl-5-((1*R*,2*R*,6*R*)-1,2,6-trimethyl-3-oxocyclohexyl)-2,4-pentadienyl]benzoic Acid. $NaH_2PO_4 \cdot 2H_2O$ (0.103 g, 0.660 mmol) and water (1.8 mL) were added to **31** (0.428 g, 0.989 mmol). *tert*-Butyl alcohol (7.2 mL), 2-methyl-2-butene (0.309 mL, 2.92 mmol), and 90% sodium chlorite (0.200 g, 1.99 mmol) were added to the mixture, and the mixture was stirred for 15 min at room temperature. The reaction mixture was diluted with water and extracted with CH_2Cl_2. The organic layer was washed with brine, dried ($MgSO_4$), and evaporated in vacuo to give 0.290 g of crude product. The product was purified by silica gel column chromatography (CH_2Cl_2-*i*-PrOH) to give **5** (0.211 g, 64%) as a colorless gum. IR (KBr disk): 3450, 1781, 1735, 1712 cm^{-1}. ^{1}H NMR (CD_3OD, 500 MHz): 0.71 (3H, s), 0.81 (3H, d, J = 6.8 Hz), 0.83 (3H, d, J = 6.8 Hz), 1.62 (1H, qd, J = 13.0, 4.8 Hz), 1.86 (3H, s), 1.93−1.99 (1H, m), 1.99−2.06 (1H, m), 2.23 (3H, s), 2.27 (1H, ddd, J = 13.2, 4.8, 2.0 Hz), 2.28 (3H, s), 2.42 (3H, s), 2.51−2.58 (2H, m), 3.33 (2H, d, J = 6.9 Hz), 5.25 (1H, t, J = 6.9 Hz), 5.50 (1H, d, J = 16.0 Hz), 5.89 (1H, d, J = 16.0 Hz). Anal. ($C_{27}H_{33}ClO_7$) C, H.

2,4-Di-*O*-acetylascochlorin Bis-ethyleneacetal (34). 3-Acetoxy-4-chloro-6-(1,3-dioxolan-2-yl)-5-methyl-2-[(2*E*,4*E*)-3-methyl-5-((6*R*,7*R*,8*R*)-6,7,8-trimethyl-1,4-dioxaspiro[4.5]dec-7-yl)-2,4-pentadienyl]phenyl Acetate. Ethylene glycol bis-TMS ether (1.05 mL), and trimethylsilyl trifluoromethanesulfonate (10 μL) were added to a solution of **31** (694 mg, 1.42 mmol) in CH_2Cl_2 at −78 °C. The mixture was stirred for 2 h, while gradually raising the temperature to 0 °C, and then quenched with pyridine (12 μL) diluted with a saturated $NaHCO_3$ aqueous solution, and extracted with ether. The organic layer was washed with water and brine, dried (Na_2SO_4), and concentrated in vacuo. The residue was subjected to silica gel column chromatography (hexanes−EtOAc, 3:1) to give **34** (267 mg, 33%). ^{1}H NMR ($CDCl_3$, 500 MHz): 0.72 (3H, d, J = 7.0 Hz), 0.74 (3H, d, J = 7.0 Hz), 0.86 (3H, s), 1.35−1.41 (1H, m), 1.43 1.48 (3H, m), 1.65 (1H, q, J = 7.0 Hz),

1.78−1.81 (1H, m), 1.81 (3H, s), 2.25 (3H, s), 2.29 (3H, s), 2.48 (3H, s), 3.18−3.34 (2H, br), 3.76−3.81 (1H, m), 3.88−3.94 (2H, m), 3.96−4.01 (3H, s), 4.14−4.18 (2H, m), 5.22 (1H, t, J = 7.0 Hz), 5.25 (1H, d, J = 16.0 Hz), 5.86 (1H, d, J = 16.0 Hz), 5.88 (1H, s). Anal. ($C_{30}H_{40}ClO_8$) C, H.

2,4-Di-*O*-acetylascochlorin Bis-ethyleneacetal Oxide (35). 3-Acetoxy-4-chloro-6-(1,3-dioxolan-2-yl)-5-methyl-2-[(*E*)-2,3-epoxy-3-methyl-5-((6*R*,7*R*,8*R*)-6,7,8-trimethyl-1,4-dioxaspiro[4.5]dec-7-yl)-4-pentenyl]phenyl Acetate. $NaHCO_3$ (97.5 mg) and *m*-chloroperbenzoic acid (70%, 137 mg) were added to a solution of **34** (267 mg, 0.464 mmol) in CH_2Cl_2 (6 mL), and the mixture was stirred on ice for 1.5 h. The reaction was quenched with a 10% $Na_2S_2O_3$ aqueous solution, stirred for an additional 15 min, diluted with water, and then extracted with ether. The organic layer was washed with a 10% $Na_2S_2O_3$ aqueous solution, a saturated $NaHCO_3$ solution, and brine, dried (Na_2SO_4), and concentrated in vacuo to give **35** (302 mg).

