Structural Development of Liver X Receptor (LXR) Antagonists Derived from Thalidomide-Related Glucosidase Inhibitors

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Following our previous discovery of LXR antagonistic activity of 2′-substituted phenylphthalimides derived from thalidomide-related glucosidase inhibitors, structure–activity studies and further structural development led to 5-chloro-*N*-2′-*n*-pentylphenyl-1,3-dithiophthalimide (5CPPSS-50), with IC_{50} values of about 10 and 13 μM for LXRα and LXRβ, respectively.

Key words liver X receptor; thalidomide; phthalimide skeleton; antagonist; glucosidase inhibitor

Nuclear receptors (NRs) are ligand-dependent transcription factors which regulate the expression of responsive genes and thereby affect diverse processes, including cell growth, development, differentiation and metabolism.[1] Based on the elucidated human genome sequence, 48 NRs are thought to exist in humans.[1] NRs are divided into four subfamilies, including (1) classical steroid hormone receptors, (2) retinoid/vitamin D_3/thyroid hormone receptors, (3) metabolic receptors, and (4) orphan receptors. So far, the ligands of only 20—25 of them have been identified, including (1) classical steroid hormone receptors [estrogen receptors (ERs)/estradiol, progesterone receptor (PR)/progesterone, androgen receptor (AR)/testosterone, glucocorticoid receptor (GR)/cortisone, and mineral corticoid receptor (MR)/aldosterone], (2) retinoid/vitamin D_3/thyroid hormone receptors [retinoic acid receptors (RARs)/all *trans*-retinoic acid and retinoid X receptors (RXRs)/9-*cis* retinoic acid, thyroid hormone receptors (TRs)/thyroxine, vitamin D receptor (VDR)/1,25-dihydroxyvitamin D_3], and (3) metabolic receptors [peroxisome proliferator-activated receptors (PPARs)/fatty acid, liver X receptor (LXRs)/oxysterol, farnesoid X receptor (FXR)/bile acid, and steroid xenobiotic receptor (SXR)/steroids].[1] The LXRs are members of the metabolic receptor subfamily of the NR superfamily, and consist of two subtypes, LXRα and LXRβ. The physiological ligands of LXRs are considered to be oxysterols, such as 24(*S*),25-epoxycholesterol (**1**, EPC),[1—3] and several synthetic agonists, including GW3965 (**2**) and T0901317 (**3**), have been reported (Fig. 1).[4,5]

LXRs function as heterodimers with other nuclear receptors, the retinoid X receptors (RXRα, RXRβ and RXRγ), to regulate important aspects of cholesterol homeostasis by controlling expression of their target genes, including ATP binding cassette ABCA1 and CYP7A genes.[6,7] LXRs also regulate the expression of several genes involved in glucose metabolism.[8,9] Thus, LXRs have been regarded as members of the metabolic subfamily of nuclear receptors, participating in the regulation of both lipid and sugar metabolism. Mitro *et al.* reported that LXRs act as glucose sensors, *i.e.*, D-glucose and D-glucose-6-phosphate act as ligands for LXRs and activate their transcription activity with EC_{50} values of 3141 μM for LXRα and 308 μM for LXRβ.[10] This finding indicates that LXRs recognize both oxysterols and glucose derivatives as their physiological ligands.

We have been engaged in structural development studies of thalidomide, a drug first launched as a sedative/hypnotic agent, but withdrawn from the market because of its severe teratogenicity, focusing on its potential for the treatment of a range of diseases, including cancers, diabetes, and rheumatoid arthritis.[11—15] We have developed a series of potent α-glucosidase inhibitors, including CP0P (**4**), CP4P (**5**) and PPS-33 (**6**) (Fig. 2).[11—18] CP0P (**4**) is a non-competitive inhibitor of α-glucosidase, whereas CP4P (**5**) is a competitive inhibitor.[16—18] PPS-33 (**6**) is another competitive α-glucosidase inhibitor, but it also inhibits other enzymes, including maltase and dipeptidylpeptidase type IV.[19] The competitive inhibition of α-glucosidase by CP4P (**5**) and PPS-33 (**6**) indicated that these compounds might be structural mimics of glucose. On this basis, we found that CP4P (**5**) and PPS-33 (**6**) act as antagonists for LXRs.[20] Our former structural development studies based on CP4P (**5**) suggested that 2′-hydrophobic substituents enhance the LXR antagonistic activity of the compounds, and PP2P (**7**) and PP-60 (**8**) have been reported to be moderate LXR antagonists.[20]

