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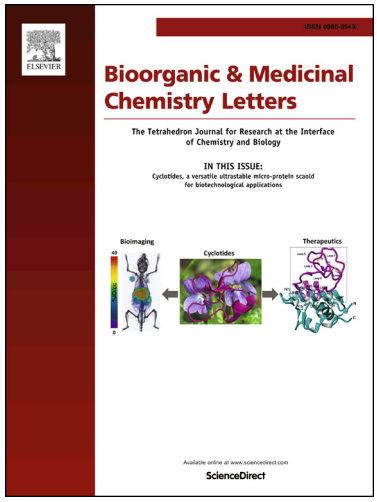

Structural Development of Tetrachlorophthalimides as Liver X Receptor β (LXRβ)-Selective Agonists with Improved Aqueous Solubility

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Structural Development of Tetrachlorophthalimides as Liver X Receptor β (LXRβ)-Selective Agonists with Improved Aqueous Solubility

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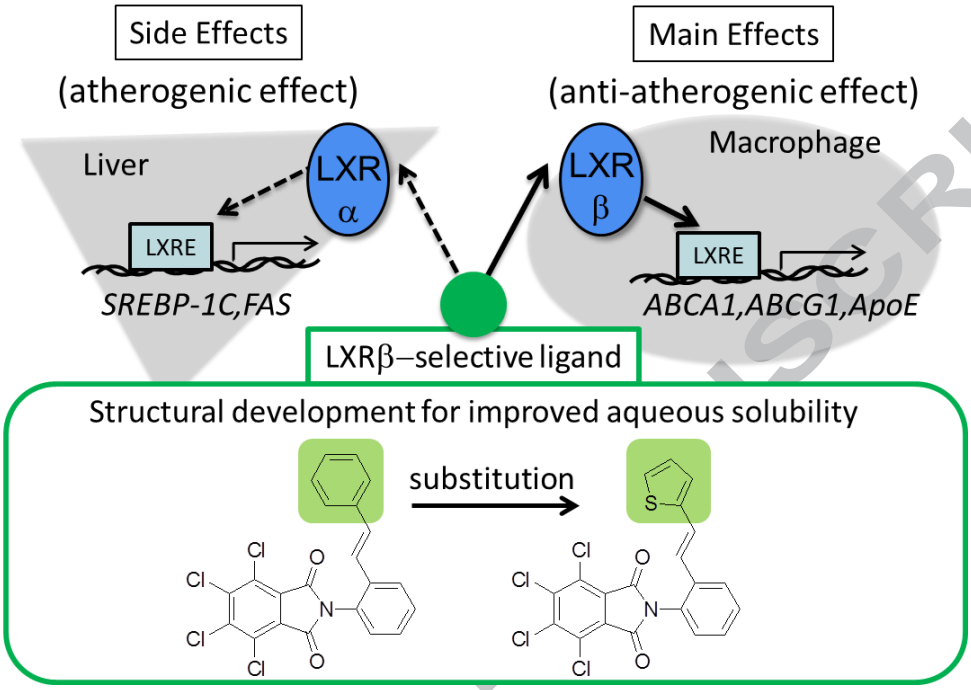


Abstract

LXRβ-selective agonists are promising candidates to improve atherosclerosis without increasing plasma or hepatic TG levels. We have reported a series of tetrachlorophthalimide analogs as an LXRβ-selective agonist. However, they exhibited poor aqueous solubility probably due to its high hydrophobicity and highly rigid and plane structure. In this report, we present further structural development of tetrachloro(styrylphenyl)phthalimides as the LXRβ-selective agonists with improved aqueous solubility.

Liver X receptors (LXRs) are members of the nuclear receptor (NR) superfamily,[1,2] and ligand-dependent transcription factors. The physiological ligands for LXRα/β are oxysterols, including 22(*R*)-hydroxycholesterol (**1**) and 24(*S*),25-epoxycholesterol (**2**) (Figure 1).[3] Upon binding of an agonist to the ligand-binding domain (LBD) of LXR, gene transcription occurs. The products of LXR-regulated genes, such as *ABCA1*, *ABCG1*, *ABCG5*, *ABCG8*, *ApoE* and *GLUT4*[4-6] are involved in lipid metabolism, reverse cholesterol transport,[7] and glucose transport, so LXRs are considered to be potential drug targets for atherosclerosis, hyperlipidemia, and metabolic syndrome.[8] However, LXRs agonists also induce genes involved in lipogenesis, such as *SREBP-1c* (sterol regulatory binding element protein 1c)[9] and *FAS* (fatty acid synthase),[8] resulting in increased plasma and hepatic triglyceride levels,[10] which in turn might lead to fatty liver and atherosclerosis as possible side effects.

