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Design and discovery of 2-oxochromene derivatives as liver X receptor β-selective agonists

Takayuki Matsuda, Ayumu Okuda, Yuichiro Watanabe, Tohru Miura, Hidefumi Ozawa, Ayako Tosaka, Koichi Yamazaki, Yuki Yamaguchi, Sayaka Kurobuchi, Minoru Koura, Kimiyuki Shibuya *

Tokyo New Drug Research Laboratories, Pharmaceutical Division, Kowa Co., Ltd, 2-17-43, Noguchicho, Higashimurayam, Tokyo 189-0022, Japan

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ABSTRACT

In an attempt to molecularly design liver X receptor (LXR) β-selective agonists, we discovered that the combination of the 2-oxochromene moiety (head) and the imidazoline-2,4-dione moiety (tail) plays an important role in the expression potency and selectivity toward LXRβ. We synthesized a series of 2-oxochromene derivatives and identified **43** as a LXRβ-selective agonist that increased the HDL-C level without significantly elevating the TG level and resulted in a decreased lipid-accumulation area in the aortic arch in a high-fat-and-cholesterol-fed Bio F_1B hamster. In this manuscript, we report the design, synthesis and pharmacology of these 2-oxochromene derivatives.

Atherosclerotic cardiovascular disease (CVD), which includes myocardial infarction and ischemic stroke, is a major cause of morbidity and mortality in developed countries.[1] CVD is characterized by the accumulation of low-density lipoprotein (LDL) particles in the arterial wall, which leads to the formation of cholesterol-laden foam cells. Therefore, lipid-lowering drugs, such as statins, are widely used to lower the circulating LDL as therapeutics for dyslipidemia, and thus far, these drugs have contributed to the suppression of such diseases.[2] However, this therapy does not satisfactorily reduce cardiovascular events.[3] Therefore, we have dedicated our efforts to developing a novel drug for the treatment of atherosclerosis.

Liver X receptors (LXRs) are ligand-activated transcription factors involved in cholesterol metabolism, glucose homeostasis, inflammation and lipogenesis.[4] LXRα (known as NR1H3) is the dominant subtype in the liver, small intestine, and macrophage, whereas LXRβ (known as NR1H2) is distributed ubiquitously. LXR activation is known to regulate the expression levels of ATP-binding cassette transporter A1 (ABCA1), ABCG1 and G8 and cholesterol metabolism, increasing the high-density lipoprotein particle cholesterol (HDL-C) level and providing cholesterol efflux. These actions induce an anti-atherogenic effect in the peripheral blood vessels and can yield a synergistic effect when in combination with statin therapy.[5] We hypothesized that this reverse cholesterol transport (RCT) plays an important role in regulating LXRs in clinical trials.

The synthetic agonists T0901317 and GW3965 have been reported as valuable tools for LXR studies (Fig. 1).[6,7] In murine and hamster models of atherosclerosis, LXR agonists increase the plasma HDL-C levels and decrease atherosclerotic lesions.[8] These findings indicate the potential utility of LXR agonists for the treatment of cardiovascular disease. However, significant side effects, such as elevations in the plasma and hepatic triglyceride (TG) levels, were observed as a result of lipogenesis due to the induction of the sterol regulatory element-binding protein-1c (SREBP-1c) by LXRα activation in the liver.

To develop an LXR agonist as an anti-atherosclerotic agent, we should eliminate this undesirable side effect, which relies on differentiating between these two LXR subtypes and discovering

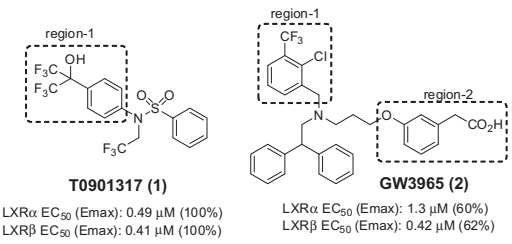

Figure 1. Structure of the LXR agonists from Tularik and GlaxoSmithKline.

* Corresponding author. Tel.: +81 42 391 6211; fax: +81 42 395 0312.
E-mail address: k-sibuya@kowa.co.jp (K. Shibuya).