In this paper, we describe the structure–activity relationship of the 2′-alkyl group and further structural development of LXR antagonists based on the *N*-2′-alkylphenylphthalimide skeleton.

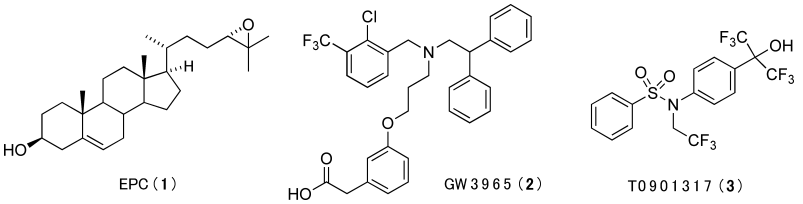

Fig. 1. Structures of Known LXR Agonists, EPC (**1**), GW3965 (**2**) and T0901317 (**3**)[1—5]

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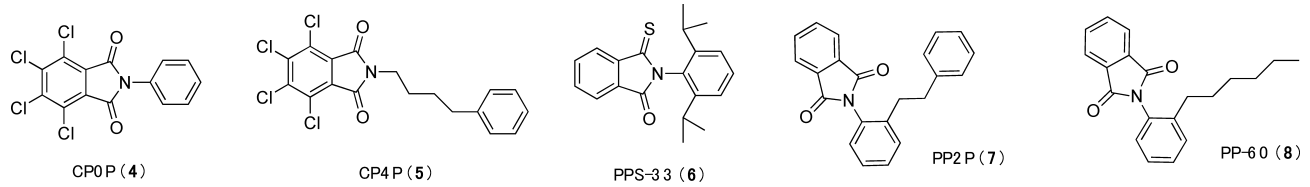
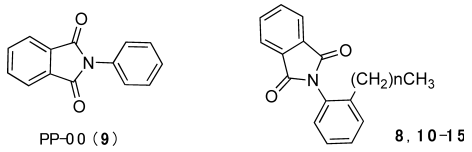


Fig. 2. Structures of Thalidomide-Related α-Glucosidase Inhibitors with (**5**, **6**) or without (**4**) LXR Antagonistic Activity[10—12] and LXR Antagonists (**7**, **8**) Derived from These α-Glucosidase Inhibitors[20]

Table 1. LXR Antagonistic Activities of 2′-Alkylated Phenylphthalimides (**8**—**15**)



	n	% Inhibition at 100 μM	
		LXRα	LXRβ
PP-00 (**9**)	—	29	33
PP-10 (**10**)	0	26	31
PP-20 (**11**)	1	42	29
PP-30 (**12**)	2	50	44
PP-40 (**13**)	3	64	52
PP-50 (**14**)	4	89	81
PP-60 (**8**)	5	88	75
PP-70 (**15**)	6	88	77

Effect of the 2′-Alkyl Group As previously reported, PP-60 (**8**) shows moderate antagonistic activity toward LXRs, while the corresponding non-substituted phenylpthalimide (PP-00: **9**) has only very weak activity.[20] To find the optimum alkyl chain length of the 2′-alkyl substituent, we prepared derivatives with different alkyl chain length, *i.e.*, PP-10 (**10**), PP-20 (**11**), PP-30 (**12**), PP-40 (**13**), PP-50 (**14**) and PP-70 (**15**) (Table 1). All of the compounds were prepared by condensation of an appropriate *o*-alkylaniline with phthalic anhydride. The LXR antagonistic activity of the prepared compounds was evaluated using a reporter gene assay method with CMX-GAL4N-hLXR as the recombinant receptor gene, TK-MH100x4-LUC as the reporter gene and the CMX β-galactosidase gene for normalization, as previously reported,[21—23] and the results are shown in Table 1. None of the prepared compounds exhibited any LXR agonistic activity (data not shown).