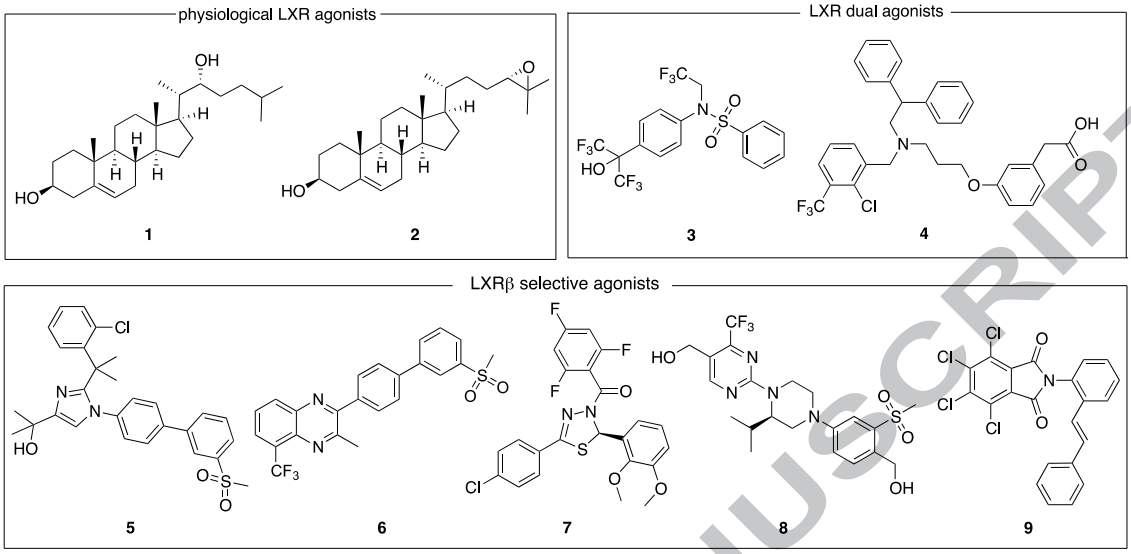
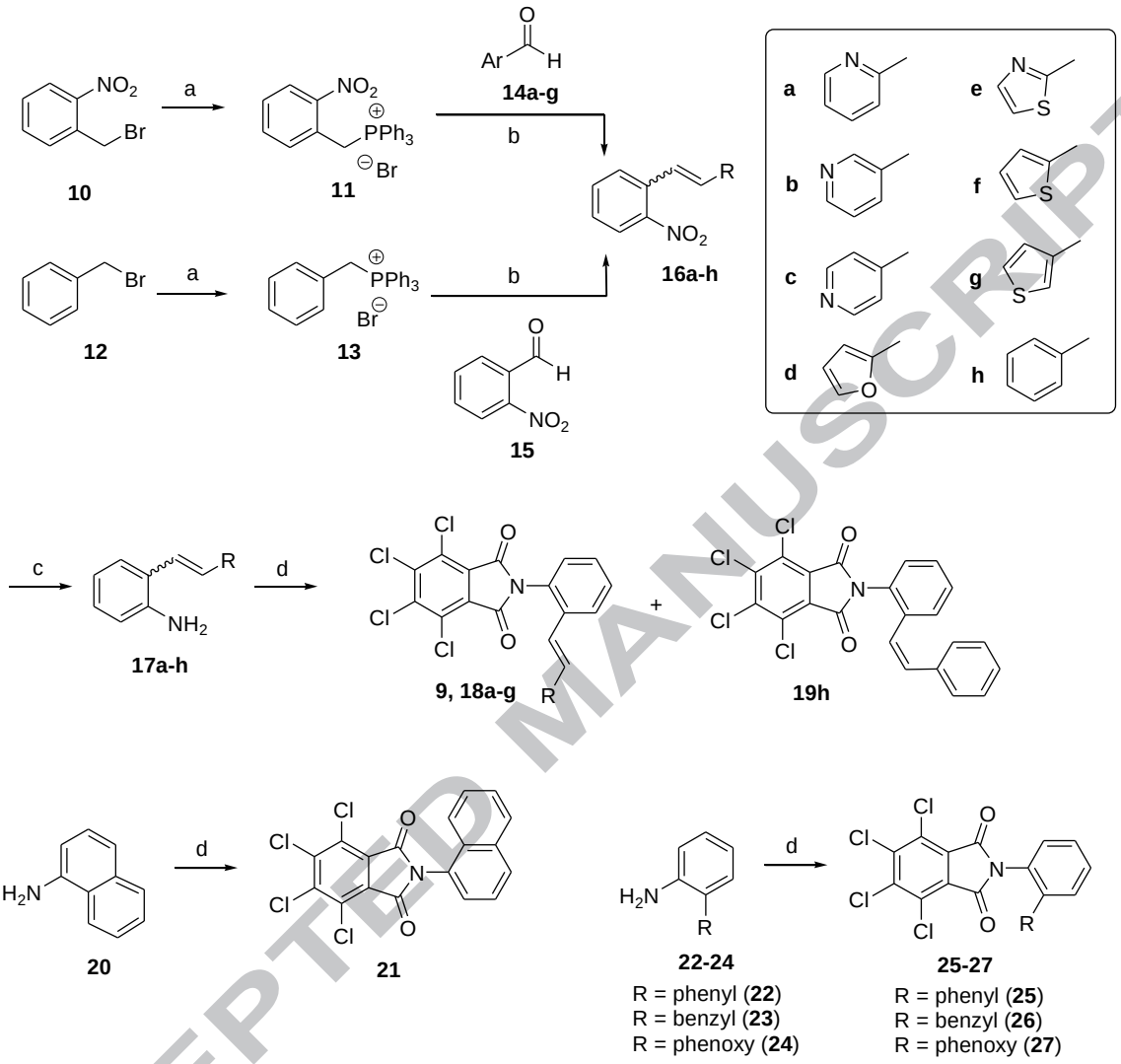