Compound 17. 5-Chloro-4-hydroxy-6-methyl-2-[(*E*)-1-Hydroxy-1-methyl-3-((6*R*,7*R*,8*R*)-7,8,9-trimethyl-1,4-dioxaspiro[4.5]dec-7-yl)-2-propenyl]-2,3-dihydrobenzo-[*b*]furan-7-carbaldehyde. A 30% methanolic potassium methoxide solution (0.262 mL, 1.12 mmol) was introduced into a flask under nitrogen atmosphere, and methanol was removed in vacuo. To the solid potassium methoxide was added dropwise a solution of the epoxide **35** and 18-crown-6 (13.5 mg, 0.051 mmol) in tetrahydrofuran (THF; 4.5 mL) and hexamethyldisilane (0.157 mL, 0.767 mmol). The mixture was heated at reflux for 10 h, acidified with an oxalic acid aqueous solution, and extracted with ether. The organic layer was washed with water and brine successively, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes−EtOAc, 5:2) to afford **17** (117 mg, 56% in two steps). ^{1}H NMR ($CDCl_3$, 500 MHz): 0.68−0.72 (6H, m), 0.816 (1.5H, s), 0.822 (1.5H, s), 1.36−1.60 (3H, m), 1.63−1.69 (1H, m), 1.75−1.82 (1H, m), 1.87 (1H, s, OH), 2.66 (3H, s), 3.06−3.17 (2H, m), 3.76−3.81 (1H, m), 3.88−3.94 (2H, m), 3.96−4.01 (1H, m), 4.84 4.88 (1H, m), 5.33 (1H, d, J = 16.0 Hz), 5.490 (0.5H, d, J = 16.0 Hz), 5.493 (1H, d, J = 16.0 Hz), 6.16 (1H, s), 10.30 (1H, s). Anal. ($C_{23}H_{29}ClO_5$) C, H.

Compound 21. 3-Chloro-6-hydroxy-4-methoxy-2-methyl-5-[(2*E*,6*E*)-7-((*S*)-2,2-dimethyl-3-oxotetrahydrofuran-5-yl)-3-methyl-2,6-octadienyl]benzaldehyde. A mixture of **20** (4.20 g, 9.99 mmol), methyl iodide (1.0 mL, 16.1 mmol), and potassium carbonate (1.4 g, 10.1 mmol) in acetone (50 mL) was heated at reflux for 1 h. The resultant mixture was filtered, and evaporation of the filtrate gave **21** (450 mg, 87%) after purification by silica gel column chromatography (hexane-acetone, 95:5). IR (KBr disk): 1730, 1635 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): 1.21 (3H, s), 1.27 (3H, s), 1.62 (3H, s), 1.79 (3H, s), 1.99−2.07 (2H, m), 2.09−2.20 (2H, m), 2.34 (1H, dd, J = 18.0, 10.0 Hz), 2.42 (1H, dd, J = 18.0, 6.5 Hz), 2.63 (3H, s), 3.38 (2H, d, J = 7.0 Hz), 3.86 (3H, s), 4.50 (1H, dd, J = 10.0, 6.5 Hz), 5.18 (1H, tm, J = 7.0 Hz), 5.50 (1H, t, J = 7.0 Hz), 10.26 (1H, s), 12.51 (1H, s). Anal. ($C_{24}H_{31}ClO_5$) C, H.

Compound 22. 2-Chloro-6-[(2*E*,6*E*)-7-((*S*)-2,2-dimethyl-3-oxotetrahydrofuran-5-yl)-3-methyl-2,6-octadienyl]-4-formyl-5-hydroxy-3-methylphenyl Acetate. Acetic anhydride (1.0 mL, 10.6 mmol) was added to a solution of **20** (4.0 g, 9.5 mmol) in pyridine (5 mL). The mixture was stirred overnight at room temperature, poured into ice water, and extracted with ether. The organic layer was washed with water and brine successively, dried ($MgSO_4$), and concentrated in vacuo. The residue was crystallized from hexane−acetone (95:5) to give **22** (3.0 g, 68%); mp 99 °C. IR (KBr disk): 1775, 1730, 1635 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): 1.21 (3H, s), 1.28 (3H, s), 1.63 (3H, s), 1.75 (3H, s), 1.99 2.06 (2H, m), 2.10−2.17 (2H, m), 2.34 (1H, dd, J = 18.0, 10.0 Hz), 2.37 (3H, s), 2.41 (1H, dd, J = 18.0, 6.5 Hz), 3.29 (2H, d, J = 7.0 Hz), 4.51 (1H, dd, J = 10.0, 6.5 Hz), 5.09 (1H, t, J = 7.0 Hz), 5.50 (1H, t, J = 7.0 Hz), 10.30 (1H, s), 12.52 (1H, s). Anal. ($C_{25}H_{31}ClO_6$) C, H.