As shown in Table 1, the effect of 2′-alkyl chain length on the LXR antagonistic activity of the compounds was clear. The compounds with no alkyl group or an alkyl group shorter than an ethyl group, *i.e.*, PP-00 (**9**) and PP-10 (**10**), showed only very weak antagonistic activity toward both LXRα and LXRβ. The ethyl analog, PP-20 (**11**), showed also very weak antagonistic activity toward LXRβ, but it had moderate antagonistic activity toward LXRα. The antagonistic activity of compounds with a 2′-alkyl chain longer than a methyl group increased in the order of: PP-20 (**11**)<PP-30 (**12**)<PP-40 (**13**)<PP-50 (**14**), for both LXRα and LXRβ. Further elongation of the 2′-alkyl chain, *i.e.*, PP-60 (**8**) and PP-70 (**15**), scarcely affected (in the case of LXRα), or seemed to slightly decrease (for LXRβ) the activity. Thus, the 2′-*n*-pentyl group [PP-50 (**14**)] seemed to be the best substituent for LXR antagonistic activity. The substituent effect on antagonistic activity seemed to be greater for LXRα than for LXRβ. The IC_{50} values of PP-50 (**14**) were calculated to be 42—45 μM and 69—82 μM for LXRα and LXRβ, respectively (Fig. 4, Table 2).

Table 2. IC_{50} Values of PP-50 (**14**), 5CPP-50 (**20**), PPS-50 (**21**), PPSS-50 (**22**) and 5CPPSS-50 (**23**) for LXR Antagonistic Activity (μM)

	PP-50 (**14**)	5CPP-50 (**20**)	PPS-50 (**21**)	PPSS-50 (**22**)	5CPPSS-50 (**23**)
LXRα	42—45	23—25	24—28	26—28	9.9—10
LXRβ	69—82	41—44	67—71	31—40	12—14

Effects of 5-Chloro Substitution and Thiocarbonylation Our previous and preliminary results, *i.e.*, (i) 5CP4P (**16**) possesses more potent antagonistic activity toward both LXRα and LXRβ than 56CP4P (**17**) or CP4P (**18**), and (ii) PPS-33 (**6**) is a moderately potent LXR antagonist while PP-33 (**19**) is not (Fig. 3), suggested that 5-chlorination and/or thiocarbonylation of the phthalimide moiety enhance the activity.[20] Therefore, we examined the effects of 5-chlorination and/or thiocarbonylation of PP-50 (**14**), *i.e.*, 5CPP-50 (**20**), PPS-50 (**21**), PPSS-50 (**22**), and 5CPPSS-50 (**23**) (Fig. 3). All of the compounds were prepared by usual organic synthetic methods, and their LXR antagonistic activity was evaluated as described in Experimental. None of these compounds showed LXR agonistic activity.

As expected, 5-chlorination enhanced the antagonistic activity toward both LXRα and LXRβ (Fig. 4, Table 2): 5CPP-50 (**20**) is a more potent LXR antagonist than PP-50 (**14**). Monothiocarbonylation of PP-50 (**14**) also resulted in enhancement of the activity, as expected: PPS-50 (**21**) is more active than PP-50 (**14**) (Table 2). Further thiocarbonylation of PPS-50 (**21**), *i.e.*, PPSS-50 (**22**), had little effect on LXRα antagonistic activity, but enhanced the LXRβ antagonistic activity. Based on these results, 5-chloro-*N*-2′-*n*-pentylphenyl-1,3-dithiophthalimide (5CPPSS-50: **23**) was designed and prepared, and was found to be the most potent LXR antagonist among the prepared compounds, with IC_{50} values of 9.9—10 μM and 12—14 μM for LXRα and LXRβ, respectively (Table 2).