Figure 1. Chemical structures of LXR agonists

LXRs include two subtypes with different tissue distribution, LXRα and LXRβ. LXRα is highly expressed in liver, intestine and macrophages, while LXRβ has a more widespread pattern of expression, being almost ubiquitous. LXRα contributes to lipogenesis in liver, while selective LXRβ activation improves RCT in LXRα-knockout mouse.[11,12] Therefore, LXRβ-selective agonists are expected to improve atherosclerosis via induction of RCT and cholesterol efflux from liver, without increasing plasma or hepatic TG levels. However, LXRα and LXRβ are highly related and share 78% amino acid sequence identity in the ligand-binding domains (LBDs), especially in the vicinity of the ligand-binding pocket.[13] Consequently, most LXR ligands, including T0901317

(**3**)[1] and GW3965 (**4**)[14,15] do not show subtype selectivity.

To date, a few LXRβ-selective agonists **5-8** have been reported (Figure 1).[16] During our continual research of LXR ligands,[17] we have also found that **9** exhibited >100-fold selective LXRβ agonistic activity in a full-length LXRβ reporter gene assay system.[18] Compound **9** showed high selectivity over other NRs, and induced only *ABCA1* mRNA expression but not *SREBP-1c* mRNA expression. However, **9** exhibited poor aqueous solubility. In this report, we present further structural development of tetrachloro(styrylphenyl)phthalimides as the LXRβ-selective agonists with improved aqueous solubility.



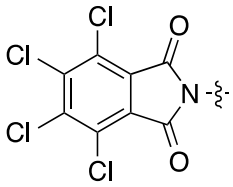
Scheme 1. Reagents and conditions: (a) PPh_3, CH_3CN, reflux; (b) benzaldehydes, 18-crown-6, K_2CO_3, DCM, reflux; (c) $SnCl_2 \cdot 2H_2O$, AcOEt, reflux; (d) tetrachlorophthalic anhydride, AcOH, reflux.

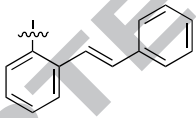
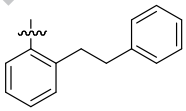
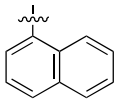
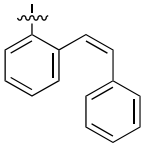
Styrylphenylphthalimide analogs were synthesized as shown in Scheme 1. Benzyl bromides **10**, **11** were treated with PPh_3 to generate phosphonium ylides **11**, **13**. Wittig

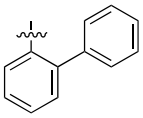
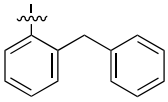
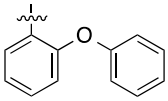
reaction of ylides **11** and aldehydes **14a-g**, and ylide **13** and aldehyde **15** afforded *ortho*-stilbenes **16a-h**. Reduction of the nitro group of **16a-h** with $SnCl_2 \cdot 2H_2O$, cyclization with tetracholorophthalic anhydride, and separation of the *EZ* isomers gave the *E*-isomers **9** and **18a-g, and** *Z*-isomer **19h**. Various amines **20**, **22-24** were cyclized with tetracholorophthalic anhydride to give **21, 25-27**.