Compounds 28 and 27. 2-Chloro-6-[(2*E*,6*E*)-7-((*S*)-2,2-dimethyl-3-oxotetrahydrofuran-5-yl)-3-methyl-2,6-octadienyl]-4-formyl-5-methoxy-3-methylphenyl Acetate and 3-Chloro-4-hydroxy-6-methoxy-2-methyl-5-[(2*E*,6*E*)-7-((*S*)-2,2-dimethyl-3-oxotetrahydrofuran-5-yl)-3-methyl-2,6-octadienyl]benzaldehyde. A mixture of **22** (0.301 g, 0.651 mmol), methyl iodide (0.0484 mL, 0.781 mmol), and potassium carbonate (99 mg, 0.716 mmol) in acetone (2 mL) was heated at reflux for 2 h. The resultant mixture was filtered, and evaporation of the filtrate gave a crude product. The product was purified by silica gel column chromatography to afford **28** (75.5 mg, 24%) as a colorless gum. ^{1}H NMR ($CDCl_3$, 500 MHz): 1.22 (3H, s), 1.28 (3H, s), 1.64 (3H, s), 1.77 (3H, br s), 2.04 (2H, t, J = 7.8 Hz), 2.09−2.20 (2H, m), 2.35 (3H, s), 2.39 (1H, dd, J = 18.2, 10.0 Hz), 2.47 (1H, dd, J = 18.0, 6.2 Hz), 2.63 (3H, s), 3.33 (2H, d, J = 6.5 Hz), 3.83 (3H, s), 4.52 (1H, dd, J = 10.0, 6.2 Hz), 5.08 (1H, tq, J = 6.5, 1.3 Hz), 5.52 (1H, t, J = 6.6 Hz), 10.44 (1H, s). Anal. ($C_{26}H_{33}ClO_6$) C, H; H: calcd, 6.97; found, 7.40.

A 1% NaOH aqueous solution (4.2 mL, 1.05 mmol) was added to a solution of **28** (63.5 mg, 0.133 mmol) in MeOH (4.2 mL), and the mixture was stirred for 4 h at room temperature. The reaction mixture was neutralized with 2 N HCl, and MeOH was removed in vacuo. The residue was diluted with water and extracted with EtOAc. The organic layer was washed with water, a saturated $NaHCO_3$ solution, and brine successively, dried with $MgSO_4$, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes−EtOAc) to give **27** as a colorless gum (52.1 mg, 90%). IR (film): 3390, 1756, 1686, 1564 cm^{-1}. ^{1}H NMR ($CDCl_3$, 500 MHz): 1.22 (3H, s), 1.29 (3H, s), 1.64 (3H, br s), 1.80 (3H, br s), 2.06 (2H, t, J = 7.5 Hz), 2.12−2.22 (2H, m), 2.40 (1H, dd, J = 18.0, 10.0 Hz), 2.47 (1H, dd, J = 18.0, 6.5 Hz), 2.66 (3H, s), 3.42 (2H, d, J = 6.6 Hz), 3.82 (3H, s), 4.53 (1H, dd, J = 10.0, 6.5 Hz), 5.20 (1H, tq, J = 6.9, 1.2 Hz), 5.51 (1H, t, J = 6.9 Hz), 6.35 (1H, s), 10.37 (1H, s). Anal. ($C_{24}H_{31}ClO_5$) C, H; H: calcd, 7.18; found, 7.59.

4-*O*-Ethoxycarbonylmethylascofuranone (36). Ethyl {2-Chloro-6-[(2*E*,6*E*)-7-((*S*)-2,2-dimethyl-3-oxotetrahydrofuran-5-yl)-3-methyl-2,6-octadienyl]-4-formyl-5-hydroxy-3-methylphenoxy}acetate. Sodium hydride (60%, dispersion in mineral oil, 31.4 mg, 1.31 mmol) was washed with dry hexane and suspended in DMF (1 mL). To the above suspension was added dropwise **20** (0.50 g, 1.19 mmol) in DMF (5 mL). The mixture was stirred at room temperature for 10 min, and ethyl bromoacetate (0.145 mL, 1.31 mmol) was added. The resulting solution was stirred for an additional 5.5 h at 40 °C, poured into a mixture of a saturated NH_4Cl solution and ice, and extracted with ether. The organic layer was washed with water and brine successively, dried ($MgSO_4$), and concentrated in vacuo. The residue was subjected to silica gel column chromatography (hexanes−EtOAc, 16:1) to give **36** (0.527 g, 88%). NMR ($CDCl_3$, 500 MHz): 1.21 (3H, s), 1.27 (3H, s), 1.33 (3H, d, J = 7.0 Hz), 1.62 (3H, s), 1.86 (3H, s), 1.99−2.07 (2H, m), 2.08−2.18 (2H, m), 2.35 (1H, dd, J = 18.0, 10.0 Hz), 2.42 (1H, dd, J = 18.0, 6.5 Hz), 2.63 (3H, s), 3.45 (1H, d, J = 7.0 Hz), 4.51 (1H, dd, J = 10.0, 6.5 Hz), 4.56 (2H, s), 5.17 (1H, d, J = 7.0 Hz), 5.50 (1H, t, J = 7.0 Hz), 10.26 (1H, s), 12.51 (1H, s). Anal. ($C_{27}H_{35}ClO_7$) C, H.