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LXRβ-selective agonists.[9] Considering the high homology of the LXRα and LXRβ ligand-binding domains (LBD; 77% amino acid identity), it appears difficult to create an LXRβ-selective agonist. In our drug-discovery program, we first compared the differences in the profiles for T0901317 and GW3965. The former agonist has no selectivity between LXRα and LXRβ, whereas the latter shows three-fold increased selectivity for LXRβ.[10] Through X-ray analysis, we then found that the hydroxyl group of 1,1-bistrifluoromethylcarbinol (region-1) in T0901317 and 2-chloro-3-trifluorophenyl group (region-1) in GW3965 can interact with His435 in the LXR ligand-binding pocket.[11] In contrast, the interaction of the phenyl acetic acid (region-2) in GW3965 with Arg319, Ser242 and Leu330 could also be observed. These results led us to hypothesize that the structural unit of region-2 may play an important role in activating LXRβ. Our molecular design was based on the combination of head-to-tail structures (corresponding to region-1 and region-2). To explore the head core structure, we performed a high-throughput screening of our chemical library for the assay of the up-regulation of ABCA1 mRNA in a THP-1 human macrophage cell line and of SREBP-1c mRNA in a HepG2 cell line. After the screening, compounds **3**, **4** and **5** were identified as hit compounds with a common 2-oxochromene core structure and a lipophilic substituent at the 4-position. As shown in Table 1, **3** and **5** showed moderate induction of ABCA1 mRNA compared with that of SREBP-1c mRNA, whereas **4** showed lower induction. The reference compound GW3965 showed greater potency for ABCA1 up-regulation than **5**.

Based on the structure of **5**, we speculated that the trifluoromethyl group or the carbonyl oxygen can interact with the His435-Trp457 activation switch[11] to express LXR agonistic action and that another lipophilic substituent at the 8- or 10-position may be located in the highly hydrophobic LXR ligand-binding pocket. However, the analysis of the tetracyclic structure of **5** revealed a highly planar structure, indicating possible intercalation of the DNA chain.[12] Therefore, we disconnected the C–N bond of the quinoline ring of **5** to dismantle the tetracyclic system. We then referred to the Merck compound[13] as the head-to-tail design to denote the arrangement of the 3-trifluoromethyl group and the 7-*n*-propyl group on the benzisoxazole moiety (head) and the thiazolidine-2,4-dione moiety (tail). We also incorporated a similar head-and-tail combination into our molecular design presented in Figure 2 with an optimal linker. Based on this concept, we synthesized two types of bis-*n*-propyl-2-oxochromen **6** and mono-*n*-propyl-2-oxochromen **7** and studied their structure–activity relationships.

7-Hydroxy-6,8-di-*n*-propyl-4-(trifluoromethyl)-*2H*-chromen-2-one (**12**) was prepared as depicted in Scheme 1. 1,3-Diallyloxybenzene (**9**) was obtained by the bis-allylation of resorcinol (**8**). A Claisen rearrangement reaction of **9** was performed to yield 2,4-diallylbenzene-1,3-diol (**10**), which was subsequently hydrogenated to yield **11**. Finally, **11** was condensed with ethyl 4,4,4-trifluoro-3-oxobutanoate to furnish **12**.

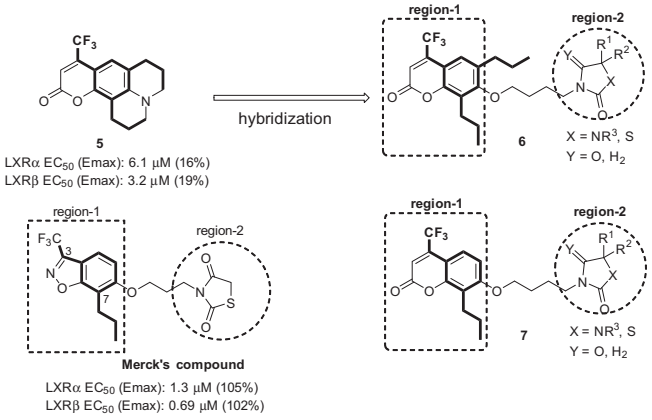
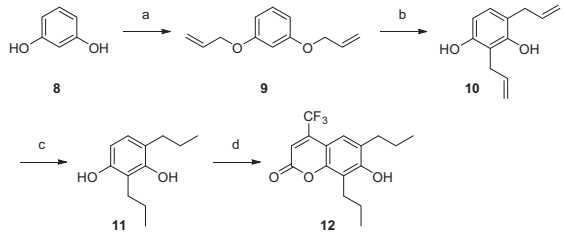


Figure 2. Drug design of the LXR agonists.