Our previous SAR studies indicated that chloro atoms at phthalimide are necessary for selective LXRβ agonistic activity. In addition, introduction of various substituents or changing position of methoxy substituent of the terminal benzene ring at styryl group did not lead to improve LXRβ agonistic activity. These results indicated that substituent(s) at the terminal benzene ring would interfere binding to LXRβ binding pocket because of its bulkiness. Therefore, other approaches except for introduction of substituent(s) are required for further structural development. On the other hand, the % efficacy for LXRβ varied depending on the space that was occupied by the terminal benzene ring. These results led us to change the space that was occupied by the terminal benzene ring by changing the linker.

Table 1. SAR for the suitable spatial arrangement of terminal benzene ring



Compound	Ar	agonistic activity		
		LXRα	LXRβ	
		EC_{50} (μM)[a]	EC_{50} (μM)[a]	% efficacy[b]
3		0.006	0.021	100
9		N.A.	0.27±0.02	143
28		N.A.	4.98±0.29	117
21		N.A.	N.A.	-
19h		N.A.	>10	104[c]

25		N.A.	1.73±0.78	153
26		N.A.	8.00±0.59	109
27		N.A.	8.98±1.39	99

[a] N.A.: no activity at 30 μM; data are the mean±SD.

[b] % efficacy:percent of maximal activity relative to **3**.

[c] % efficacy at 30 μM

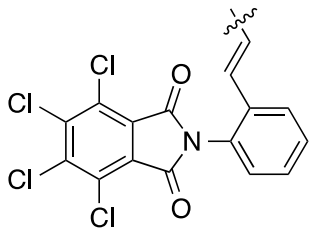
We fixed the non-substituted terminal phenyl group, and synthesized analogs with C0-C2 linker length to examine the suitable spatial arrangement of terminal benzene ring. Naphthyl analog **25** and (*Z*)-styrylphenyl analog **19h** lacked LXRβ agonistic activity indicating that LXRβ would not have enough space near *meta*-position. This hypothesis was supported by our previous SAR that *meta*-phenethyl analog lacked LXRβ agonistic activity. As for analogues possessing C0-C2 linker (**25**, **26** and **28**), the order of EC_{50} value was C0 (**25**) < C2 (**28**) < C1 (**26**). However, (*E*)-styrylphenyl analog **9** showed more potent EC_{50} than biphenyl analog **25**. On the other hand, linker with heteroatom (**27**) showed less potent EC_{50} and % efficacy than the carba-analog **26**.

This result might suggest that hetero linker does not contribute to LXRβ agonistic activity. These results mentioned above led us to fix the spacer as (*E*)-vinyl linker.

Second approach for structural development was to substitute the terminal benzene ring with hetero aromatic rings to improve aqueous solubility. Compared to pyridine analogues (**18a**-**18c**), 2-pyridinyl (**18a**) > 3-pyridinyl (**18b**) > 4-pyridinyl (**18c**) analogues showed potent activity (EC_{50}) and % efficacy in the indicated order, but these analogues were weaker than phenyl analogue (**9**). We also synthesized analogs bearing five-membered heterocycles **18d-g**. This idea based on our previous reports which showed the disruption of molecular symmetry (bending molecules) can increase the aqueous solubility of molecules even if their hydrophobicity is not concomitantly increased.[19,20] 2-Furyl (**18d**), 2-thiazolyl (**18e**) analogues also exhibited weaker LXRβ agonistic activity than compound **9**. These results suggest that decrease of hydrophobicity of molecules by introduction of the heteroatom(s) decreased LXRβ agonistic activity. Then, we hypothesized that substitution of the terminal phenyl ring with a heterocycle possessing higher hydrophobicity might maintain LXRβ agonistic

activity. Actually, 2-thienyl (**18f**) and 3-thienyl (**18g**) analogues, especially, **18f** exhibited potent activity (EC_{50}: 0.559 μM, 149% efficacy) close to phenyl analogue (**9**). Next, we analyzed the solubility of compound **9** and **18f** in EtOH: 1/15 M phosphate buffer (pH7.4) 1:1 by HPLC. Compound **18f** showed about 4 times improved solubility (0.015 ug/mL) over **9** (0.0036 ug/mL). Melting point of **18f** (257-258ºC) was lower than that of **9** (264-265ºC). This data support our idea that converting the terminal benzene ring to five-membered hetero aromatic ring could disrupt the molecular symmetry that increase the improvement of aqueous solubility.

