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Discovery of a 2-hydroxyacetophenone derivative as an outstanding linker to enhance potency and β-selectivity of liver X receptor agonist

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ABSTRACT

Our research found that the 2-hydroxyacetophenone derivative is an outstanding linker between the 1,1-bistrifluoromethylcarbinol moiety and the imidazolidine-2,4-dione moiety to enhance the potency and β-selectivity of liver X receptor (LXR) agonist in our head-to-tail molecular design. The incorporation of this linker is 20-fold more potent than our previous compound (**2**) for LXRβ agonistic activity (EC_{50}) in a GAL-4 luciferase assay. Furthermore, we also identified 5-[5-(1-methylethoxy)pyridyl-2-yl]-5-methylimidazoline-2,4-dione (**54**), which lowers the lipophilicity of 2-hydroxyacetophenone derivative. We revealed that a combination of our newly developed linker and hydantoin (**54**) plays a pivotal role in improving the potency and selectivity of LXRβ. The optically separated **(−)-56** increases high-density lipoprotein cholesterol levels without elevating plasma triglyceride levels and results in a decrease of the lipid accumulation area in the aortic arch in a high-fat- and cholesterol-fed low-density lipoprotein receptor knock-out mice. In this manuscript, we report that **(−)-56** is a highly potent and β-selective LXR agonist for use in the treatment of atherosclerosis.

1. Introduction

In our drug discovery program, we recently reported the 2-oxochromene molecule **1** and the 1,1-bistrifluoromethylcarbinol molecule **2** as liver X receptor (LXR) β-selective agonists and demonstrated their potential pharmacological uses.[1,2] Both compounds have anti-atherogenic and hypolipidemic effects that do not elevate triglycerides (TG) in the F_1B hamster. These compounds have a common feature in their molecular design, a butylene linker between their head moieties (2-oxochromene and 1,1-bistrifluoromethylcarbinol) and their tail moieties (imidazolidine-2,4-diones), as shown in Figure 1.

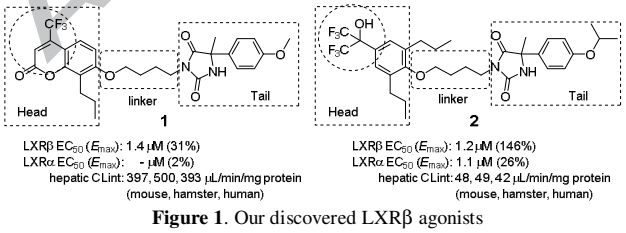

Figure 1. Our discovered LXRβ agonists

Liver X receptors (LXRs) are ligand-activated transcription factors involved in cholesterol metabolism, glucose homeostasis, inflammation and lipogenesis.[3,4] LXRα (known as NR1H3) is the dominant subtype in the liver, small intestine and in macrophages, whereas LXRβ (known as NR1H2) is distributed ubiquitously. LXR activation is known to regulate the expression of the ATP-binding cassette transporter A1 (ABCA1), ABCG1 and ABCG8, cholesterol metabolism, increasing high-density lipoprotein cholesterol (HDL-C) levels and cholesterol efflux.[5-11] The agonistic actions of LXR would be expected to have an anti-atherogenic effect in peripheral blood vessels. However, elevations in the plasma and hepatic TG occurred.[12,13] The activation LXRα induces the expression of the sterol regulatory element-binding protein-1c (SREBP-1c), and leads to the observed lipogenesis.[12,13] To suppress the undesirable effect of increased lipogenesis in an LXR agonist, our efforts were focused on differentiating between the two LXR subtypes.[14-15] Although we have demonstrated the pharmacologically useful effects of **1** and **2**, there remain pharmacokinetic (PK) issues with oral administration at higher doses. We could not detect the plasma drug concentrations of **1** at doses of 30 and 100 mg/kg due to the high clearance (CLs) of hamster hepatic microsomes

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(500 μL/min/mg protein). Similarly, we monitored the lower plasma drug concentration of **2** and the higher plasma concentration of its metabolite, despite the improvement in the CL of hamster hepatic microsome (49 μL/min/mg protein) after oral administration at a dose of 100 mg/kg.[2] We identified the major metabolite of **2** as the carboxylate derivative **3** with the oxidative cleavage of the C-N bond between the butylene unit and the imidazolidine-2,4-dione unit as shown in Figure 2. Therefore, our purpose in this study is to modify the chemical structure of **2** and optimize the metabolic stability, potency and selectivity of LXRβ. In this manuscript, we report our optimization process and the discovery of a highly potent and β-selective LXR agonist for the treatment of atherosclerosis.

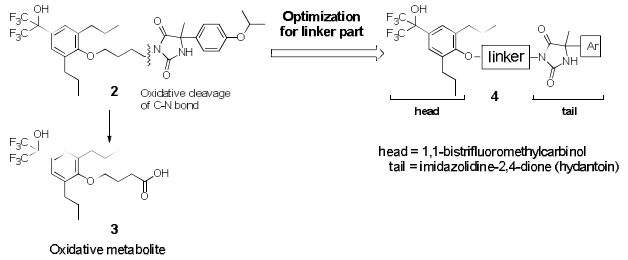
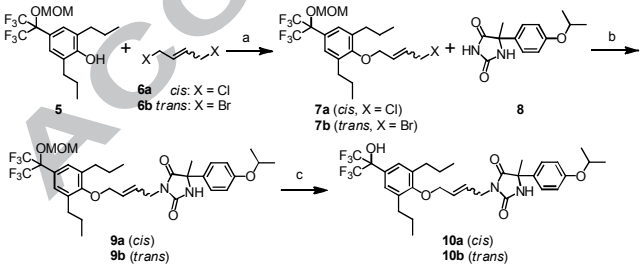


Figure 2. Plan for the improvement of metabolic stability, potency (EC_{50}) and selectivity for LXRβ through the modification of the structure of **2**.

2. Results and discussion

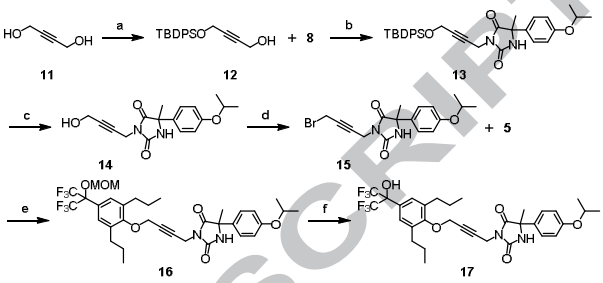
2.1 Synthesis of the unsaturated alkyl linker derivatives

First, we focused on the linker between the 1,1-bistrifluoromethylcarbinol moiety (head) and the imidazolidine-2,4-dione moiety (tail), which could be a metabolic target (first pass effect) in the liver. We then investigated how much the agonistic activities change after modifying the structure of a butylene unit. We prepared *cis* and *trans*-2-butene **10a-b** as depicted in Scheme 1. The alkylation of 4-(1,1,1,3,3,3-hexafluoro-2-(methoxymethoxy)propan-2-yl)-2,6-di-*n*-propylphenol (**5**)[2] with *cis*-1,4-dichloro-2-butene (**6a**) and *trans*-1,4-dibromo-2-butene (**6b**) under basic conditions produced the allylhalides **7a** and **7b**, followed by alkylation with 5-(4-(1-methylethoxy)phenyl)-5-methylimidazolidine-2,4-dione (**8**)[2] under basic conditions to produce **9a** and **9b**. Finally, under acidic conditions, the methoxymethyl (MOM) ethers of **9a-b** were deprotected to yield the desired compounds in 3 steps of **10a** and **10b** at yields of 71% and 14%, respectively.



Scheme 1. Reagents and conditions: (a) *cis*-1,4-dichloro-2-butene (**6a**) or *trans*-1,4-dibromo-2-butene (**6b**), K_2CO_3, DMF, rt, overnight, 42-89%; (b) K_2CO_3, DMF, rt, overnight, 21-73%; (c) HCl/EtOAc, rt, 1 h, 80%.