Scheme 1. Reagents and conditions: (a) allyl chloride, K_2CO_3, DMF, 70 °C, 24 h, 85%; (b) *N*,*N*-dimethylaniline, 200 °C, 10 h, 57%; (c) H_2, Pd/C, MeOH, rt, 18 h, 98%; (d) ethyl 4,4,4-trifluoro-3-oxobutanoate, $ZnCl_2$, 110 °C, 18 h, 59%.

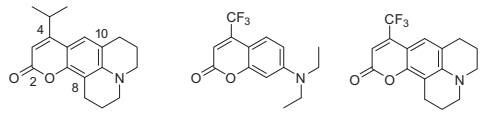
7-Hydroxy-8-*n*-propyl-4-(trifluoromethyl)-*2H*-chromen-2-one (**16**) was prepared as depicted in Scheme 2. Lithiation of 1,3-dimethoxybenzene (**13**) with *n*-BuLi followed by alkylation with *n*-propyl iodide gave 1,3-dimethoxy-2-*n*-propylbenzene (**14**). After demethylation of **14** with BBr_3, the 2-*n*-propylbenzene-1,3-diol (**15**) obtained was condensed with ethyl 4,4,4-trifluoro-3-oxobutanoate to furnish **16**.

Imidazolidine-2,4-dione **18**[14] was prepared for a Bucherer–Bergs reaction[15] of ketone **17** using NaCN and $(NH_4)_2CO_3$, as depicted in Scheme 3.

The preparation of 2-oxochromene derivatives **6** and **7** is depicted in Scheme 4.[16] 6,8-Bis-*n*-propyl-2-oxochromene **12** and 8-*n*-propyl-2-oxochromene **16** were reacted with 1,4-dibromobutane to obtain the alkylbromide intermediates, which were then reacted with imidazolidine-2,4-dione, thiazolidine-2,4-dione, succinimide or oxazolidin-2-one to produce **6** and **7**, respectively.

The activities of the series of 2-oxochromene derivatives **6** and **7** were evaluated through GAL4-LXRα/β luciferase assays.[17] We investigated which atoms X and Y are favorable for compound **6.** The results are summarized in Table 2. Thiazolidine-2,4-dione **19** and imidazolidine-2,4-dione **20** showed moderate activities (LXRβ EC_{50} 1.4 μM, 1.3 μM; LXRβ E_{max} 45%, 47%) and exhibited seven- and

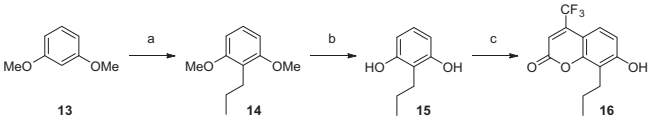
Table 1
Structure and cellular activity of the hit compounds[a]



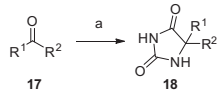
3 **4** **5**

Compound	ABCA1[b]	SREBP-1c[b]	ABCA1/SREBP-1c
3	5.0	1.4	3.6
4	1.3	3.3	0.40
5	9.1	3.9	2.3
GW3965	15	4.9	3.1

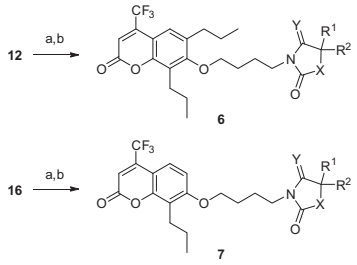
[a] The assays were conducted at 5 μM.
[b] The value is the ratio of the activation relative to that of the control (DMSO).



Scheme 2. Reagents and conditions: (a) *n*-BuLi, *n*-PrI, THF, rt, overnight, 45%; (b) BBr_3, CH_2Cl_2, −70 °C, 1 h to rt, 2 h, 76%; (c) ethyl 4,4,4-trifluoro-3-oxobutanoate, $ZnCl_2$, 110 °C, 18 h, 77%.

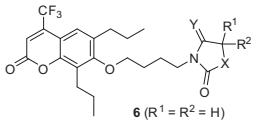


Scheme 3. Reagents and conditions: (a) NaCN, $(NH_4)_2CO_3$, EtOH aq, 100 °C, 50–85%



Scheme 4. Reagents and conditions: (a) 1,4-dibromobutane, K_2CO_3, DMF, rt, overnight, 80–96%; (b) amide or imide, K_2CO_3, DMF, rt, overnight, 51–99%.