Table 2. SAR of the terminal hetero aromatic rings



		agonistic activity			cLogP[d]
Compound	Ar	LXRα	LXRβ		
		EC_{50} (μM)[a]	EC_{50} (μM)[a]	% efficacy[b]	
9		N.A.	0.27±0.02	143	7.0

18a		N.A.	4.77±2.51	148	5.6
18b		N.A.	4.96±2.21	141	5.7
18c		N.A.	>10	35[c]	5.5
18d		N.A.	4.67±1.53	167	6.6
18e		N.A.	3.61±0.89	151	5.2
18f		N.A.	0.559±0.12	149	6.8
18g		N.A.	0.915±0.02	133	6.8

[a] N.A.: no activity at 30 μm; data are the mean:SD.

[b] % efficacy:percent of maximal activity relative to **3**.

[c] % efficacy at 30 μM

[d] ACD/Chem sketch 2012 (ver 14.01)

To understand the relationships between LXRβ agonistic activity and hydrophobicity of the compounds, CLogP and $-\text{Log}EC_{50}$ of the compounds shown in Table 2 was plotted. As shown in Figure 2, CLogP and LXRβ agonistic activity showed a high correlation

(R^2 = 0.63). This result might indicate that the binding pocket of LXRβ occupied with the terminal aromatic ring is hydrophobic character.

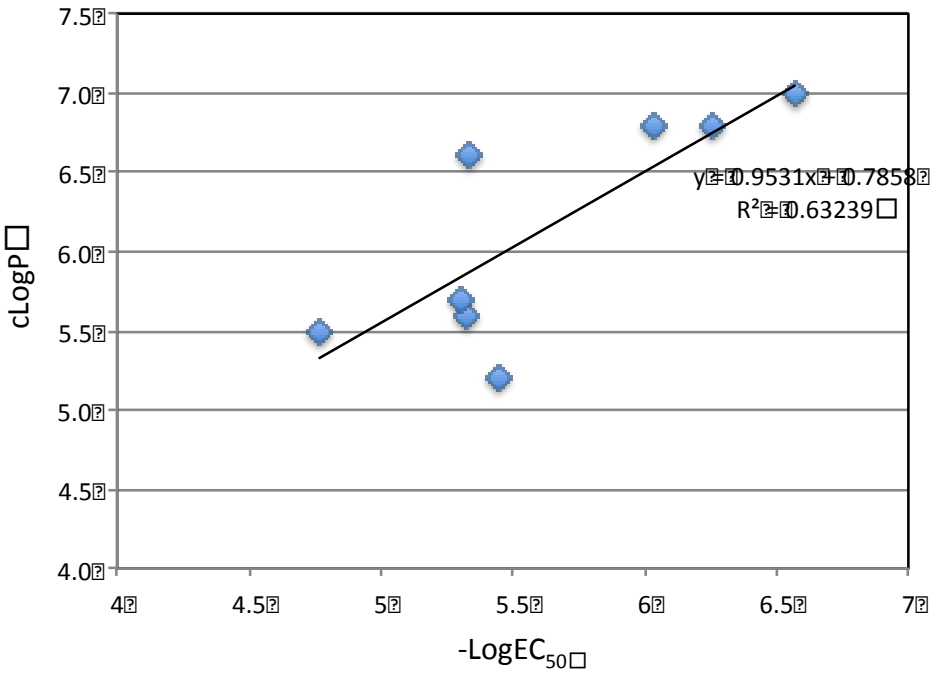
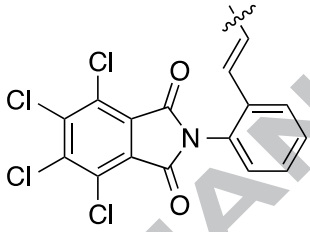


Figure 2. Relationships between LXRβ agonistic activity and hydrophobicity.

Next, we investigated further biological studies whether **18f** exhibit similar activity to parent **9**. First, we considered the difference of agonistic activities towards human and mouse LXRs by means of mouse full-length LXRs reporter gene assay. Compounds **26** and **27** showed 3-fold weaker LXRβ-agonistic activity than **9** but showed no

LXRα-agonistic activity at 30 μM. And % efficacies of **18f** and **18g** against LXRβ were similar to that of **9**. Thus, **9**, **18f** and **18g** are LXRβ selective agonists for both human and mouse.

Table 3. Selectivity for LXRβ in mouse LXRs.



	agonistic activity		
	mouse LXRα	mouse LXRβ	
Compound	EC_{50} (μM)[a]	EC_{50} (μM)[a]	% efficacy[b]
9	N.A.	0.30±0.01	26
18f	N.A.	0.79±0.18	24
18g	N.A.	0.82±0.09	23
18e	N.A.	2.93±0.15	22

[a] N.A.: no activity at 30 μm; data are the mean:SD.
[b] % efficacy:percent of maximal activity relative to **3**.