We then prepared the but-2-yne derivative **17**, as depicted in Scheme 2. We performed the protection of but-2-yne-1,4-diol (**11**) with *tert*-butyl(chloro)diphenylsilane (TBDPSCl) in the presence of imidazole to produce 4-((*tert*-butyldiphenylsilyl)oxy)but-2-yn-1-ol (**12**), which was followed by a Mitsunobu reaction[16] of **8** to produce **13**. Next, we carried out the deprotection of **13** by *tetra*-*n*-butylammonium fluoride (TBAF) to release the alcohol **14**, which reacted with a combination of triphenylphosphine (PPh_3) and carbon tetrabromide (CBr_4) to release the propalgyl bromide **15**. The alkylation of **15** with **5** in the presence of K_2CO_3 gave the product **16**. Finally, we deprotected the MOM ether of **16** under acidic conditions to yield the desired compound **17**.

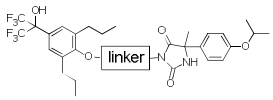


Scheme 2. Reagents and conditions: (a) TBDPSCl, imidazole, DMF, rt, overnight, 29%; (b) DEAD, PPh_3, THF, rt, overnight, 55%; (c) TBAF, THF, 1 h, 90%; (d) CBr_4, PPh_3, CH_2Cl_2, rt, 1 h, 87%; (e) K_2CO_3, DMF, rt, overnight, 77%; (f) HCl/EtOAc, rt, 1 h, 88%.

2.2 Evaluation of the unsaturated alkyl linker derivatives

To evaluate the impact of the unsaturated alkyl linker on function, we conducted a GAL4-LXR luciferase assay on **10a**, **10b** and **17**. Interestingly, the 2-*cis*-butene derivative **10a** lost LXR activity while the 2-*trans*-butene derivative **10b** has slightly improved potency (EC_{50} for LXRβ = 0.80 μM) and selectivity (EC_{50} ratio for LXRα/LXRβ = 1.5) compared with **2** (EC_{50} for LXRβ = 1.15 μM, EC_{50} ratio for LXRα/LXRβ = 1.0). Furthermore, the butyne derivative **17** is more potent than **10b**. As can be seen, even the small modification of the linker influences the activity and the selectivity towards LXRβ. Our results are summarized and shown in Table 1.

Table 1. LXR activity of the 1,1-bistrifluoromethylcarbinol derivatives containing various linkers **4** (**2**, **10a**, **10b**, **17**)[a]



Compound	linker	LXRα EC_{50}[b] (%)[c]	LXRβ EC_{50}[b] (%)[c]	EC_{50} α/β	*Clog*P
2		1.12 (26)	1.15 (146)	0.97	7.63
10a		ia (0)	ia (1)	-	7.58
10b		1.23 (40)	0.80 (182)	1.54	7.58
17		0.86 (27)	0.61 (149)	1.41	7.33

ia = inactive at 10 μM.

[a] The GAL4-LXR luciferase assay was performed at a maximum dose of 10 μM. The results are given as the mean of two independent experiments.

[b] EC_{50} data are reported in μM.

[c] The E_{max} (%) is defined as the percentage ratio between maximum fold induction for the test compound and fold induction for T0901317 at 10 μM in the same experiment.

These results led us to conclude that the orientation and the distance of the linker between the head and tail moieties plays a pivotal role in the agonistic actions of LXRs. We next replaced the unsaturated alkyl with an aromatic part as a rigid linker to fix the orientation of the head and tail moieties.

2.3 Synthesis of the aromatic linker derivatives

Considering the increasing lipophilicity of the newly created molecule after incorporation of an aromatic substituent into the linker part, we used the mono-propyl phenol **18**[2] as a starting material instead of **5**. To examine the impact of the orientation of the linker between the head and tail moieties, we introduced the benzyl part as a rigid linker and prepared the **22** derivative in a retro-synthetic plan. The diaryl ether formation between **18** and **19** produced **20**, then alkylation of **20** with hydantoin **8** produced **21** and finally, the deprotection of MOM ether **21** gave the desired molecule **22** as shown in Figure 3.

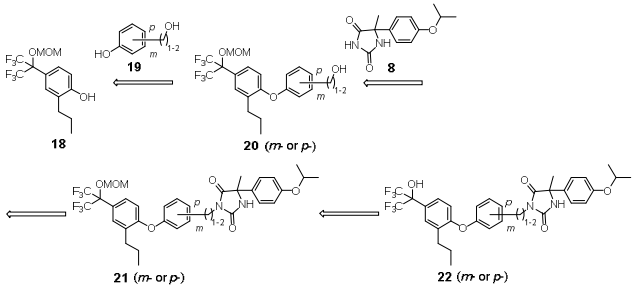


Figure 3. Outline of our retro-synthetic plan.

First, we prepared the benzyl derivatives **28a-b** as depicted in Scheme 3. Copper-promoted arylation[17] of **18** with 3- or 4-formylphenylboronic acid (**23a** or **23b**) produced 3- or 4-phenoxybenzaldehyde (**24a** or **24b**), followed by a reduction with $NaBH_4$ gave the benzyl alcohol **25a-b** product. The bromination of **25a-b** using a combination of PPh_3 and CBr_4 produced the benzyl bromide **26a-b**. The resultant **26a-b** reacted with the hydantoin **8** in the presence of K_2CO_3 to produce the bis-aryl ether **27a-b**. Finally, the MOM ether of **27a-b** was deprotected under acidic conditions to yield the desired compound **28a-b**.

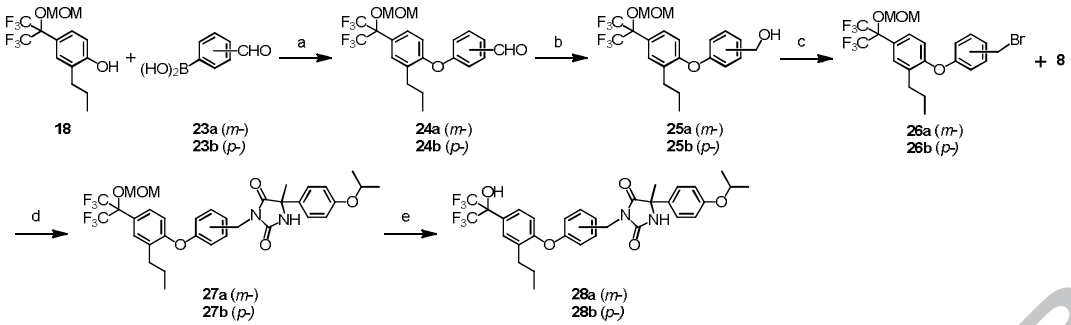
Second, to introduce the phenethyl into the linker, we prepared the phenethyl derivatives **34a-b** as depicted in Scheme 4. Similarly, we used the copper-promoted arylation between **18** and 3- or 4-vinylphenylboronic acid (**29a** or **29b**) to produce the stylene **30a-b**. Hydroboration of **30a-b** with borane-THF complex, followed by oxidation with $NaBO_3$-$4H_2O$, resulted in the phenethyl alcohol **31a-b**. We performed the reaction process depicted in Scheme 2 (step d-f) to obtain the desired compound **34a-b**.

Third, to introduce a substituent at the 2-potision of the phenethyl moiety and examine the effects of the substituents, we prepared the 2-substituted phenethyl derivatives **44a-d** as depicted in Scheme 5. The phenol **35** was reacted with methyl iodide and benzyl bromide to give the phenyl ethers **36b** and **36c**, respectively. The substitution of 4-fluoro-2-substituted-1-nitrobenzene (**36a** R= Me, **36b** R = OMe, **36c** R = OBn) with **18** was carried out under basic conditions to give the 4-aryloxy-2-substituted-1-nitrobenzene **37a-c**, at yields of 71%, 91% and 96%, respectively. Hydrogenation of **37a-b** with Pd/C under a hydrogen atmosphere gave the aniline **38a-b.** In contrast, to avoid deprotection of the benzyl ether, the reduction of **37c** was performed with iron powder under acid conditions to produce the aniline **38c**. We used a Sandmeyer reaction[18,19] of **38a-c** in the presence of KI to produce the iodobenzene **39a-c**. We also conducted a Suzuki–Miyaura coupling reaction[20] of **39a-c** with 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane to produce the stylene **40a-c**, at yields of 99%, 64% and 75%, respectively. We performed the procedure described in Scheme 4 (step b) to give the phenethyl alcohol **41a-c**. The bromination of **41a** failed because of its chemical instability; thus, we conducted the tosylation of **41a** with TsCl and pyridine to produce the tosylate **42a** in 50% yield. We obtained the bromides **41b-c**, which resulted in lower yields than those produced by Scheme 2 (step d). We prepared the desired compounds **44a-c** via the cleavage of the MOM ether **43a-c**, which was obtained from **42a** and **42b-c** by alkylation of **8** with overall yields of 17%, 4% and 3% with 8, 9 and 10 steps, respectively. The desired compound **44d** was prepared by the hydrogenolysis of **44c**.