Compound 23. {2-Chloro-6-[(2*E*,6*E*)-7-((*S*)-2,2-dimethyl-3-oxotetrahydrofuran-5-yl)-3-methyl-2,6-octadienyl]-4-formyl-5-hydroxy-3-methylphenoxy}acetic Acid. A solution of potassium carbonate (0.456 mg, 3.30 mmol) in 2 mL of water was added to a solution of **36** (0.448 g, 0.943 mmol) in methanol (15 mL), and the mixture was stirred for 2 h at room temperature. The reaction mixture was acidified with 3 N aqueous HCl solution, diluted with brine, and extracted with ether. The organic layer was washed with water and brine, dried ($MgSO_4$), and evaporated in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2−MeOH, 15:1) to give **23** (0.387 g, 91%). ^{1}H NMR ($CDCl_3$, 500 MHz): 1.28 (3H, s), 1.32 (3H, s), 1.62 (3H, s), 1.77 (3H, s), 2.03−2.09 (2H, m), 2.09−2.17 (1H, m), 2.18−2.27 (1H, m), 2.44 (1H, dd, J = 18.0, 10.0 Hz), 2.51 (1H, dd, J = 18.0, 6.5 Hz), 2.64 (3H, s), 3.42−3.51 (2H, m), 4.57 (1H, d, J = 16.0 Hz), 4.66 (1H, d, J = 16.0 Hz), 4.69 (1H, dd, J = 10.0, 6.5 Hz), 5.03 (1H, t, J =

7.0 Hz), 5.52 (1H, t, J = 7.0 Hz), 10.27 (1H, s), 12.50 (1H, s). Anal. ($C_{25}H_{31}ClO_7$) C, H.

Compound 24. {2-Chloro-6-[(2*E*,6*E*)-7-((*S*)-2,2-dimethyl-3-oxotetrahydrofuran-5-yl)-3-methyl-2,6-octadienyl]-4-formyl-5-hydroxy-3-methylphenoxy}carbonylacetic Acid. 4-(Dimethylamino)pyridine (2.9 mg, 0.04 mmol) and glutaric anhydride (0.033 g, 0.490 mmol) were added to a solution of **20** (0.100 g, 0.238 mmol) in dry pyridine (1 mL). The reaction mixture was stirred at 50 °C overnight, acidified to pH 2 with 1 N HCl, and extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes−EtOAc) to produce **24** as a colorless gum (0.040 g, 32%). IR (film): 3000, 1752, 1713, 1638 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 1.23 (3H, s), 1.29 (3H, s), 1.63 (3H, br s), 1.74 (3H, br s), 2.02 (2H, t, J = 7.5 Hz), 2.11 (2H, qui, J = 7.0 Hz), 2.12−2.17 (2H, m), 2.37 (1H, dd, J = 18.2, 10.0 Hz), 2.44 (1H, dd, J = 18.2, 6.3 Hz), 2.54 (2H, t, J = 7.0 Hz), 2.65 (3H, s), 2.76 (2H, t, J = 7.0 Hz), 3.24−3.31 (2H, br), 4.55 (1H, dd, J = 10.0, 6.3 Hz), 5.07 (1H, tq, J = 7.0, 1.5 Hz), 5.50 (1H, br t, J = 7.0 Hz), 10.30 (1H, s), 12.52 (1H, s). Anal. ($C_{28}H_{35}ClO_8$) C, H.

Compound 25. 2-Chloro-6-[(2*E*,6*E*)-7-((*S*)-2,2-dimethyl-3-oxotetrahydrofuran-5-yl)-3-methyl-2,6-octadienyl]-4-formyl-5-hydroxy-3-methylphenyl Pyridine-3-carboxylate. Nicotinoyl chloride hydrochloride (5.0 g, 29.4 mmol) was added to a solution of **20** (4.2 g, 9.99 mmol) in dry pyridine (20 mL). The reaction mixture was stirred overnight at room temperature and poured into ice water. The resulting mixture was extracted with ether. The organic layer was washed with water and brine successively, dried ($MgSO_4$), and concentrated in vacuo. The residue was subjected to silica gel column chromatography (hexane−acetone, 95:5) to give **25** (3.5 g, 67%). IR (KBr disk): 1750, 1700, 1640, 1590 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 1.20 (3H, s), 1.27 (3H, s), 1.55 (3H, s), 1.62 (3H, s), 1.90−1.97 (2H, m), 2.03−2.12 (2H, m), 2.34 (1H, br dd, J = 18.0, 10.0 Hz), 2.41 (1H, dd, J = 18.0, 6.5 Hz), 2.69 (3H, s), 3.23−3.33 (1H, br), 3.36−3.46 (1H, br), 4.51 (1H, dd, J = 10.0, 6.5 Hz), 5.10 (1H, t, J = 7.0 Hz), 5.48 (1H, d, J = 7.0 Hz), 7.51 (1H, dd, J = 8.0, 5.0 Hz), 8.46 (1H, dt, J = 8.0, 2.0 Hz), 8.90 (1H, dd, J = 5.0, 2.0 Hz), 9.41 (1H, d, J = 2.0 Hz), 10.35 (1H, s), 12.59 (1H, s). Anal. ($C_{29}H_{32}ClNO_6$) C, H, N.