The selectivity of **18f** over other NRs (PPARα/γ, RARα/β/γ, RXRα/β/γ, FXR) was evaluated. Compound **18f** showed weak agonistic activity towards FXR (9% efficacy)

and RARα (6% efficacy), but did not show activity towards other NRs. (supplementary Figure S1).

We next investigated whether **18f** binds directly to LXRα/β LBD as **9** does by means of TR-FRET assay[18] (Figure 3). Compound **18f** showed dose-dependent LXRβ-partial agonistic activity (EC_{50} = 40.4 nM, 14% efficacy compared to T0901317 (**3**) at 3 μM). On the other hand, compound **18f** showed very weak agonistic activity towards LXRα (6% efficacy at 0.3 μM compared to T0901317 (**3**) 3 μM). These EC_{50} values and % efficacies were similar to those of **9**. These results may indicate that **18f** binds directly to both the LXRβ LBD and LXRα LBD, but recruits the coactivator peptide preferentially to LXRβ rather than LXRα, at least under our conditions. There is another possibility that T0901317 (**3**) and **18f** recruit the different coactivators. In that case, **18f** could show the partial agonistic activity in TR-FRET assay, whereas the full agonistic activity in reporter gene assay. The difference of recruited coactivators may cause the selective activation of the target gene transcription.

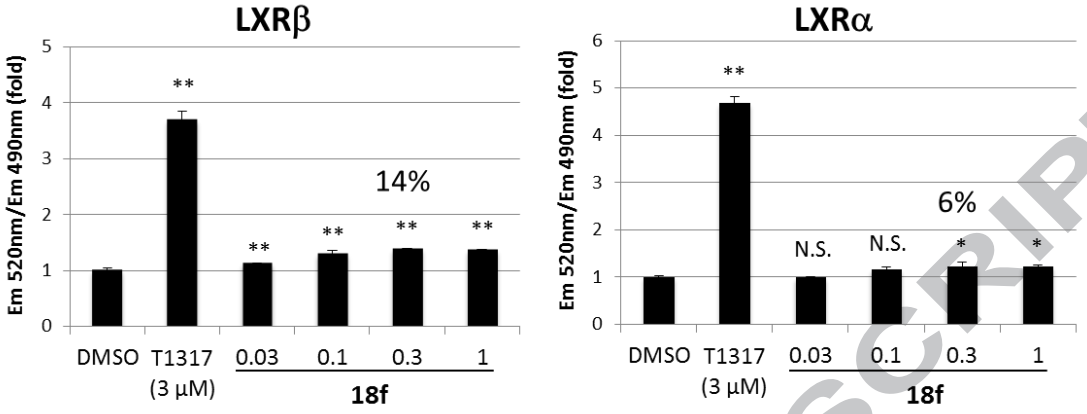


Figure 3. Results of TR-FRET LXRα/β binding assays. Data are the mean ± SD. The binding efficacy was compared using an unpaired, two-sided Student's t-test. The P-value is indicated by asterisk *p<0.05, **p<0.01, N.S.=not significant relative to DMSO control. %eff was calculated by comparison to T0901317.