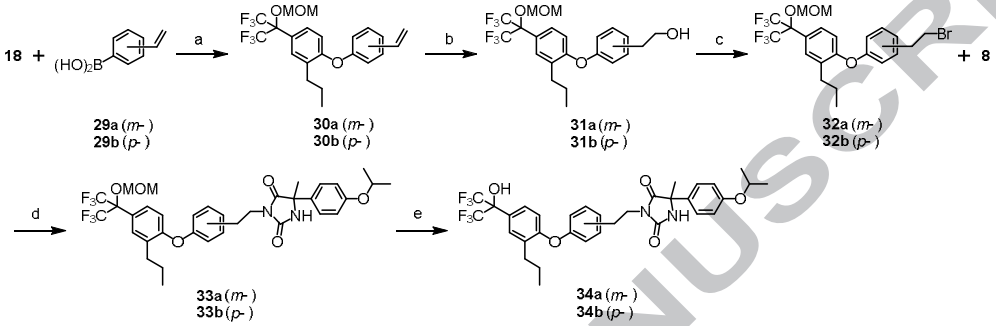
Finally, we also produced the acetophenone derivatives **49a-e** as depicted in Scheme 6. A Grignard reaction of **24b** with MeMgBr produced the 2-alcohol **45**, followed by oxidation with MnO_2 to give the acetophenone **46**. A Stille coupling reaction[21] of **38a-c** with tributyl(1-ethoxyvinyl)stannane yielded the acetophenone derivatives **47a-c**. The direct tosylation of the methyl group of **46** and **47a-c** was performed by treating with ((hydroxy)(tosyloxy)-iodo)benzene (PhI(OH)OTs)[22] to produce the tosylate **48a-d** at yields of 24%, 82%, 37% and 18%, respectively. The connection between **48a-d** and **8** was carried out under basic conditions in the same manner (Scheme 5, step h) to produce **49a-d**. The hydrogenolysis of **49d** yielded the desired phenol **49e**.

2.4 Evaluation of the aromatic linker derivatives

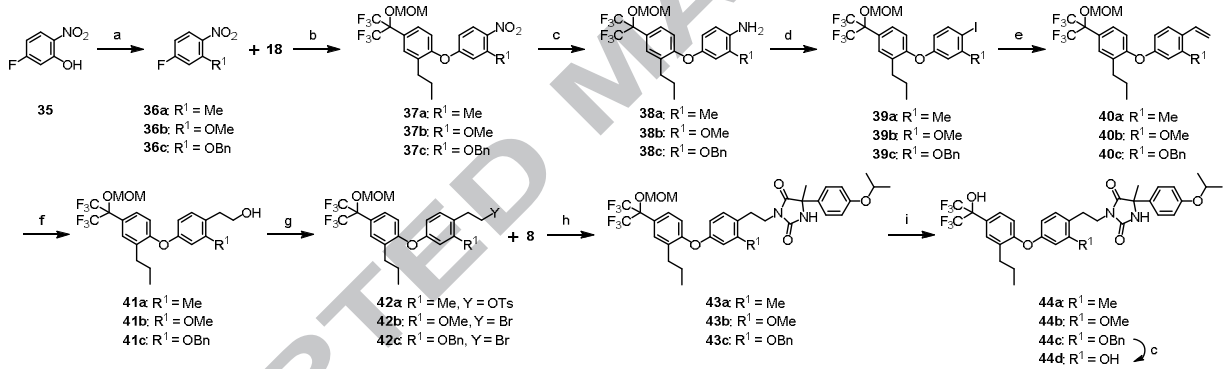
The results of the LXR transactivation assay are summarized in Table 2. The benzyl derivatives **28a-b** and the phenethyl derivatives **34a-b** have moderate potency (EC_{50}) toward LXRβ. The *para*-substitution system has the tendency to express higher subtype selectivity than the *meta*-substitution system (selectivity for LXRα EC_{50}/ LXRβ EC_{50}; **28b =** 5.5 *vs* **28a** = 3.9; **34b** = 5.3 *vs* **34a** = 1.0). Based on **34b,** which has good potency and selectivity (EC_{50} for LXRβ = 1.88 μM, E_{max} = 223%), we conducted further structural modifications and introduced substituents at the 3-position of the phenethyl **34b**. The methyl group in **44a** significantly enhanced the E_{max} value of LXRβ (EC_{50} = 0.70 μM, E_{max} = 615%), but has the higher *C*log*P* value (8.12). To address this issue, we incorporated a polar substituent group into the benzene ring. Interestingly, the methoxy and the hydroxyl groups increased the agonistic activities of LXRβ (**44b**; EC_{50} = 1.66 μM, E_{max} = 534%, **44d**; EC_{50} = 2.87 μM, E_{max} = 284%) and slightly lowered its lipophilicity (*C*log*P* = 7.50 and 7.24). Having obtained the products **28a-44d**, we turned our attention to the benzylic position because it can be metabolically labile. Introduction of the hydroxyl group at the benzylic position of **34b** (yielding **50**[23]) results in slightly lower potency (EC_{50} for LXRβ = 2.33 μM, E_{max} = 176%). Next, the conversion of the alcohol **50** into the ketone **49a** remarkably improves potency and lipophilicity (EC_{50} for LXRβ = 0.12 μM, E_{max} = 236%, *C*log*P* = 6.54), but has only moderate LXRα agonistic activity (EC_{50} for LXRα = 0.36 μM, E_{max} = 46%). Successively, we examined whether substitution at the 2-position of the acetophenone could ameliorate the subtype selectivities of **44b** and **44d**. We obtained satisfactory results of LXRβ activity (**49b** : **49c** : **49e**; EC_{50} = 0.064 : 0.36 : 0.11 μM; E_{max} = 264 : 306 : 348%), but could not suppress LXRα activity (**49b** : **49c** : **49e**; EC_{50} = 0.40 : 3.74 : 0.34 μM; E_{max} = 71 : 38 : 55%). Accordingly, we noted the lower lipophilicity of **49e** and the fixed 2-hydroxyacetophenone linker, and then explored how to modify the structure of 5-(4-(1-methylethoxy)phenyl)-5-methylimidazolidine-2,4-dione (**8**). To this end, we incorporated a nitrogen atom into the benzene ring of **8**.



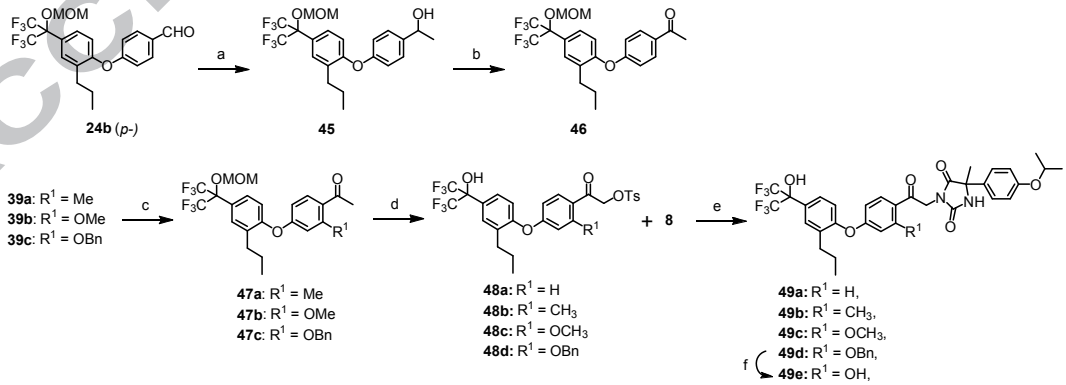
Scheme 3. Reagents and conditions: (a) $Cu(OAc)_2$, pyridine, MS4A, CH_2Cl_2, rt, overnight, 68-75%; (b) $NaBH_4$, MeOH, 0 °C, 1 h, 99%; (c) CBr_4, PPh_3, CH_2Cl_2, rt, 0.5-2 h, 18-94%; (d) K_2CO_3, DMF, rt, overnight, 21-73%; (e) HCl/EtOAc, rt, 1 h, 80%.