Conclusion

In conclusion, LXR antagonists based on *N*-2′-alkylphenylphthalimide were structurally developed. As the 2′-alkyl group, an *n*-pentyl group was determined to be the best substituent. Further structural development resulted in 5CPPSS-50 (**23**), which has IC_{50} values of around 10 μM for antagonistic activity towards both LXRs. Further structural development and biological studies of 5CPPSS-50 (**23**) are in progress.

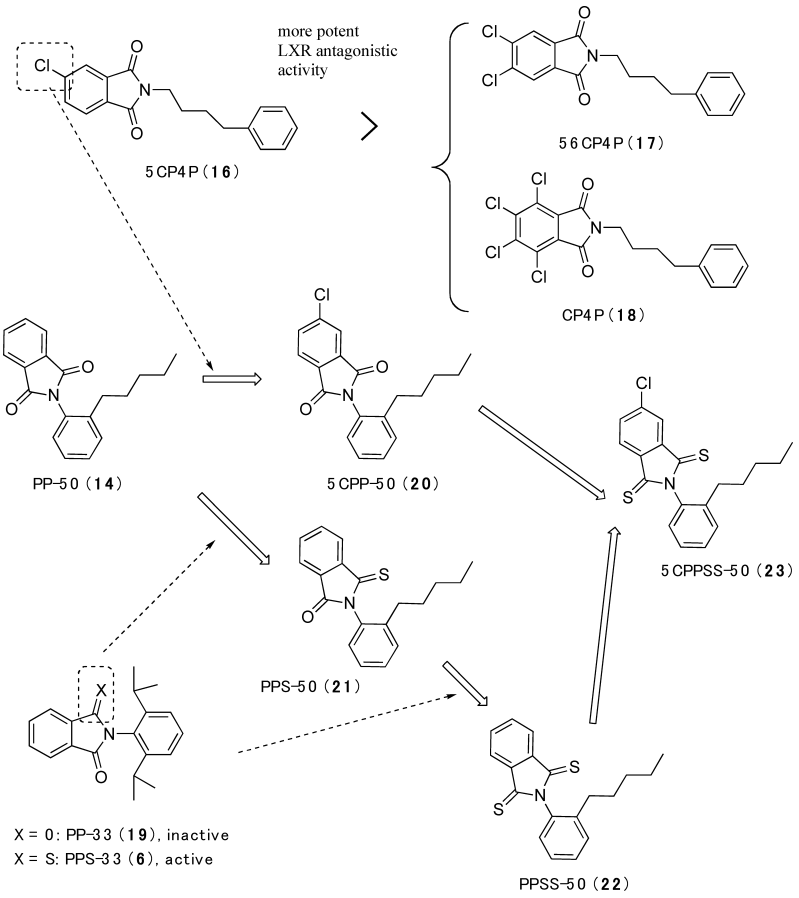


Fig. 3. Effects of 5-Chlorination and Thiocarbonylation,[20] and Design of Novel LXR Antagonists (**20**—**23**)