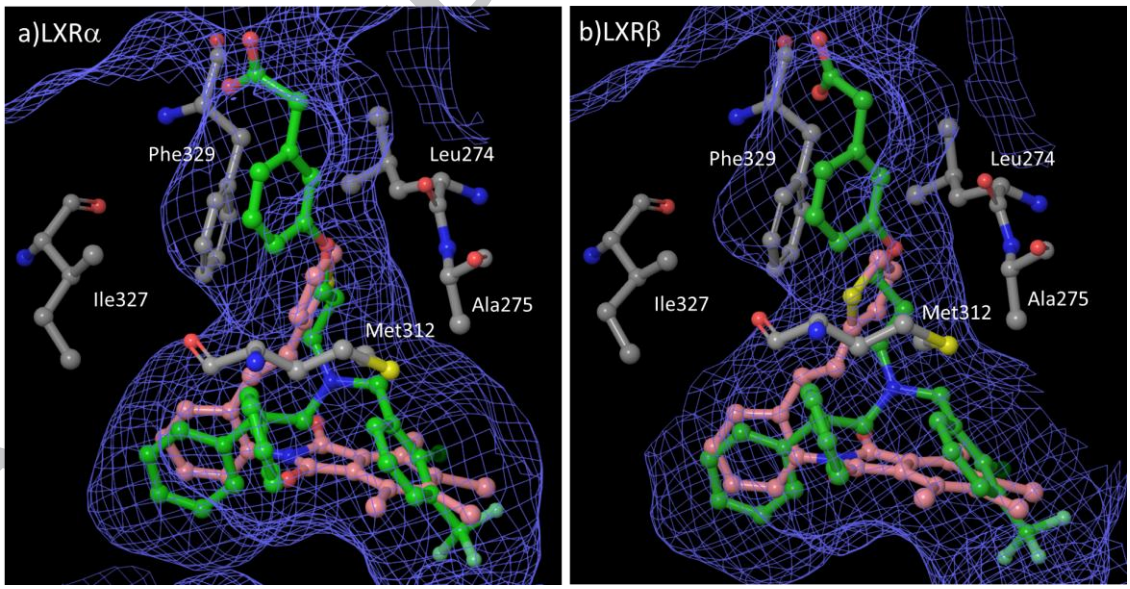


Figure 4. Docking simulation of LXRs and **18f**. Compound **18f** (pink) was docked into the X-ray crystal structures of complexes between GW3965 (**4**, green) and the LBDs (violet mesh) of a) LXRα (PDB ID: 3IPQ) and b) LXRβ (PDB ID: 1PQ6).

To understand the binding mode between **18f** and LXR, **18f** was computationally docked into the cocrystal structures of LXRα LBD and LXRβ LBD taken from the complexes with GW3965 (**4**) (Figure 4). The most favorable conformation had a free energy of binding of -12.02 kcal/mol (LXRα) or -11.62 kcal/mol (LXRβ), indicating that **26** would bind to both LXRs. In addition, the results indicated that **26** would bind similarly to the binding pockets of both LXRs but the thiophene ring in **26** faced to the opposite direction, which might cause the selectivity. These results were also consistent with the idea that the LXRβ selective agonistic activity of **26** might be a result of post-binding conformation change or differential coactivator recruitment, rather than binding preference.[18] Our SARs indicated that the hydrophobic terminal aromatic ring would be suitable for LXRβ agonistic activity. The amino acids located near the bound thiophene ring in **26** were also hydrophobic character, that is, Leu274, Ala275, Met312, Ile327 and Phe329. Thus, the docking study supported this SAR, and the hydrophobic terminal aromatic ring might be able to have hydrophobic interaction with these hydrophobic amino acids.

We next examined the agonistic activity of **18f** by means of mRNA expression analysis of *ABCA1* and *SREBP-1c*. In THP-1 cells, **18f** induced expression of only *ABCA1* mRNA, but not *SREBP-1c* mRNA expression. Compound **18f** showed more potent activity than our previous reported **9** in 1 μM treatment, which reflect the result of %eff of reporter gene assay (Figure 5). This is consistent with the idea that **18f** works as an LXRβ specific agonist which would improve atherosclerosis without lipogenic side effects.

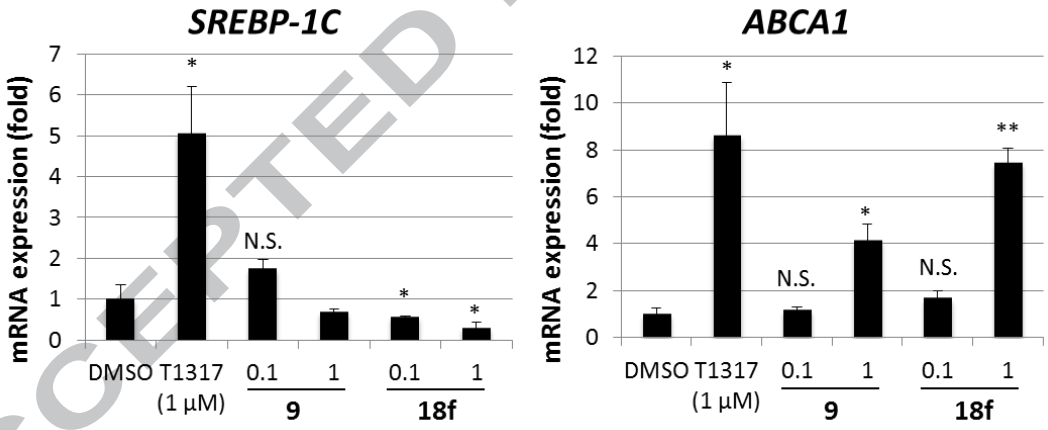


Figure 4. Real-time PCR analysis of *ABCA1* and *SREBP-1c* mRNA expression in THP-1 cells. Data are the mean ± SD. The mRNA level was compared using an unpaired, two-sided Student's t-test. The P-value is indicated by asterisk *p<0.05, **p<0.01, N.S.=not significant relative to DMSO control.