Scheme 4. Reagents and conditions: (a) $Cu(OAc)_2$, pyridine, MS4A, CH_2Cl_2, rt, overnight, 68-75%; (b) BH_3-THF, THF, rt, 1 h then $NaBO_3$-$4H_2O$, H_2O, rt, overnight, 46-85%; (c) CBr_4, PPh_3, CH_2Cl_2, rt, 0.5-2 h, 18-94%; (d) K_2CO_3, DMF, rt, overnight, 21-73%; (e) HCl/EtOAc, rt, 1 h, 80%.

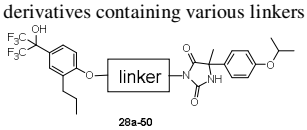


Scheme 5. Reagents and conditions: (a) MeI or BnBr, K_2CO_3, DMF, 60 °C, 1 h, 99%; (b) K_2CO_3, DMF, 80 °C, 2 h, 71-96%; (c) i) H_2, Pd/C, MeOH, rt, 1 h, 92%; or Fe, *aq*. AcOH, rt, 4 h, 99%; (d) $NaNO_2$, *p*-TsOH-H_2O, KI, MeCN, rt, overnight, 30-67%; (e) 4,4,5,5-tetramethyl-2-vinyl-1,3,2-dioxaborolane, $Pd(PPh_3)_4$, Na_2CO_3, DMF-H_2O, microwave, 80 °C, 20 min, 64-99%; (f) BH_3-THF, THF, rt, 1 h then $NaBO_3$-$4H_2O$, H_2O, rt, overnight, 62-73%; (g) i) TsCl, pyridine, CH_2Cl_2, 40 °C, 2 h, 50% (**42a**) or ii) CBr_4, PPh_3, CH_2Cl_2, rt, 0.5-2 h, 10-31% (**42b**, **42c**); (h) K_2CO_3, DMF, rt, overnight, 21-73%; (i) HCl/EtOAc, rt, 1 h, 99%.



Scheme 6. Reagents and conditions: (a) MeMgBr, THF, 0 °C, 0.5 h then rt, 1 h; (b) MnO_2, CH_2Cl_2, rt, overnight, 77% for 2 steps; (c) $(CH_2CH(OEt))SnBu_3$, $Pd(PPh_3)_4$, PhMe, reflux, 3 h, then 1 N HCl, rt, overnight, 50-80%; (d) PhI(OH)OTs, CH_3CN, reflux, 6 h, 18-82%; (e) K_2CO_3, DMF, rt, overnight, 21-73%; (f) H_2, Pd/C, MeOH, rt, 3 h, 88%.

Table 2. LXR activity of the 1,1-bistrifluoromethylcarbinol derivatives containing various linkers **28-50**[a]



28a-50

Compound	linker	LXRα EC_{50}[b] (%)[c]	LXRβ EC_{50}[b] (%)[c]	EC_{50} α/β	*C*log*P*
28a		1.96 (53)	0.50 (429)	3.92	7.35
28b		ia (0)	1.81 (22)	>5.52	7.35
34a		2.89 (23)	2.85 (126)	1.01	7.63
34b		ia (1)	1.88 (223)	>5.32	7.63
44a		3.14 (53)	0.70 (615)	4.89	8.12
44b		ia (0)	1.66 (534)	6.02	7.50
44d		ia (1)	2.87 (284)	3.48	7.24
50		ia (5)	2.33 (176)	4.29	6.81
49a		0.36 (46)	0.12 (236)	3.00	6.54
49b		0.40 (71)	0.064 (264)	6.25	7.02
49c		3.74 (38)	0.36 (306)	10.4	6.41
49e		0.34 (55)	0.11 (348)	3.09	6.15

ia = inactive at 10 µM.

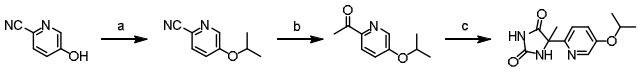
[a] The GAL4-LXR luciferase assay was performed at a maximum dose of 10 µM. The results are given as the mean of two independent experiments.

[b] EC_{50} data are reported in µM.

[c] The E_{max} (%) is defined as the percentage ratio between maximum fold induction for the test compound and fold induction for T0901317 at 10 µM in the same experiment.

2.5 Synthesis of the pyridylhydantoin derivative 56

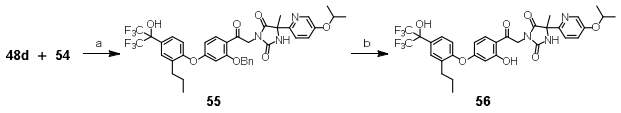
We prepared the pyridylhydantoin **54** from 5-hydroxy-picolinonitrile (**51**) as depicted in Scheme 7. We performed the alkylation of **51** with 2-iodopropane to yield 5-isopropoxy-picolinonitrile (**52**) at 59%. Upon exposure of **52** to MeMgBr, followed by the work-up produced the ketone **53**. Finally, we conducted a Bucherer-Bergs reaction[24-27] of **53** using NaCN and $(NH_4)_2CO_3$ in *aq*. EtOH at 70 °C to produce **54** at 85% yield.



51 52 53 54

Scheme 7. Reagents and conditions: (a) *i*-PrI, K_2CO_3, DMF, rt, 6 h, 59%; (b) MeMgBr, THF, 0 °C, 1 h, then HCl *aq*., 1 h, 91%; (c) NaCN, $(NH_4)_2CO_3$, *aq*. EtOH, 70°C, 85%.

We performed the same procedure described in Scheme 6 (step e-f) to give the desired compound **56**, as depicted in Scheme 8.



Scheme 8. Reagents and conditions: (a) K_2CO_3, DMF, rt, overnight, 21%; (b) H_2, Pd/C, MeOH, rt, 3 h, 80%.

2.6 Evaluation of the pyridylhydantoin derivative 56

Interestingly, the pyridylhydantoin derivative **56** remarkably improved the potency and selectivity of LXRβ (EC_{50} for LXRβ = 0.058 μM, E_{max} = 329%, selectivity EC_{50} ratio for LXRα/LXRβ = 5.69, *C*log*P* = 5.23) as shown in the dose-response curves of **T0901317**, **2**, **49e** and **56** in Gal4-LXRα and LXRβ luciferase assays (Figure 4).

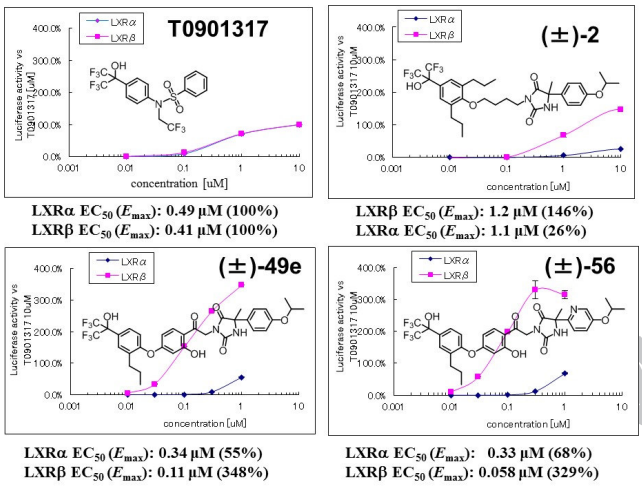


Figure 4. Dose-response curves of **T0901317**, (±)-**2**, (±)-**49e** and (±)-**56**

Successively, we evaluated an *in vitro* liver CL of **56** compared to the prototype compounds **2** and **49e**. To our delight, the metabolic stability of **56** has an improved value in all species examined as shown in Table 3.