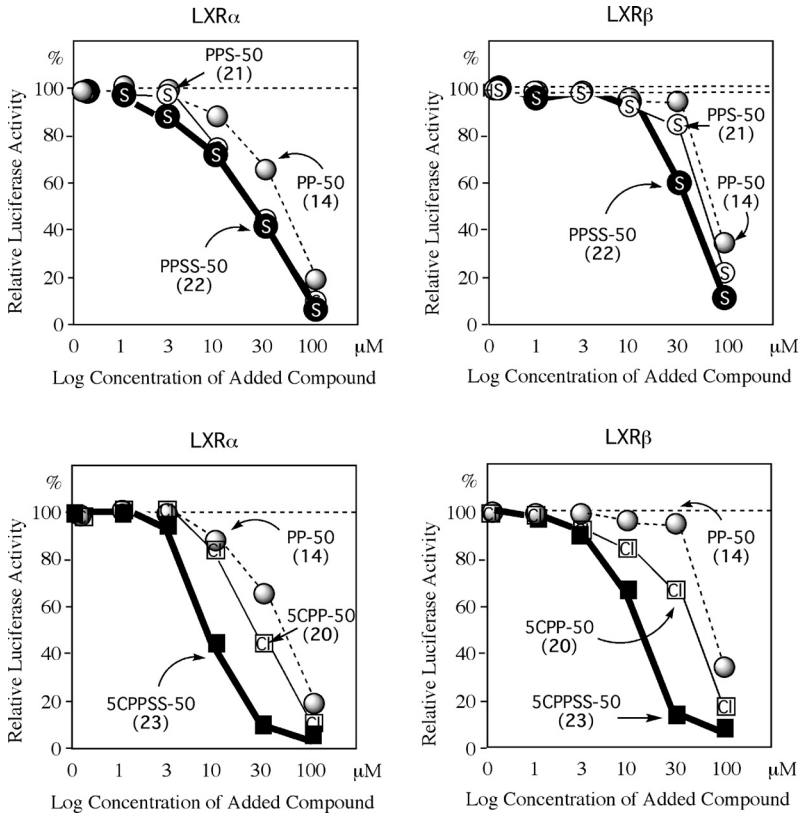


Fig. 4. LXR Antagonistic Activities of PP50 (**14**) and Its Derivatives (**20**—**23**) Measured by Means of Reporter Gene Assay

Various concentration of the test compounds were added in the presence of 0.1 μM T0901317 (**3**). The relative luciferase activity induced with 0.1 μM T0901317 (**3**) alone was defined as 100%.

Experimental

General Melting points were determined by using a Yanagimoto hot-stage melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a JEOL JNM-GX500 (500 MHz) spectrometer. Chemical shifts are expressed in parts per million relative to tetramethylsilane. Mass spectra were recorded on a JEOL JMS-DX303 spectrometer. For silica gel chromatography, Silica Gel 60 (Cica-Reagent Co. Ltd.) was used.

***N*-2′-Methylphenylphthalimide (PP-10: 10)** mp 180—181 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.99 (2H, m), 7.83 (2H, m), 7.40 (2H, m), 7.35 (1H, m), 7.23 (1H, d, J=5.9 Hz), 2.24 (3H, s). *Anal.* Calcd for $C_{15}H_{11}NO_2$: C, 75.94; H, 4.67; N, 5.90. Found: C, 76.03; H, 4.72; N, 5.86.

***N*-2′-Ethylphenylphthalimide (PP-20: 11)** mp 132—133 °C. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 7.62—8.10 (4H, m), 6.96—7.50 (4H, m), 2.52 (2H, q, J=7.5 Hz), 1.18 (3H, t, J=7.5 Hz). *Anal.* Calcd for $C_{16}H_{13}NO_2$: C, 76.48; H, 5.21; N, 5.57. Found: C, 76.65; H, 5.26; N, 5.54.