Compound 26. 2-Chloro-6-[(2*E*,6*E*)-7-((*S*)-2,2-dimethyl-3-oxotetrahydrofuran-5-yl)-3-methyl-2,6-octadienyl]-4-formyl-5-hydroxy-3-methylphenyl Pyridine-4-carboxylate. Isonicotinoyl chloride hydrochloride (0.233 g, 1.31 mmol) was added to a solution of **20** (0.200 g, 0.476 mmol) in dry pyridine (1 mL). The reaction mixture was stirred for 1 h at room temperature, quenched with water, and stirred for an additional 30 min. The resulting mixture was extracted with ether. The extract was washed with an aqueous $CuSO_4$ solution, water, saturated aqueous $NaHCO_3$ solution, and brine successively, dried ($MgSO_4$), and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes−EtOAc) to give **26** as a colorless gum (0.135 g, 54%). IR (film): 1756, 1644 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 1.21 (3H, s), 1.28 (3H, s), 1.53 (3H, br s), 1.62 (3H, br s), 1.88−1.97 (2H, m), 2.02−2.12 (2H, m), 2.35 (1H, dd, J = 18.0, 10.0 Hz), 2.42 (1H, dd, J = 18.0, 6.3 Hz), 2.69 (3H, s), 3.21−3.33 (1H, br), 3.33−3.46 (1H, br), 4.51 (1H, dd, J = 10.0, 6.3 Hz), 5.09 (1H, br t, J = 7.0 Hz), 5.48 (1H, br t, J = 7.0 Hz), 8.00−8.02 (2H, m), 8.89−8.91 (2H, m), 10.35 (1H, s), 12.60 (1H, s). Anal. ($C_{29}H_{32}ClNO_6$) C, H, N.

Compound 29. 3-Chloro-4,6-dihydroxy-2-methyl-5-[(2*E*,6*E*)-7-(2,2-dimethyl-3-oxo-2,3-dihydrofuran-5-yl)-3-methyl-2,6-octadienyl]benzaldehyde. A solution of $AgNO_3$ (0.340 g, 2.00 mmol) in deionized water (0.7 mL) was added dropwise to a solution of NaOH (0.160 g, 2.00 mmol) in deionized water (0.7 mL) while cooling in a water bath. To the resultant suspension was added a solution of **20** (0.2 g, 0.476 mmol) in dioxane (1.4 mL). The mixture was stirred for 3 h at room temperature and diluted with a mixture of water and dioxane (1:1). The solution was then filtered through a Cerite pad. The filtrate was acidified with 6 N HCl and extracted with CH_2Cl_2. The organic layer was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The residue was subjected to silica gel column chromatography (CH_2Cl_2−acetone, 60:1) to afford **29** (0.047 g, 24%); mp 139.5−140.5 °C. IR: 1680, 1635, 1555 cm^{-1}. ^1H NMR ($CDCl_3$, 500 MHz): 1.35 (6H, s), 1.82 (3H, br s), 1.84 (3H, br s), 2.13 (2H, br t, J = 7.5 Hz), 2.33 (2H, br q, J = 7.5 Hz), 2.60 (3H, s), 3.40 (2H, d, J = 7.2 Hz), 5.24 (1H, tm, J = 7.2 Hz), 5.38 (1H, s), 6.36 (1H, br s), 6.51 (1H, tm, J = 7.5 Hz), 10.14 (1H, s), 12.69 (1H, s). ^{13}C NMR (ppm): 12.93, 14.44, 16.13, 22.03, 23.03 (probably two overlapping peaks), 27.05, 38.29, 88.10, 98.52, 113.14, 113.59, 114.21, 121.90, 125.76, 135.03, 137.71, 138.12, 156.17, 162.15, 184.66, 193.30, 207.20. Anal. ($C_{23}H_{27}ClO_5$) C, H.

Cell Culture. U2OS, a human osteosarcoma, was provided by C.-L. Wu (MGH Cancer Center, Charlestown, MA). C3H10T1/2 was obtained from the Institute of Fermentation (Osaka, Japan). These cell lines were cultured in Dulbecco's modified Eagle's medium supplemented with 5% fetal bovine serum at 37 °C in a humidified incubator under 10% CO_2 atmosphere.

Plasmids. Expression vectors of intact human nuclear receptors, corresponding Gal4-fusion nuclear receptors, and reporter plasmids for the corresponding nuclear receptors, as well as Gal4-fusion nuclear receptors, were kind gifts from S. Kato (University of Tokyo, Tokyo, Japan).[45−47] pCMV-β-galactosidase was provided by N. H. Heintz (University of Vermont, Burlington, VT).