Table 3. hepatic CLint (μL/min/mg protein) of each animal obtained using an *in vitro* assay.*

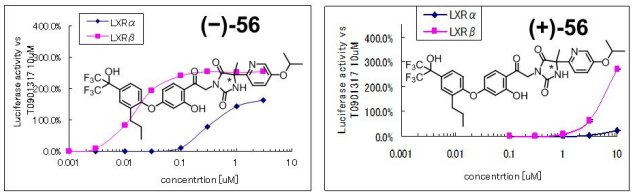
Compound	Mouse	Hamster	Human
2	84	206	71
49e	76	27	12
56	24	11	7

*The hepatic CLint values of compounds **2**, **49e** and **56** were assessed using hepatic microsomes from each animal (mouse, hamster and human).[28]

We concluded that **56** would be a well-balanced LXRβ agonist and warrants further evaluation.

2.7. Synthesis and evaluation of both enantiomers of 56

We conducted the chiral separation of **(±)-54** by optical resolution using a CHIRALPAK AY-H column and obtained both enantiomers of **(+)-54** and **(−)-54**.[29] Having prepared **(+)-56** and **(−)-56** in the same manner in Scheme 8 (steps a-b), we evaluated the compounds in Gal4-LXRα and LXRβ luciferase assays. We found that **(−)-56** is the desired LXRβ agonist (EC_{50} for LXRβ; **(−)-56**: 0.007 μM, EC_{50} α/β selectivity: 28.5), while **(+)-56** has almost no LXRα agonistic activity and has significantly decreased LXR β agonistic activity (EC_{50} for LXRβ; **(+)-56**: 2.9 μM) as shown in Figure 5.



LXRα EC_{50} (E_{max}): 0.20 μM (164%)
LXRβ EC_{50} (E_{max}): 0.007 μM (255%)

LXRα EC_{50} (E_{max}): ia (25%)
LXRβ EC_{50} (E_{max}): 2.9 μM (269%)

Figure 5. Dose-response curves of **(−)-56** and **(+)-56**

To examine systemic PK, we measured the drug concentration of **(−)-56** after oral administration in mice at a dose of 10 mg/kg using PEG400 as a delivery vehicle. Our results show that the plasma drug concentration levels of **(−)-56** have nearly equivalent levels of **2** at a dose of 100 mg/kg (Table 4).[30]

Table 4 PK profile of **(±)-2** and **(−)-56**

Compound	Dose (mg/kg)	Cmax (ng/mL)	AUC (h*ng/mL)
(±)-**2**	100	406	3419
(−)-**56**	10	503	3027

Considering the enhanced potency of **(−)-56**, we then conducted an *in vivo* test in another established animal model. We changed animal model from high-fat- and cholesterol-fed F_1B hamsters to high-fat and cholesterol-fed low-density lipoprotein (LDL) receptor knock-out mice, which is one of the most widely used and promising animal models for atherosclerosis.[31,32] We evaluated **(−)-56** and T0901317 in this mouse model.[33] At a dose of 10 mg/kg, T0901317 significantly decreased the area of lipid accumulation in the aortic arch (40% relative to the control) with a decrease of LDL-C, but significantly elevated the plasma TG level (465%) and decreased HDL-C (47%). In contrast, even at doses of 1 and 3 mg/kg, **(−)-56** remarkably decreased the area of lipid accumulation (62 and 59%, respectively) and increased in HDL-C (115 and 116%, respectively) without altering LDL-C. From these results, we speculate that the increase in HDL-C by **(−)-56** substantially contributes the cholesterol efflux from atherogenic lesions.[34] On the other hand, the anti-atherosclerotic effect of T0901317 could be attributed to a significant lowering of LDL-C (55%) as shown in Table 5.

Table 5. *In vivo* study of T0901317 and **(−)-56**[34]

Compound	Dose (mg/kg)	TC (%)	HDL-C (%)	LDL-C (%)	Plasma TG (%)	Area of lipid accumulation (%)
T0901317	10	96	47*	55*	465*	40*
(−)-56	1	111	115*	108	152	62*
	3	102	116*	97	166*	59*

*$p < 0.05$; Statistical analysis was conducted using Dunnett's test. The % value is calculated relative to control.

3 Conclusion

In conclusion, we discovered a 2-hydroxyacetophenone derivative that has an outstanding linker between the 1,1-bistrifluoromethylcarbinol moiety and imidazolidine-2,4-dione moiety in our head-to-tail molecular design. Our novel **(−)-56** compound increased HDL-C without significantly affecting TG levels and leaded to a decrease in the lipid accumulation area of the aortic arch in high-fat and cholesterol-fed LDL receptor knock-out mice. However, the identification of the absolute configuration of **(−)-56** and its efficient synthesis requires further study. Further development of this compound is currently underway in our laboratory.

4 Experimental Section

4.1. Chemistry

4.1.1. General comments

Commercially available reagents and solvents were used without further purification. Thin layer chromatography (TLC) analyses were performed on silica gel 60 F254 plates (Merck). ^{1}H NMR and ^{13}C NMR spectra were obtained on a JEOL JNM-LA 400 MHz spectrometer using $CDCl_3$ or CD_3OD as the solvent with tetramethylsilane as the internal standard. Infrared (IR) spectra were recorded on a Thermo Nicolet 370 FT-IR (ATR) spectrometer. Mass spectra were obtained on a JEOL MS-BU20 mass spectrometer. Elemental analyses (C, H, N) were performed using a Yanaco MT-5 instrument. Melting points were determined in open glass capillaries on a Buchi B-545 melting point apparatus. Optical rotations were measured on a JASCO P2200 polarimeter operating at the sodium D line at room temperature. The chiral HPLC analyses were performed on a Shimazu LC-2010A HT liquid chromatograph.

4.1.2. Representative synthetic procedure of (–)-56

2-Benzyloxy-4-fluoro-1-nitrobenzene (36c):
To a stirred suspension of 4-fluoro-2-hydroxy-1-nitrobenzene (**35**) (5.0 g, 31.8 mmol) and K_2CO_3 (5.28 g, 38.2 mmol) in *N, N*-dimethylformamide (DMF) (50 mL) was added benzyl bromide (5.99 g, 35.0 mmol) at room temperature. The reaction mixture was stirred at 60 °C for 1 h. After the reaction was completed, the reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 5/1) to give the title compound (9.47 g, 99%) as a pale yellow crystal; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.23 (2H, s), 6.74 (1H, ddd, *J* = 2.4, 7.3, 9.0 Hz), 6.83 (1H, dd, *J* = 2.4, 10.2 Hz), 7.33-7.47 (5H, m), 7.97 (1H, dd, *J* = 6.1, 9.0 Hz); MS (EI) *m/z* 247 $[M]^+$; Anal. Calcd for $C_{13}H_{10}FNO_3$: C, 63.16; H, 4.08; N, 5.67. Found: C, 63.23; H, 4.11; N, 5.61.

2-(Benzyloxy)-4-(4-(1,1,1,3,3,3-hexafluoro-2-(methoxy-methoxy)propan-2-yl)-2-*n*-propylphenoxy)-1-nitrobenzene (37c):
To a stirred suspension of 4-(1,1,1,3,3,3-hexafluoro-2-(methoxy-methoxy)propan-2-yl)-2-*n*-propylphenol (**18**) (5.30 g, 15.3 mmol) and K_2CO_3 (2.64 g, 19.1 mmol) in DMF (25 mL) was added **36c** (3.15 g, 12.7 mmol) at room temperature. The reaction mixture was stirred at 80 °C for 3 h. After the reaction was completed, the reaction mixture was diluted with water and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/acetone = 10/1) to give the title compound (8.31 g, 99%) as a pale yellow crystal; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.89 (3H, t, *J* = 7.1 Hz), 1.55 (2H, qt, *J* = 7.1, 7.8 Hz), 2.50 (2H, t, *J* = 7.8 Hz), 3.58 (3H, s), 4.89 (2H, s), 5.18 (2H, s), 6.50 (1H, dd, *J* = 2.2, 8.6 Hz), 6.58 (1H, d, *J* = 2.2 Hz), 6.92 (1H, d, *J* = 8.8 Hz), 7.31-7.39 (5H, m), 7.46 (1H, d, *J* = 8.8 Hz), 7.54 (1H, s), 7.97 (1H, d, *J* = 8.6 Hz); MS (EI) *m/z* 573 $[M]^+$; Anal. Calcd for $C_{27}H_{25}F_6NO_6$: C, 56.55; H, 4.39; N, 2.44. Found: C, 56.57; H, 4.33; N, 2.42.