***N*-2′-*n*-Propylphenylphthalimide (PP-30: 12)** Ethyl triphenyl phosphonium bromide (1229 mg, 3.31 mmol) in THF (25 ml) was treated with 1 eq BuLi (1.6 M *n*-hexane solution, 2.04 ml) under ice cooling (stirred for 20 min). Then, 2-nitrobenzaldehylde (500 mg) in THF (1 ml) was added in aliquots, and stirred for 1 h. The resulted mixture was washed with water and separated by silica gel chromatography (AcOEt : *n*-hexane=1 : 1.15 v/v) to give 271 mg pale yellow oil (crude yield: 50%). The oil was dissolved in 10 ml EtOH and hydrogenated in the presence of 10% Pd/C (20 mg) under an H_2 atmosphere for 2 h to give 2-*n*-propylaniline quantitatively. The obtained 2-*n*-propylaniline was mixed with phthalic anhydride (121 mg) and heated at 160 °C to melt under an Ar atmosphere for 1 h. The resulted mixture was separated by silica gel chromatography (AcOEt : *n*-hexane=1 : 5 v/v) to give PP-30 (376 mg, y: 92%). The total yield was 46%. mp 94—95 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.95—7.99 (2H, m), 7.78—7.83 (2H, m), 7.42 (1H, dt, J=7.33, 1.22 Hz), 7.39 (1H, dd, J=7.79, 2.14 Hz), 7.33 (1H, dt, J=7.33, 2.14 Hz), 7.17 (1H, dd, J=7.79, 1.22 Hz), 2.47 (2H, t, J=7.79 Hz), 1.57 (2H, tq, J=7.79, 7.32 Hz), 0.86 (3H, t, J=7.32 Hz). *Anal.* Calcd for $C_{17}H_{15}NO_2$: C, 76.96; H, 5.70; N, 5.28. Found: C, 77.03; H, 5.56; N, 5.37.

***N*-2′-*n*-Butylphenylphthalimide (PP-40: 13)** PP-40 (**13**) was prepared in the same manner as described for the synthesis of PP-30 (**12**) in a total yield of 38%. mp 75—76 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.94—7.99 (2H, m), 7.78—7.83 (2H, m), 7.42 (1H, dt, J=1.22, 7.86 Hz), 7.39 (1H, dd, J=7.25, 2.44 Hz), 7.33 (1H, dt, J=2.44, 7.25 Hz), 7.17 (1H, dd, J=1.22, 7.86 Hz), 2.49 (2H, t, J=7.78 Hz), 1.52—1.56 (2H, m), 1.19—1.23 (2H, m), 0.79—0.81 (3H, t, J=7.02 Hz). *Anal.* Calcd for $C_{18}H_{17}NO_2$: C, 77.40; H, 6.13; N, 5.01. Found: C, 77.51; H, 6.53; N, 4.99.

***N*-2′-*n*-Pentylphenylphthalimide (PP-50: 14)** PP-50 (**14**) was prepared in the same manner as described for the synthesis of PP-30 (**12**) in a total yield of 52%. mp 77—78 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.95—7.99 (2H, m), 7.78—7.83 (2H, m), 7.42 (1H, dt, J=1.22, 7.94 Hz), 7.39 (1H, dd, J=7.25, 2.44 Hz), 7.33 (1H, dt, J=2.44, 7.25 Hz), 7.17 (1H, dd, J=7.94, 1.22 Hz), 2.48 (2H, t, J=7.94 Hz), 1.51—1.56 (2H, m), 1.19—1.23 (4H, m), 0.79—0.81 (3H, t, J=7.02 Hz). *Anal.* Calcd for $C_{19}H_{19}NO_2$: C, 77.79; H, 6.53; N, 4.77. Found: C, 77.70; H, 6.55; N, 4.88.

***N*-2′-*n*-Hexylphenylphthalimide (PP-70: 15)** PP-70 (**15**) was prepared in the same manner as described for the synthesis of PP-30 (**12**) in a total yield of 48%. mp 40 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.94—7.99 (2H, m), 7.78—7.83 (2H, m), 7.41 (1H, dt, J=1.22, 7.21 Hz), 7.39 (1H, dd, J=8.02, 2.14 Hz), 7.32 (1H, dt, J=2.14, 7.21 Hz), 7.16 (1H, dd, J=8.02, 1.22 Hz), 2.48 (2H, t, J=7.94 Hz), 1.49—1.58 (2H, m), 1.10—1.27 (8H, m), 0.79 (3H, t, J=7.02 Hz). *Anal.* Calcd for $C_{21}H_{23}NO_2$: C, 78.47; H, 7.21; N, 4.35. Found: C, 78.77; H, 7.27; N, 4.21.