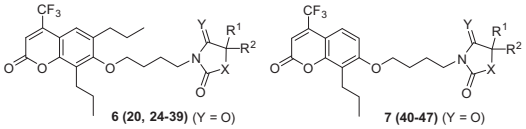
Table 2
LXR activity of 2-oxochromene derivatives **6**[a]



Compound	X	Y	LXRα EC_{50}[b] (%)[c]	LXRβ EC_{50}[b] (%)[c]
19	S	O	2.6 (6)	1.4 (45)
20	NMe	O	2.6 (10)	1.3 (47)
21	CH_2	O	nd (2)	1.4 (22)
22	O	H_2	nd (1)	2.2 (5)
23	CH_2	H_2	ia (0)	ia (0)

nd = not determined.
ia = inactive at 10 μM.
[a] The GAL4-LXR luciferase assay was performed with a maximal dose of 30 μM. The results are provided as the means of two independent experiments.
[b] EC_{50} data are reported in μM.
[c] The % efficacy is defined as the percentage ratio between the maximal fold induction for the test compound and the fold induction for T0901317 at 10 μM from the same experiment.[18]

Table 3
LXR activity of the 2-oxochromene derivatives in the series **6** (**20**, **24**–**39**) and **7** (**40**–**47**)[a]



Compound	X	R^1	R^2	LXRα EC_{50}[b] (%)[c]	LXRβ EC_{50}[b] (%)[c]
20	NMe	H	H	2.6 (10)	1.3 (47)
24	NMe	H	Me	1.7 (16)	1.3 (52)
25	NMe	Me	Me	11 (21)	4.7 (70)
26	NH	H	Me	1.5 (5)	1.3 (14)
27	NMe	Me	Ph	ia (0)	ia (0)
28	NH	Me	Ph	ia (0)	5.3 (6)
29	NH	Me	4-MePh	ia (0)	2.4 (9)
30	NH	Me	2-MeOPh	ia (0)	ia (0)
31	NH	Me	3-MeOPh	ia (0)	ia (0)
32	NH	Me	4-MeOPh	ia (0)	0.96 (8)
33	NH	Me	3,4-diMeOPh	ia (0)	ia (0)
34	NH	Me	3,4-OCH_2OPh	nd (2)	1.2 (32)
35	NH	Me	4-Me_2NPh	ia (0)	ia (0)
36	NH	Me	4-CF_3Ph	ia (0)	nd (1)
37	NH	Me	4-NO_2Ph	ia (0)	8.0 (7)
38	NH	Me	4-HO_2CPh	ia (0)	ia (0)
39	NH	Me	4-HOPh	ia (0)	ia (0)
40	NMe	H	H	nd (4)	1.3 (26)
41	NMe	Me	Me	2.4 (7)	1.02 (51)
42	NH	Me	Ph	ia (0)	2.0 (8)
43	NH	Me	4-MeOPh	nd (2)	1.4 (31)
44	NH	Me	3,4-diMeOPh	ia (0)	3.0 (9)
45	NH	Me	3,4-OCH_2OPh	nd (4)	1.1 (39)
46	NH	Me	4-HO_2CPh	ia (0)	ia (0)
47	NH	Me	4-HOPh	ia (0)	nd (2)

nd = not determined.
ia = inactive at 10 μM.
[a] The GAL4-LXR luciferase assay was performed with a maximal dose of 30 μM. The results are provided as the means of two independent experiments.
[b] EC_{50} data are reported in μM.
[c] The% efficacy is defined as the percentage ratio between the maximal fold induction for the test compound and the fold induction for T0901317 at 10 μM from the same experiment.[18]

five-fold selectivities for LXRβ (E_{max} ratio), respectively. In contrast, succinimide **21**, oxazolidin-2-one **22** and pyrrolidin-2-one **23** presented significantly decreased and even negligible activities.