Proliferation Assay. U2OS cells (5×10^3 cells) were cultured with drugs for 48 h in microplate wells, and [^{3}H]-thymidine (0.5 μCi/well) was added 4 h before harvesting. The radioactivity incorporated was determined by a liquid scintillation counter.[48]

Reporter Assay. U2OS cells (1×10^6 cells in a 10 cm dish) were transfected with pCMV-β-galactosidase (4 μg), an expression vector of the nuclear receptor (2 μg), and a corresponding reporter plasmid (4 μg) by calcium precipitation method, as described previously.[18] Cells were cultured for 12 h and washed with phosphate-buffered saline three times to remove DNA precipitates. After they were cultured in fresh medium for 4 h, cells were trypsinized and suspended in fresh medium. Aliquots of cells were further cultured with drugs for 20 h. Luciferase activity, β-galactosidase activity, and chloramphenicol acetyltransferase expression in the cell lysates were determined with commercial assay kits.

Adipocyte Differentiation. C3H10T1/2 cells (1×10^5 cells/well) were grown for 48 h in a 24 well until reaching a confluent state. Cells were treated with 1 μM dexamethasone and 0.5 mM 3-isobutyl-1-methylxanthine for 48 h followed by 1 μM insulin and drugs for 5 days. At the end of the culture, GPDH activity and protein concentration in the cell lysate were determined.[18,49]

Acknowledgment. We are grateful to S. Kato, N. H. Heintz, and C.-L. Wu for kindly providing plasmids and a cell line. This work was supported in part by a grant for the regional Consortium program of New Energy and Industrial Technology.

References

(1) Tamura, G.; Suzuki, S.; Takatsuki, A.; Ando, K.; Arima, K. Ascochlorin, a new antibiotic, found by paper-disc agar diffusion method. I) isolation, biological and chemical properties of ascochlorin. *J. Antibiot.* **1968**, *21*, 539−544.

(2) Sawada, M.; Hosokawa, T.; Okutomi, T.; Ando, K. Hypolipidemic property of ascofuranone. *J. Antibiot.* **1973**, *26*, 681−686.

(3) Hosokawa, T.; Suzuki, K.; Okutomi, T.; Sawada, N.; Ando, K. Effect of ascofuranone on serum lipids of rats fed a cholesterol rich diet. *Jpn. J. Pharmacol.* **1975**, *25*, 35−39.

(4) Hosokawa, T.; Sawada, M.; Ando, K.; Tamura, G. Enhanced excretion of fecal neutral sterols and the hypocholesterolemic properties of 4-O-methylascochlorin. *Agric. Biol. Chem.* **1980**, *44*, 2461−2468.

(5) Hosokawa, T.; Sawada, M.; Ando, K.; Tamura, G. Alteration of cholesterol metabolism by 4-O-methylascochlorin in rats. *Lipids* **1981**, *16*, 433−438.