2-(Benzyloxy)-4-(4-(1,1,1,3,3,3-hexafluoro-2-(methoxy-methoxy)propan-2-yl)-2-propylphenoxy)aniline (38c):
To a stirred solution of **37c** (8.31 g, 12.7 mmol) in acetic acid (64 mL) and water (4.0 mL) was added iron powder (10.7 g, 191 mmol) at room temperature. The reaction mixture was stirred at room temperature for 4 h. After completing the reaction, 1 N NaOH *aq*. was added to the reaction mixture. The resulting mixture was filtered through a pad of Celite and rinsed with EtOAc. The reaction mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo* to give the title compound (8.49 g, 99%) as a yellow amorphous; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.97 (3H, t, *J* = 7.3 Hz), 1.68 (2H, qt, *J* = 7.3, 7.3 Hz), 2.70 (2H, t, *J* = 7.3 Hz), 3.55 (3H, s), 4.84 (2H, s), 5.04 (2H, s), 6.50 (1H, dd, *J* = 2.4, 8.3 Hz), 6.61 (1H, d, *J* = 2.4 Hz), 6.67 (1H, d, *J* = 8.8 Hz), 6.72 (1H, d, *J* = 8.3 Hz), 7.33-7.40 (7H, m); MS (EI) *m/z* 543 $[M]^+$; Anal. Calcd for $C_{27}H_{27}F_6NO_4$: C, 59.67; H, 5.01; N, 2.58. Found: C, 59.66; H, 4.87; N, 2.65.

2-(Benzyloxy)-4-(4-(1,1,1,3,3,3-hexafluoro-2-(methoxy-methoxy)propan-2-yl)-2-*n*-propylphenoxy)-1-iodobenzene (39c):
To a stirred suspension of **38c** (8.49 g, 12.7 mmol) and *p*-toluene sulfonic acid monohydrate (7.27 g, 38.2 mmol) in CH_3CN (64 mL) was added the solution (water 8.5 mL) of a mixture of $NaNO_2$ (1.76 g, 25.5 mmol) and KI (5.28 g, 31.8 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 10 min, and stirred at room temperature overnight. To the reaction mixture was added saturated $Na_2S_2O_3$ *aq.* and saturated $NaHCO_3$ *aq.* The aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 10/1) to give the title compound (4.86 g, 58%) as a yellow crystal; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92 (3H, t, *J* = 7.3 Hz), 1.61 (2H, qt, *J* = 7.3, 7.6 Hz), 2.60 (2H, t, *J* = 7.6 Hz), 3.56 (3H, s), 4.86 (2H, s), 5.11 (2H, s), 6.36 (1H, dd, *J* = 2.4, 8.6 Hz), 6.52 (1H, d, *J* = 2.4 Hz), 6.78 (1H, d, *J* = 8.9 Hz), 7.31-7.43 (6H, m), 7.47 (1H, s), 7.70 (1H, d, *J* = 8.6 Hz); MS (EI) *m/z* 654 $[M]^+$; Anal. Calcd for $C_{27}H_{25}F_6IO_4$: C, 49.56; H, 3.85. Found: C, 49.74; H, 3.79.

1-(2-(Benzyloxy)-4-(4-(1,1,1,3,3,3-hexafluoro-2-(methoxy-methoxy)propan-2-yl)-2-propylphenoxy)phenyl)ethan-1-one (47c):
To a stirred solution of **39c** (852 mg, 1.3 mmol) in toluene (13 mL) was added ethoxyvinyltributyl tin (2.35 g, 6.5 mmol) and tetrakis(triphenylphosphine)palladium (0) ($Pd(PPh_3)_4$) (300 mg, 0.26 mmol) under an argon atmosphere. The reaction mixture was refluxed for 1 h under an argon atmosphere. The reaction mixture was diluted with 5% HCl at 0 °C, and then stirred overnight at room temperature. The reaction mixture was filtered through a pad of Celite and rinsed with EtOAc. The aqueous layer was extracted with EtOAc. The organic layer was washed with saturated $NaHCO_3$ *aq.* and brine, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 10/1) to give the title compound (348 mg, 47%) as a yellow

oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ0.93 (3H, t, *J* = 7.3 Hz), 1.61 (2H, qt, *J* = 7.3, 7.8 Hz), 2.59 (2H, t, *J* = 7.8 Hz), 2.62 (3H, s), 3.60 (3H, s), 4.90 (2H, s), 5.13 (2H, s), 6.54 (1H, dd, *J* = 2.4, 8.6 Hz), 6.60 (1H, d, *J* = 2.4 Hz), 6.93 (1H, d, *J* = 8.9 Hz), 7.34-7.45 (6H, m), 7.53 (1H, s), 7.83 (1H, d, *J* = 8.6 Hz); MS (EI) *m/z* 570 $[M]^+$; Anal. Calcd for $C_{29}H_{28}F_6O_5$: C, 61.05; H, 4.95. Found: C, 60.91; H, 4.94.

2-(2-(Benzyloxy)-4-(4-(1,1,1,3,3,3-hexafluoro-2-hydroxy-propan-2-yl)-2-*n*-propylphenoxy)phenyl)-2-oxoethyl 4-methyl benzenesulfonate (48d):
To a stirred solution of **47c** (70 mg, 0.12 mmol) in CH_3CN (0.65 mL) was added ((hydroxy)(tosyloxy)iodo)benzene (PhI(OH)OTs) (96 mg, 0.25 mmol). The reaction mixture was refluxed for 6 h and then concentrated *in vacuo*. The residue was diluted with water at 0 °C. The aqueous layer was extracted with EtOAc. The organic layer was washed with brine, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 3/1) to give the title compound (80 mg, 93%) as a yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ0.89 (3H, t, *J* = 7.3 Hz), 1.57 (2H, qt, *J* = 7.3, 7.3 Hz), 2.43 (3H, s), 2.52 (2H, t, *J* = 7.3 Hz), 5.07 (2H, s), 5.14 (2H, s), 6.50-6.54 (2H, m), 6.94 (1H, d, *J* = 8.6 Hz), 7.24-7.43 (7H, m), 7.54 (1H, d, *J* = 8.6 Hz), 7.62 (1H, s), 7.67 (2H, d, *J* = 8.6 Hz), 7.92 (1H, d, *J* = 9.2 Hz); MS (EI) *m/z* 696 $[M]^+$.

5-(1-Methylethoxy)picolinonitrile (52):
To a stirred suspension of 5-hydroxypicolinonitrile (**51**) (616 mg, 5.13 mmol) and K_2CO_3 (1.42 g, 10.3 mmol) in DMF (3.0 mL) was added 2-iodopropane (1.31 g, 7.7 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 5.5 h. To the reaction mixture was added water (3.0 mL). The aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 10/1) to give the title compound (491 mg, 59%) as a yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (6H, d, *J* = 6.2 Hz), 4.66 (1H, sept, *J* = 6.2 Hz), 7.19 (1H, dd, *J* = 2.7, 8.6 Hz), 7.62 (1H, d, *J* = 8.6 Hz), 8.32 (1H, d, *J* = 2.7 Hz); MS (EI) *m/z* 162 $[M]^+$.

1-(5-(1-Methylethoxy)pyridin-2-yl)ethan-1-one (53):
To a stirred solution of **52** (491 mg, 3.0 mmol) in THF (20 mL) was added methylmagnesium bromide (MeMgBr) (0.97 M in THF solution, 9.3 mL, 9.1 mmol) at 0 °C under an argon atmosphere. The reaction mixture was stirred 0 °C for 2 h. To the reaction mixture was added 1 N HCl (10 mL). Then, the reaction mixture was neutralized with saturated $NaHCO_3$ *aq.* at 0 °C. The aqueous layer was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 10/1) to give the title compound (418 mg, 77%) as a yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.40 (6H, d, *J* = 5.9 Hz), 2.68 (3H, s), 4.68 (1H, sept, *J* = 5.9 Hz), 7.22 (1H, dd, *J* = 3.0, 8.6 Hz), 8.03 (1H, d, *J* = 8.6 Hz), 8.28 (1H, d, *J* = 3.0 Hz); MS (EI) *m/z* 179 $[M]^+$.