In summary, we achieved further structural development of tetrachloro(styrylphenyl)phthalimides as the LXRβ-selective agonists with improved

aqueous solubility compared to compound **9** which we have reported. 2-Thienyl analogue **18f** exhibited potent LXRβ selective agonistic activity (EC_{50}: 0.559 μM, 149% efficacy) close to phenyl analogue **9** and similar biological identity in several assays. In addition, Compound **18f** showed about 4 times improved solubility (0.015 μg/mL) over **9** (0.0036 μg/mL). Then, Compound **18f** could to be a promising lead compound for the development of agents to treat atherosclerosis without the side effects.

References and notes

(1) Annicotte JS, Schoonjans K, Auwerx J. *Anat. Rec., Part A.* 2004;277A;312-316.

(2) Schultz JR, Tu H, Luk A, et al. *Genes Dev*. 2000;14;2831-2838.

(3) (a) Janowski BA, Grogan MJ, Jones SA, et al. *Proc. Natl. Acad. Sci. U.S.A.* 1999;96;266-271.
(b) Lehmann JM, Kliewer SA, Moore LB, et al. *J. Biol. Chem*. 1997;272;3137-3140.

(4) Venkateswaran A, Laffitte BA, Joseph SB, et al. *Proc. Natl. Acad. Sci. U.S.A.* 2000;97;12097-12102.

(5) Kennedy MA, Venkateswaran A, Tarr PT, et al. *J. Biol. Chem.* 2002;277;17375.

(6) Repa JJ, Berge KE, Pomajzl C, et al. *J. Biol. Chem.* 2002;277;18793-18800.

(7) Zhao C, Dahlman-Wright K. *J. Endocrinol.* 2010;204;233-240.

(8) Joseph SB, Laffitte BA, Patel PH, et al. *J. Biol. Chem.* 2002;277;11019-11025.

(9) Repa JJ, Mangelsdorf DJ. *Nat. Med.* 2002;8;1243-1248.

(10) Schultz JR, Tu H, Luk A,et al. *Genes Dev.* 2000;14;2831-2838.

(11) Bradley MN, Hong C, Chen M, et al. *Clin. Invest.* 2007;117;2337-2346.

(12) Lund EG, Peterson LB, Adams AD, et al. *Biochem. Pharmacol.* 2006;71;453-463.

(13) (a) Faernegardh M, Bonn T, Sun S, et al. *J. Biol. Chem.* 2003;278;38821-38828.
(b) Williams S, Bledsoe RK, Collin J, et al. *J. Biol. Chem.* 2003;278;27138-27143.

(14) Collins JL, Fivush AM, Watson MA, et al. *J. Med. Chem.* 2002;45;1963-1966.

(15) Joseph SB, McKilligin E, Pei L, et al. *Proc. Natl. Acad. Sci. U.S.A* 2002;99;7604-7609.

(16) (a) Kick E, Martin R, Xie Y, et al. *Bioorg. Med. Chem. Lett.* 2015;25;372-377. (b) Hu B, Unwalla RJ, Goljer I, et al. *J. Med. Chem.* 2010;53;3296. (c) Molteni, V, Li X, Nabakka J, et al. *J. Med. Chem.* 2007;50;4255-4259. (d) Zheng Y, Zhuang L, Fan KY, et al. *J. Med. Chem.* 2016;59;3264-3271.

(17) (a) Noguchi-Yachide T, Miyachi H, Aoyama H, et al. *Chem. Pharm. Bull.* 2007;55;12;1750-1754. (b) Motoshima K, Noguchi-Yachide T, Sugita K, et al. *Bioorg. Med. Chem.* 2009;17;14;5001-5014. (c) Aoyama A, Endo-Umeda K, Kishida K, et al. *J. Med. Chem.* 2012;55;7360-7377. (d) Nomura, S., Endo-Umeda, K, Aoyama A, Makishima M, et al. *ACS Med. Chem. Lett.* 2015;6; 902-907.

18 Nomura S, Endo-Umeda K, Makishima M, Hashimoto Y, Ishikawa M. ChemMedChem. 2016;11;2347-2360.

19 Ishikawa M, Hashimoto Y. *J. Med. Chem.* 2011;54;1539–1554.

20 Ishikawa M, Hashimoto Y. *in Pract. Med. Chem.* (Eds.: C. G. Wermuth, D. Aldous, P. Raboisson, D. Rognan), Academic Press, Massachusetts, 2015;pp;747–765.