(6) Hosokawa, T.; Ando, K.; Tamura, G. Alteration of cholesterol metabolism and hypocholesterolemic property of 4-*O*-methylascochlorin on controlled reverse-phase feeding rats. *Agric. Biol. Chem*. **1982**, *46*, 775−781.
(7) Hosokawa, T.; Ando, K.; Tamura, G. An ascochlorin derivative, AS-6, potentiates insulin action in streptozotocin diabetic mice and rats. *Agric. Biol. Chem*. **1982**, *46*, 775−781.
(8) Hosokawa, T.; Ando, K.; Tamura, G. An ascochlorin derivative, AS-6, reduces insulin resistance in the genetically obese diabetic mouse, db/db. *Diabetes* **1985**, *34*, 267−274.
(9) Hosokawa, T.; Ando, K.; Tamura, G. Modification of fatty acid composition in adipocyte plasma membranes by treatment with a new antidiabetic agent, AS-6, in genetically obese diabetic mice, db/db. *Biochim. Biophys. Acta* **1985**, *834*, 130−133.
(10) Hosokawa, T.; Ando, K.; Tamura, G. Structural and functional abnormalities in the adipocyte plasma membrane from db/db mouse, and the effect on the abnormalities of oral treatment with AS-6. *Biochem. Biophys. Res. Commun*. **1984**, *125*, 64−69.
(11) Hosokawa, T.; Ando, K.; Tamura, G. Treatment with an ascochlorin derivative, AS-6 increases $^{45}Ca^{2+}$ binding on the plasma membrane of adipocytes in db/db mice. *Biochem. Biophys. Res. Commun*. **1985**, *127*, 247−253.
(12) Hosokawa, T.; Okutomi, T.; Sawada, M.; Ando, K.; Tamura, G. Unusual concentration of urine and prevention of polydipsia by fungal metabolites in DOCA hypertensive rats. *Eur. J. Pharmacol*. **1981**, *69*, 429−438.
(13) Magae, J.; Hosokawa, T.; Ando, K.; Nagai, K.; Tamura, G. Antitumor protective property of an isoprenoid antibiotic, ascofuranone. *J. Antibiot*. **1982**, *35*, 1547−1552.
(14) Magae, J.; Hayasaki, J.; Matsuda, Y.; Hotta, M.; Hosokawa, T.; Suzuki, S.; Nagai, K.; Ando, K.; Tamura, G. Antitumor and antimetastatic activity of an antibiotic, ascofuranone, and activation of phagocytes. *J. Antibiot*. **1988**, *41*, 959−965.
(15) Magae, J.; Suzuki, S.; Nagai, K.; Yamasaki, M.; Ando, K.; Tamura, G. In vitro effects of an antitumor antibiotic, ascofuranone, on the murine immune system. *Cancer Res*. **1986**, *46*, 1073−1078.
(16) Magae, J.; Hotta, M.; Nagai, K.; Suzuki, S.; Ando, K.; Yamasaki, M.; Tamura, G. Activation of natural cytotoxic activity and concomitant reduction of triglyceride content of murine spleen, treated with an antitumor antibiotic, ascofuranone. *J. Antibiot*. **1986**, *39*, 676−681.
(17) Magae, J.; Nagai, K.; Ando, K.; Tamura, G. Differentiation of mouse and human myeloid leukemia cells induced by an antitumor antibiotic, ascofuranone. *Agric. Biol. Chem*. **1988**, *52*, 3143−3147.
(18) Togashi, M.; Masuda, H.; Kawada, T.; Tanaka, M.; Saida, K.; Ando, K.; Tamura, G.; Magae, J. PPARγ activation and adipocyte differentiation induced by AS-6, a prenyl-phenol antidiabetic antibiotic. *J. Antibiot*. **2002**, *55*, 417−422.
(19) Rosen, E. D.; Walkey, C. J.; Puigserver, P.; Spiegelman, B. M. Transcriptional regulation of adipogenesis. *Genes Dev*. **2000**, *14*, 1293−1307.
(20) Rosen, E. D.; Spiegelman, B. M. PPARγ: a nuclear receptor of metabolism, differentiation, and cell growth. *J. Biol. Chem*. **2001**, *276*, 37731−37734.
(21) Perimann, T.; Evans, R. M. Nuclear receptors in Sicily: all in the famiglia. *Cell* **1997**, *90*, 391−397.
(22) Chawla, A.; Repa, J. J.; Evans, R. M.; Mangelsdorf, D. J. Nuclear receptors and lipid physiology: Opening the X-files. *Science* **2001**, *294*, 1866−1870.
(23) Olefsky, J. M. Nuclear receptor minireview series. *J. Biol. Chem*. **2001**, *276*, 36863−36864.
(24) Kliewer, S. A.; Lehman, J. M.; Willson, T. M. Orphan nuclear receptors: Shifting endocrinology into reverse. *Science* **1999**, *284*, 757−760.
(25) Nuclear receptors nomenclature committee. A unified nomenclature system for the nuclear receptor superfamily. *Cell* **1999**, *97*, 161−163.
(26) Nolte, R. T.; Wisely, G. B.; Westin, S.; Cobb, J. E.; Lambert, M. H.; Kurokawa, R.; Rosenfeld, M. G.; Willson, T. M.; Glass, C. K.; Milburn, M. V. Ligand binding and co-activator assembly of the peroxisome proliferator-activated receptor-gamma. *Nature* **1998**, *395*, 137−143.
(27) Westin, S.; Kurokawa, R.; Nolte, R. T.; Wisely, G. B.; McInerney, E. M.; Rose, D. W.; Milburn, M. V.; Rosenfeld, M. G.; Glass, C. K. Interactions controlling the assembly of nuclear-receptor heterodimers and co-activators. *Nature* **1998**, *395*, 199−202.
(28) Camp, H. S.; Li, O.; Wise, S. C.; Hong, Y. H.; Frankowski, C. L.; Shen, X.; Vanbogelen, R.; Leff, T. Differential activation of peroxisome proliferator-activated receptor-gamma by troglitazone and rosiglitazone. *Diabetes* **2000**, *49*, 539−547.