5-Chloro-*N*-2′-*n*-pentylphenylphthalimide (5CPP-50: 20) 5CPP-50 (**20**) was prepared in the same manner as described for the synthesis of PP-30 (**12**) in a total yield of 83%. mp 88—89 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.94 (1H, d, J=1.30 Hz), 7.90 (1H, d, J=8.12 Hz), 7.77 (1H, dd, J=8.12, 1.30 Hz), 7.39—7.42 (2H, m), 7.32—7.35 (1H, m), 7.15 (1H, d, J=7.27 Hz), 2.46 (2H, t, J=7.27 Hz), 1.53—1.56 (2H, m), 1.21—1.23 (4H, m), 0.80 (3H, t, J=7.27 Hz). *Anal.* Calcd for $C_{19}H_{18}ClNO_2$: C, 69.62; H, 5.53: N, 4.27. Found: C, 69.69; H, 5.32; N, 4.26.

***N*-2′-*n*-Pentylphenyl-3-thioxo-2,3-dihydroisoindol-1-one (PPS-50: 21) and *N*-2′-*n*-Pentylphenyl-2,3-dihydroisoindole-1,3-dithione (PPSS-50: 22)** A mixture of PP-50 (**14**) (650 mg, 2.22 mmol), 2,4-bis-(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide (Lawesson reagent, Aldrich Co. Ltd.) (900 mg, 2.23 mmol) in dehydrated toluene (40 ml) was refluxed for 8 h. The reaction mixture was concentrated and the residue was purified by silica gel column chromatography (AcOEt : *n*-hexane=1 : 10 to 1 : 5 v/v) to afford 430 mg (y: 59%) of PPSS-50 (**22**) and 100 mg (y: 15%) of PPS-50 (**21**) as a brown oil.

PPS-50 (**21**): ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 8.06—8.07 (1H, m), 7.87—7.89 (1H, m), 7.78 (1H, d, J=6.84 Hz), 7.41—7.76 (2H, m), 7.34 (1H, dt, J=7.69, 1.71 Hz), 7.16 (1H, d, J=8.10 Hz), 2.44 (1H, d, J=6.84 Hz), 2.42 (1H, d, J=6.84 Hz), 1.51—1.54 (2H, m), 1.17—1.26 (4H, m), 0.78 (3H, t, J=7.27 Hz). HR-FAB-MS: $(M+H)^+$ Calcd for $C_{19}H_{20}NOS$, 310.1266. Found 310.1292.

PPSS-50 (**22**): ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.96 (2H, dd, J=5.55, 2.99 Hz), 7.77 (2H, dd, J=5.55, 2.99 Hz), 7.42—7.46 (2H, m), 7.35 (1H, dt, J=7.69, 1.71 Hz), 7.13 (1H, dd, J=7.69, 1.71 Hz), 2.36 (3H, t, J=8.12 Hz), 1.49—1.54 (2H, m), 1.15—1.20 (4H, m), 0.77 (3H, t, J=7.27 Hz). HR-FAB-MS: $(M+H)^+$ Calcd for $C_{19}H_{20}NS_2$, 326.1037. Found 326.1063.