Both thiazolidine-2,4-dione and imidazolidine-2,4-dione were identified as effective tails with LXRβ agonistic action. In this study, we selected **20** as a lead compound that could be varied for further development of diversity. We then examined the effect of the substituent on the imidazolidine-2,4-dione ring in the series of **6** and **7**, as shown in Table 3. Interestingly, the more substituted **25** showed a tendency for improved activity (**25** > **24** > **20** (E_{max})), whereas 5-methyl-5-phenyl-substituted **27** lost activity. In the 5-phenyl-substituted **27** and **28**, the *N*-methyl-substituted **27** completely lost both LXRα and LXRβ activities, but the NH-free derivative **28** maintained weak activity and moderate selectivity. The results obtained for 1-NH-5-phenylimidazolidine-2,4-dione **28** prompted us to focus on the substituent of the phenyl group. To investigate the influence of the substituents on the benzene ring, we prepared and tested a variety of compounds (**29**–**39**). The analysis of the electron-donating groups (**29**–**35**) revealed the 4-methylphenyl group **29** prevailed over the 2-methoxy, 3-methoxy, 3,4-dimethoxy groups (**30**–**31**, **33**). 4-Methoxy and 3,4-methylenedioxy-phenyl groups (**32**, **34**) were the same or more than **29**, and the 4-dimethylaminophenyl group **35** was inactive. The analysis of the electron-withdrawing groups showed that both the 4-trifluoromethyl and 4-nitro groups (**36**–**37**) were weak and that the 4-carboxylic acid group was inactive (**38**). Considering the lipophilicity of the head moiety (e.g., *C*log*P* of **34** shows 7.57), we removed the *n*-propyl group at either position 6 or 8 from the 6,8-bis-*n*-propyl-2-oxochromenes and tested them. As a result, the 8-*n*-propyl-2-oxochromene derivatives not only maintained their activities but also improved their EC_{50} values (**41** vs **25**) and relatively decreased their LXRα activities. Molecule **43** showed prominent selectivity with maintained potency, similarly to **32**. Consequently, the hydrogen-bond-acceptor groups (4-MeO-**43**, 3,4-diMeO-**44**, and 3,4-OCH_2O-**45**) were superior to the hydrogen-bond-donor groups (4-HO_2C-**46** and 4-HO-**47**).

To confirm our initial concept of LXRβ-selective agonists, we assessed **43** despite its weak potency (E_{max} 31%) toward LXRβ and lack of LXRα activity (E_{max} 2%), as shown in Figure 3.

We examined **43** through an in vivo test using a high-fat-and-cholesterol-fed Bio F_1B hamster. The F_1B hamster was suitable for the evaluation of the lipid-accumulation area in the aortic arch in the hyperlipidemia model.[19] T0901317 at doses of 3 and 10 mg/kg decreased the lipid-accumulation area in the aortic arch (53% and 52% relative to the control, respectively) but significantly elevated the plasma TG (206% and 243%, respectively). In contrast, our LXRβ-selective agonist **43** was orally administered to the hamster

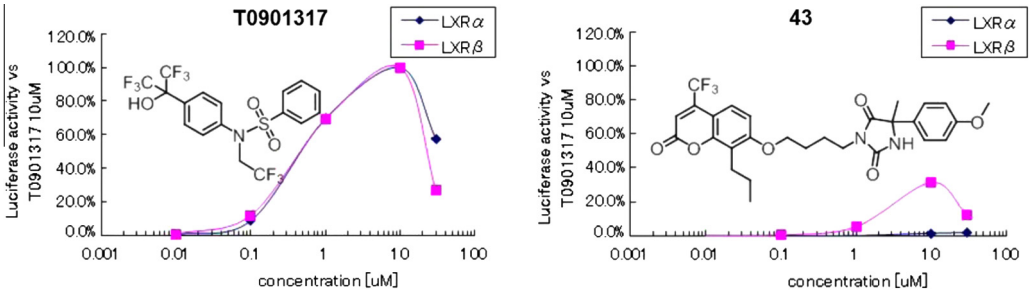


Figure 3. LXRαβ activity of T0901317 and **43** determined through an in vitro GAL4-LXR luciferase assay.

Table 4
In vivo study of the LXR agonists[22]

Compound	Dose (mg/kg)	TC (%)	HDL-C (%)	Plasma TG (%)	Area of lipid accumulation in the aortic arch (%)
T0901317	3	123	67*	206*	53*
	10	127*	59*	243*	52*
43	30	115	96	136	119
	100	101	96	131	102
	300	98	113*	96	56*

[a]The % value was calculated relative to the control.
* p <0.05; the statistical analysis was conducted using Dunnett's test.

at three-fold higher doses (30, 100 and 300 mg/kg) due to its poor PK profile.[20] Molecule **43** at a dose of 300 mg/kg increased the HDL-C (113%) level and significantly decreased the lipid-accumulation area in the aortic arch (56%) without elevating the plasma TG (96%),[21] as shown in Table 4.