***Racemic*-5-[5-(1-methylethoxy)pyridin-2-yl]-5-methyl- imidazolidine-2,4-dione ((±)-54):**
To a stirred solution of **53** (417 mg, 2.3 mmol) in EtOH (2.3 mL) was added NaCN (1.02 g, 20.9 mmol), $(NH_4)_2CO_3$ (4.03 g, 41.9 mmol) and water (2.3 mL) at room temperature. The reaction mixture was irradiated using a microwave reactor (Biotage® Initiator) at 100 °C for 1 h. The reaction mixture was concentrated to remove EtOH. The resulting precipitate was filtered off and washed with water. The solid was recrystallized from a mixture solvent of $CHCl_3$ and MeOH (20/1) to give the title compound (504 mg, 87%) as a brown amorphous; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (6H, d, *J* = 6.2 Hz), 1.79 (3H, s), 4.67 (1H, sept, *J* = 6.2 Hz), 7.36 (1H, dd, *J* = 2.7, 8.9 Hz), 7.46 (1H, d, *J* = 8.9 Hz), 8.18 (1H, d, *J* = 2.7 Hz); MS (EI) *m/z* 249 $[M]^+$.

The hydrochloric acid salt of the (±)-**54** was prepared by adding methanolic HCl to a solution of (±)-**54** in MeOH. A sample was recrystallized from MeOH to yield colorless crystals for analysis.

Hydrochloric acid salt of (±)-**54**; mp 222-225 °C; IR (KBr): 3133, 2984, 1752, 1540, 1240, 935 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 1.42 (6H, d, *J* = 5.6 Hz), 1.94 (3H, s), 4.90 (1H, sept, *J* = 5.6 Hz), 8.12 (1H, d, *J* = 9.6 Hz), 8.21 (1H, dd, *J* = 2.8, 9.6 Hz), 8.41 (1H, d, *J* = 2.8 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 21.7 (2C), 24.4, 64.9, 74.6, 127.0, 132.5, 133.6, 145.3, 157.9, 158.4, 175.2; MS (EI) *m/z* 239 $[M^+]$; Anal. Calcd for $C_{12}H_{16}ClN_3O_3$: C, 50.44; H, 5.64; N, 14.71. Found: C, 50.39; H, 5.68; N, 14.53.

Resolution of (±)-hydantoin 54 by HPLC

(±)-Hydantoin **54** was optically separated by HPLC on a CHIRALPAK AY-H column. Each elution was concentrated to give (−)-**54** and (+)-**54** as a colorless amorphous compound. The chiral HPLC conditions to separate (±)-hydantoin are described below.

Analytical chiral HPLC conditions
Column: CHIRALPAK AY-H, 5 µm, 0.46 × 150 mm; Mobile phase: *n*-hexane/*i*-PrOH = 70/30; Flow rate: 1.0 mL/min; Column temperature: 40 °C; Wavelength: 264 nm; Retention time: (−)-form 4.55 min/(+)-form 5.81 min.

Preparative chiral HPLC conditions
Column: CHIRALPAK AY-H, 5 µm, 20 × 250 mm; Mobile phase: *n*-hexane/*i*-PrOH = 70/30; Flow rate: 10 mL/min; Column temperature: 40 °C; Wavelength: 264 nm; Retention time: (−)-form 6.05 min/(+)-form 12.1 min.

Hydrochloric acid salt of (+)-**54**; mp 222-225 °C; MS (EI) *m/z* 249 [M^+]; Anal. Calcd for $C_{12}H_{16}ClN_3O_3$: C, 50.44; H, 5.64; N, 14.71. Found: C, 50.34; H, 5.60; N, 14.92; $[\alpha]_D^{20}$ = +26.5 (c = 1.0, MeOH); Optical purity: >99% ee. The IR, ^{1}H NMR, and ^{13}C NMR spectra of (+)-**54** are identical to those of (±)-**54**

Hydrochloric acid salt of (−)-**54**; mp 222-224 °C; MS (EI) *m/z* 249 [M^+]; Anal. Calcd for $C_{12}H_{16}ClN_3O_3$: C, 50.44; H, 5.64; N, 14.71. Found: C, 50.36; H, 5.60; N, 14.93; $[\alpha]_D^{20}$ = −26.6 (c = 1.0, MeOH); Optical purity: >99% ee. The IR, ^{1}H NMR and ^{13}C NMR spectra of (−)-**54** are identical to those of (±)-**54**.

***Racemic*-3-(2-(2-(Benzyloxy)-4-(4-(1,1,1,3,3,3-hexafluoro-2-hydroxy-propan-2-yl)-2-propylphenoxy)phenyl)-2-oxoethyl)-5-(5-(1-methylethoxy)pyridin-2-yl)-5-methylimidazolidine-2,4-dione (55):**
To a stirred suspension of **54** (20.4 mg, 81.8 μmol) and K_2CO_3 (22.7 mg, 164 μmol) in DMF (160 μL) was added **48d** (28 mg, 40.9 μmol) at room temperature. The reaction mixture was stirred at room temperature overnight. To the reaction mixture was added water. The mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 2/1) to give the title compound (6.5 mg, 21%) as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.88 (3H, t, *J* = 7.6 Hz), 1.36 (6H, d, *J* = 6.5 Hz), 1.54 (2H, qt, *J* = 7.6, 7.8 Hz), 1.87 (3H, s), 2.49 (2H, t, *J* = 7.8 Hz), 4.58 (1H, sept, *J* = 6.5 Hz), 4.87 (2H, s), 5.13 (2H, s), 6.29 (1H, brs), 6.48 (1H, d, *J* = 2.4 Hz), 6.53 (1H, dd, *J* = 2.4, 8.9 Hz), 6.92 (1H, d, *J* = 8.9 Hz), 7.20 (1H, dd, *J* = 2.7, 8.9 Hz), 7.29-7.36 (5H, m), 7.52 (1H, d, *J* = 8.9 Hz), 7.61 (1H, s), 7.64 (1H, d, *J* = 8.9 Hz), 7.97 (1H, d, *J* = 8.9 Hz), 8.21 (1H, d, *J* = 2.7 Hz); MS (EI) *m/z* 772 [$M]^+$.

***Racemic*-3-(2-(4-(4-(1,1,1,3,3,3-Hexafluoro-2-hydroxypropan- 2-yl)-2-propylphenoxy)-2-hydroxyphenyl)-2-oxoethyl)-5-(5-(1-methylethoxy)pyridin-2-yl)-5-methylimidazolidine-2,4-dione ((±)-56):**
To a stirred solution of **55** (3.7 mg, 4.8 μmol) in MeOH (500 μL) was added palladium carbon (1.0 mg). The reaction mixture was stirred for 3 h under a hydrogen atmosphere. The reaction mixture was filtered through a pad of Celite and rinsed with MeOH. The filtrate was concentrated *in vacuo.* The residue was purified by silica gel column chromatography (*n*-hexane/EtOAc = 2/1) to give the title compound (2.6 mg, 80%) as a colorless amorphous; IR (film) 3130, 2985, 1740, 1545, 1240, 934 cm^{-1}; ^{1}H NMR (400 MHz, CD_3OD) δ 0.90 (3H, t, *J* = 7.2 Hz), 1.33 (6H, d, *J* = 6.0 Hz), 1.61 (2H, qt, *J* = 7.2, 7.6 Hz), 1.90 (3H, s), 2.59 (2H, t, *J* = 7.6 Hz), 4.68 (1H, sept, *J* = 6.0 Hz), 4.98 (2H, s), 6.34 (1H, d, *J* = 2.4 Hz), 6.55 (1H, dd, *J* = 2.4, 9.2 Hz), 7.12 (1H, d, *J* = 8.8 Hz), 7.39 (1H, dd, *J* = 2.8, 8.8 Hz), 7.60 (1H, d, *J* = 8.8 Hz), 7.64 (1H, dd, *J* = 2.8, 8.8 Hz), 7.71 (1H, d, *J* = 2.8 Hz), 7.95 (1H, d, *J* = 9.2 Hz), 8.20 (1H, d, *J* = 2.8 Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 14.0, 22.1 (2C), 23.6, 24.3, 33.2, 45.8, 66.5, 71.9, 78.3 (sept, *J* = 29.8 Hz), 105.2, 110.0, 115.2, 122.2, 122.5, 124.2, 124.5 (2C, q, *J* = 285.7 Hz), 127.7, 129.7, 131.2, 133.4, 136.1, 139.6, 150.0, 155.0, 155.5, 158.1, 165.1, 166.1, 177.3, 195.9; MS (EI) *m/z* 683 [$M]^+$.