(29) Rocci, S.; Picard, F.; Vamecq, J.; Gelman, L.; Potier, N.; Zeyer, D.; Dubuquoy, L.; Bac, P.; Champy, M. F.; Plunket, K. D.; Leesnitzer, L. M.; Blanchard, S. G.; Desreumaux, P.; Moras, D.; Renaud, J. P.; Auwerx, J. A unique PPARgamma ligand with potent insulin-sensitizing yet weak adipogenic activity. *Mol. Cell* **2001**, *8*, 737−747.
(30) Kodera, Y.; Takeyama, K.; Murayama, A.; Suzawa, M.; Masuhiro, Y.; Kato, S. Ligand type-specific interactions of peroxisome proliferator-activated receptor gamma with transcriptional coactivators. *J. Biol. Chem*. **2000**, *275*, 33201−33204.
(31) Kliewer, S. A.; Goodwin, B.; Willson, T. M. The nuclear pregnane X receptor: a key regulator of xenobiotic metabolism. *Endocr. Rev*. **2002**, *23*, 687−702.
(32) Kliewer, S. A.; Moore, J. T.; Wade, L.; Staudinger, J. L.; Watson, M. A.; Jones, S. A.; McKee, D. D.; Oliver, B. B.; Willson, T. M.; Zetterstrom, R. H.; Perlmann, T.; Lehmann, J. M. An orphan nuclear receptor activated by pregnanes defines a novel steroid signaling pathway. *Cell* **1998**, *92*, 73−82.
(33) Bertilsson, G.; Heidrich, J.; Svensson, K.; Asman, M.; Jendeberg, L.; Sydow-Backman, M.; Ohlsson, R.; Pastlind, H.; Blomquist, P.; Berkenstam, A. Identification of a human nuclear receptor defines a new signaling pathway for CYP3A induction. *Proc. Natl. Acad. Sci. U.S.A*. **1998**, *95*, 12208−12213.
(34) Staudinger, J. L.; Goodwin, B.; Jones, S. A.; Hawkins-Brown, D.; MacKenzie, K. I.; LaTour, A.; Liu, Y.; Klassen, C. D.; Brown, K. K.; Reinhard, J.; Willson, T. M.; Koller, B. H.; Kliewer, S. A. The nuclear receptor PXR is a lithocholic acid sensor that protects against liver toxicity. *Proc. Natl. Acad. Sci. U.S.A*. **2001**, *98*, 3369−3374.
(35) Xie, W.; Radminska-Pandya, A.; Shi, Y.; Simon, C. M.; Nelson, M. C.; Ong, E. S.; Waxman, D. J.; Evans, R. M. An essential role for nuclear receptors SXR/PXR in detoxification of cholestatic bile acids. *Proc. Natl. Acad. Sci. U.S.A*. **2001**, *98*, 3375−3380.
(36) Watkins, R. E.; Wisely, G. B.; Moore, L. B.; Collins, J. L.; Lambert, M. H.; Williams, S. P.; Willson, T. M.; Kliewer, S. A. The human nuclear xenobiotic receptor PXR; structural determinations of directed promiscuity. *Science* **2001**, *292*, 2329−2333.
(37) Monolagas, S. C.; Kousteni, S.; Jilka, R. L. Sex steroids and bone. *Recent Prog. Horm. Res*. **2002**, *57*, 385−409.
(38) Behl, C. Oestrogen as a neuroprotective hormone. *Nat. Rev. Neurosci*. **2002**, *3*, 433−442.
(39) Hodgin, J. B.; Maeda, N. Minireview: estrogen and mouse models of atherosclerosis. *Endocrinology* **2002**, *143*, 4495−4501.
(40) Bhathena, S. J.; Velasquez, M. T. Beneficial role of dietary phytoestrogens in obesity and diabetes. *Am. J. Clin. Nutr*. **2002**, *76*, 1191−1201.
(41) Wiseman, H.; Duffy, R. New advances in the understanding of the role of steroids and steroid receptors in disease. *Biochem. Soc. Trans*. **2001**, *29*, 205−209.
(42) Gervois, P.; Torra, I. P.; Fruchart, J. C.; Staels, B. Regulation of lipid and lipoprotein metabolism by PPAR activators. *Clin. Chem. Lab. Med*. **2000**, *38*, 3−11.
(43) Olsen, N. J.; Kovacs, W. J. Effects of androgens on T and B lymphocyte development. *Immunol. Rev*. **2001**, *23*, 281−288.
(44) Jones, D. C.; Manning, B. M.; Daynes, R. A. A role for the peroxisome proliferator-activated receptor alpha in T-cell physiology and aging immunobiology. *Proc. Nutr. Soc*. **2002**, *61*, 363−369.
(45) Watanabe, M.; Yanagi, Y.; Masuhiro, Y.; Yano, T.; Yoshikawa, H.; Yanagisawa, J.; Kato, S. A putative tumor suppressor, TSG101, acts as a transcriptional suppressor through its coiled-coil domain. *Biochem. Biophys. Res. Commun*. **1998**, *245*, 900−905.
(46) Kato, S.; Endoh, H.; Masuhiro, Y.; Kitamoto, T.; Uchiyama, S.; Sasaki, H.; Masushige, S.; Gotoh, Y.; Nishida, E.; Kawashima, H.; Metzger, D.; Chambon, P. Activation of the estrogen receptor through phosphorylation by mitogen-activated protein kinase. *Science* **1995**, *270*, 1491−1494.
(47) Yanagisawa, J.; Yanagi, Y.; Masuhiro, Y.; Suzawa, M.; Watanabe, M.; Kashiwagi, K.; Toriyabe, T.; Kawabata, M.; Miyazono, K.; Kato, S. Convergence of transforming growth factor-b and vitamin D signaling pathways on SMAD transcriptional coactivators. *Science* **1999**, *283*, 1317−1321.
(48) Magae, J.; Illenye, S.; Chang, Y.-C.; Mitsui, Y.; Heintz, N. H. Association with E2F-1 governs intracellular trafficking and polyubiquitination of DP-1. *Oncogene* **1999**, *18*, 593−605.
(49) Grimaldi, P.; Djian, P.; Negrel, R.; Ailhaud, G. Differentiation of Ob17 preadipocytes to adipocytes: requirement of adipose conversion. *EMBO J*. **1982**, *1*, 687−692.

JM0205649