In conclusion, a combination of the 2-oxochromene moiety (head) and the imidazolidine-2,4-dione moiety (tail) in the molecular design played a pivotal role in LXR activity and β selectivity. The novel LXRβ-selective agonist **43** increased the HDL-C level without significant TG elevation and resulted in a decrease in the lipid-accumulation area in the aortic arch in a high-fat-and-cholesterol-fed Bio F_1B hamster. Although we have successfully proven our initial concept, it remains possible to further improve the potency, selectivity and pharmacokinetics of our agonist, and further developments are in progress.

Acknowledgment

We thank Dr. S. Tanabe, Director of Tokyo New Drug Research Laboratories, Kowa Co., Ltd, for his support.

Supplementary data

Supplementary data (pharmacological experimental details, pharmacokinetic experimental details, general preparation procedure and analytical data of all synthetic compounds) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2015.01.047.

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13. Int. Patent Appl. WO2004011448, 2004. In the Merck patent documentation, only concrete biological data (% cholesterol efflux) for two compounds were disclosed. We prepared the corresponding Merck compounds and evaluated them through our luciferase assay using a tail moiety as the reference structure.
14. 1,5,5-Trimethylimidazolidine-2,4-dione (CAS NO. 6851-81-6) is commercially available (Tokyo Chemical Industry Co., Ltd).
15. (a) Bergs, H. *Ger. Pat.* **1929**, *566*, 094; (b) Bucherer, H. T.; Fischbeck, H. T. *J. Prakt. Chem.* **1934**, *140*, 69; (c) Bucherer, H. T.; Steiner, W. T. *J. Prakt. Chem.* **1934**, *140*, 291; (d) Ware, E. *Chem. Rev.* **1950**, *46*, 403.
16. Int. Patent Appl. WO2009107387, 2009.
17. LXRα/GAL4 or LXRβ/GAL4 hybrid and GAL4-responsive reporter vector pG5luc-stable-expression CHOK-1 cells were seeded at 20,000 cells/well on a 96-well plate in HAM-F12 medium containing 10% immobilized bovine fetal serum, 100 units/ml of penicillin G, and 100 μg/ml of streptomycin sulfate and incubated under a wet atmosphere with 5% CO_2 at 37 °C. After 24 h, medium with the test compound at various concentrations (0.01 μM, 0.1 μM, 1 μM, 10 μM, and 30 μM) was added, and the cells were incubated for an additional 24 hours. Using Bright-Glo (Promega) as a luciferase assay substrate and measuring the luminescence intensity with a luminometer LB960 (Berthold Technologies), the effect of the test compound on the activation of luciferase transcription via the LXRα- or LXRβ-LBD was measured.
18. The luciferase activity results are shown in Tables 1–3 as activity values (%eff) at the respective concentration of the test compound relative to the T0901317 luminescence intensity of 100 at 10 μM.
19. Srivastava reported that the hyperlipidemic Bio F_1B hamster has been proven to be an adequate atherosclerosis model for the evaluation of lipid-modulating agents Srivastava, R. A. K. *Atherosclerosis* **2011**, *214*, 86; However, the above experimental condition requires 21 weeks for the evaluation. To reduce the experimental period to 10 weeks, we conducted our established protocol as described in the following paper: Ikenoy, M.; Yoshinaka, Y.; Kobayashi, H.; Kawamine, K.; Shibuya, K.; Sato, F.; Sawanobori, K.; Watanabe, T.; Miyazaki, A. *Atherosclerosis* **2007**, *191*, 290.

20. The PK profiles of **43** were measured as follows. The CLs (μL/min/mg protein) of human, mouse and hamster hepatic microsomes were 393, 397 and 500, respectively. After a solution of **43** in PEG400 was orally administered to hamsters at doses of 30 and 100 mg/kg, the drug concentration of **43** in plasma could not be detected because it is easily metabolized. Even at a dose of 300 mg/kg, **43** showed a low concentration in plasma for up to 2 h after administration and disappeared 6 h after administration, as shown in the drug concentration curve obtained for a dose of 300 mg/kg presented in the Supplementary data.
21. A reviewer suggested the possibility that the insignificant effect of **43** on plasma TG could be attributed to LXRβ partial agonism and/or poor liver exposure (site of TG synthesis). At this stage, the cause, with the exception of poor liver exposure, has not been clarified.
22. Interestingly, Srivastava reported that T0901317 did not alter the LDL-C level but increased the TG level by three-fold and the HDL-C level by 50% under the fed condition. Our pharmacological experimental details are described in the Supplementary data.

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