(−)-3-(2-(4-(4-(1,1,1,3,3,3-Hexafluoro-2-hydroxypropan-2-yl)-2-propylphenoxy)-2-hydroxyphenyl)-2-oxoethyl)-5-(5-(1-methylethoxy)pyridin-2-yl)-5-methylimidazolidine-2,4-dione ((−)-56):
The preparation of **(−)-56** was performed using **(+)-54** in the same procedure as that for racemic **(±)-56**.
MS (EI) *m/z* 683 [M^+]; Anal. Calcd for $C_{32}H_{31}N_3O_7F_6$: C, 56.22; H, 4.57; N, 6.15. Found: C, 56.07; H, 4.61; N, 6.08; $[\alpha]_D^{20}$ = −56.5 (c = 1.0, $CHCl_3$). The IR, ^{1}H NMR and ^{13}C NMR spectra of (−)-**56** were identical to those of (±)-**56**.

(+)-3-(2-(4-(4-(1,1,1,3,3,3-Hexafluoro-2-hydroxypropan-2-yl)-2-propylphenoxy)-2-hydroxyphenyl)-2-oxoethyl)-5-(5-(1-methylethoxy)pyridin-2-yl)-5-methylimidazolidine-2,4-dione ((+)-56):
The preparation of **(+)-56** was performed using **(−)-54** in the same procedure as that for racemic **(±)-56**.
MS (EI) *m/z* 683 [M^+]; Anal. Calcd for $C_{32}H_{31}N_3O_7F_6$: C, 56.22; H, 4.57; N, 6.15. Found: C, 56.06; H, 4.63; N, 6.09; $[\alpha]_D^{20}$ = +56.1 (c = 1.0, $CHCl_3$). The IR, ^{1}H NMR and ^{13}C NMR spectra of (+)-**56** were identical to those of (±)-**56**.

4.2. Pharmacology

4.2.1. *In vitro* study

The CHO K-1 cells stably transfected with LXRs/GAL4 fused protein expression vectors and the GAL4 –responsive reporter vector (pG5luc, Promega, WI) were seeded at 20,000 cells/well on a 96-well plate in HAM-F12 medium containing 10% bovine fetal serum, 100 units/mL of penicillin G, and 100 μg/mL of streptomycin sulfate and incubated in a wet atmosphere with 5% CO_2 at 37 °C. After 24 h, media with various concentrations of the test compound (0.01 μM, 0.1 μM, 1 μM and 10 μM) was added, and the cells incubated for an additional 24 h. We then used Bright-Glo (Promega) as a substrate and measured the luminescence intensity with a luminometer LB960 (Berthold Technologies) to find the effect of the test compound on the activation of luciferase transcription via LXRα- or LXRβ-LBD.

4.2.2. *In vivo* study

Low-density lipoprotein (LDL) receptor knock-out (male, 10 weeks old) mice (n = 60) (Charles River Japan, Inc., Kanagawa, Japan) were used for animal experiments. The animal room was controlled at 23±3 °C and relative humidity of 50±20%. Animals were fed a CE-2 chow diet (CLEA Japan Inc., Tokyo, Japan), followed by supplementation with western diet (TD.88137, Harlan Laboratories, WI, USA) for 10 weeks.

During fat loading, a suspension of (−)-**56** in 0.5% methyl cellulose (MC) was administered orally at 1 or 3 mg/kg/day (n = 15 for each dose group). As a comparative agent, a suspension of T0901317 in 0.5% MC was administered orally at 10 mg/kg/day (n=15 for each dose group). The control group (n = 15) received an aqueous solution of 0.5% MC instead of T0901317. Blood samples were collected to determine plasma lipid levels using a commercially available kit (total cholesterol: Cholesterol E-Test Wako; Wako Pure Chemical Industries, Osaka, Japan, triglyceride: Triglyceride E-Test Wako; Wako Pure Chemical Industries, Osaka, Japan). Plasma lipoprotein profiles were analyzed by CLiP method using a Shimadzu HPLC system (LC-20A) and Superose 6 column (10 mm×300 mm, GE Healthcare, UK). Briefly, 15 μL of plasma was diluted 10-fold in PBS containing 1 mM EDTA. Diluted plasma (20 μL) was separated by the column at 0.5 mL/min with PBS containing 1 mM EDTA and kept at 40 °C for simultaneous determination of cholesterol contents in the eluents.

For analyses of atherosclerotic lesions, the animals were anesthetized by intraperitoneal injection of pentobarbital sodium (50 mg/kg) followed by vascular perfusion for 5 min with saline containing 4% paraformaldehyde at a perfusion pressure of 120 mmH_2O.

The heart was fixed with 4% paraformaldehyde for 24 hrs. Tissues were embedded in OCT compound (Tissue-Tek) and snap frozen in liquid N_2. Cryosections of 10 μm thickness were cut from the end of the aortic sinus. Sections were stained with Oil-Red O for lipid and counterstained with hematoxylin. A positive area was quantified at 600 μm distally using an image analysis system (SP500F; Olympus, Tokyo, Japan).

4.3. Pharmacokinetics

The oral doses of **2** at 100 mg/kg and **(−)-56** at 10 mg/kg were formulated in PEG 400. A solution of **2** and **(−)-56** in PEG 400 were orally administered to a CE-2 chow diet-fed Golden Syrian hamsters. Blood samples (heparin plasma) were collected from a forearm vein 0.5, 1, 2, 3, 4, 6, 8, 10 and 24 h after administration. The drug concentrations of **2** and **(−)-56** in the supernatant was measured by HPLC-LC-MS/MS.

Supplementary Data

Supplementary data (general preparation procedure and the analytical data of all synthetic compounds) associated with this article can be found.

Acknowledgments

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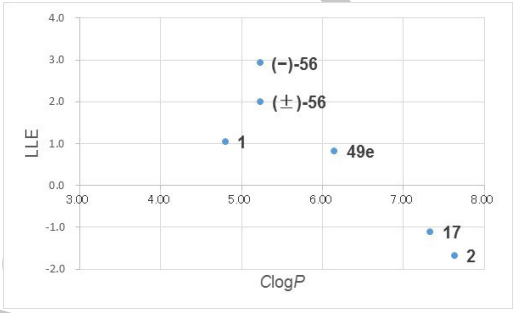
Table 6 The upregulation of ABCA1 mRNA and SREBP-1c mRNA in *in vivo* study with T0901317 and **(−)-56**

Compound	Dose (mg/kg)	ABCA1 in blood (fold)	SREBP-1c in liver (fold)
T0901317	10	2.5*	2.9*
(−)-56	1	2.1*	1.2
	3	3.0*	1.5*

*$p < 0.05$; Statistical analysis was conducted using Dunnett's test. The fold value was calculated relative to control.

35. A reviewer recommended that the authors map the correlation between the *C*Log*P* and the lipophilic ligand efficiency (LLE) values. This correlation analysis would help improve the understanding of the lipophilic binding contribution.



*LLE = pEC_{50}-CLog*P*

Graphical Abstract

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Discovery of a 2-hydroxyacetophenone derivative as an outstanding linker to enhance potency and β-selectivity of liver X receptor agonist

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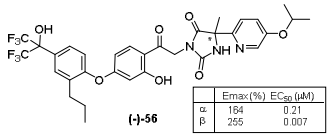

(-)-56

	Emax (%)	EC_{50} (μM)
α	164	0.21
β	265	0.007

a