5-Chloro-*N*-2′-*n*-pentylphenyl-2,3-dihydroisoindole-1,3-dithione (5CPPSS-50: 23) A mixture of 5CPP-50 (**20**) (150 mg, 0.46 mmol), 2,4-bis-(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane 2,4-disulfide (Lawesson reagent, Aldrich Co. Ltd.) (404 mg, 1.00 mmol) in dehydrated toluene (30 ml) was refluxed for 8 h. The reaction mixture was concentrated and the residue was purified by silica gel column chromatography (AcOEt : *n*-hexane=1 : 15 v/v) and recrystallized from *n*-hexane–AcOEt to afford 90.5 mg (55%) of 5CPPSS-50 (**23**) as brown powder. mp 90—91 °C. ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 7.91 (1H, d, J=1.71 Hz), 7.89 (1H, d, J=8.12 Hz), 7.71 (1H, dd, J=8.12, 1.71 Hz), 7.43—7.49 (2H, m), 7.36 (1H, dt, J=7.69, 1.71 Hz), 7.12 (1H, d, J=7.69 Hz), 2.35 (2H, t, J=7.85 Hz), 1.49—1.55 (2H, m), 1.13—1.34 (4H, m), 0.79 (3H, t, J=7.27 Hz). *Anal.* Calcd for $C_{19}H_{18}ClNS_2$: C, 63.40; H, 5.04: N, 3.89. Found: C, 63.22; H, 5.21: N, 3.91.

Reporter Gene Assay[21—23] Human embryonic kidney (HEK) 293 cells were cultured in Dulbecco's modified Eagle's medium containing 5% fetal bovine serum at 37 °C in a humidified atmosphere of 5% CO_2 in air. Transfections were performed by the calcium phosphate coprecipitation method. Test compounds with or without 0.1 mM T0901317 (**3**) (purchased from Cayman Co. Ltd.), were added 8 h after the transfection, and luciferase and β-galactosidase activities were assayed using a luminometer and microplate reader (Wallac 1420 Multilabel Couner, Peerkin Elmer Co. Ltd.). The experiment was repeated three times, and the normalized average values are presented in this paper.

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References

1) Chawla A., Pepa J. J., Evans R., Mangelsdorf D. J., *Science*, **294**, 1866—1870 (2001).
2) Janowski B. A., Willy P. J., Devi T. R., Falck J. R., Mangelsdorf D. J., *Nature* (London), **383**, 728—731 (1996).
3) Lehmann J. M., Kliewer S. A., Moore L. B., Smith-Oliver T. A., Blanchard D. E., Spencer T. A., Willson T. M., *J. Biol. Chem.*, **272**, 3137—3140 (1997).
4) Collins J. L., Fivush A. M., Watson M. A., Galardi C. M., Lewis M. C., Moore L. B., Parks D. J., Wilson J. G., Tippin T. K., Binz J. G., Plunket K. D., Morgan D. G., Beaudet E. G., Whitney K. D., Kliewer S. A., Willson T. M., *J. Med. Chem.*, **45**, 1963—1966 (2002).
5) Schults J. R., Tu H., Luk A., Repa J. J., Medina J. C., Li L., Schwendner S., Wang S., Thoolen M., Mangelsdorf D. J., Lustig K. D., Shan B., *Genes Develop.*, **14**, 2831—2838 (2000).
6) Repa J. J., Turley S. D., Lobaccaro J. M. A., Medina J., Li L., Lustig K., Shan B., Heyman R. A., Dietschy J. M., Mangelsdorf D. J., *Science*, **289**, 1524—1529 (2000).
7) Peet D. J., Turley S. D., Ma W., Janowski B. A., Lobaccaro J. M. A., Hammer R. E., Mangelsdorf D. J., *Cell*, **93**, 693—704 (1998).
8) Laffitte B. A., Chao L. C., Li J., Walczak R., Hummasti S., Joseph S. B., Castrillo A., Wilpitz D. C., Mangelsdorf D. J., Collins J. L., Saez E., Tontonoz P., *Proc. Natl. Acad. Sci. U.S.A.*, **100**, 5419—5424 (2003).
9) Cao G., Liang Y., Broderick C. L., Oldham B. A., Beyer T. P., Schmidt R. J., Zhang Y., Stayrook K. R., Suen C., Otto K. A., Miller A. R., Dai J., Foxworthy P., Gao H., Ryan T. P., Jiang X. C., Burris T. P., Eacho P. I., Etgen G. J., *J. Biol. Chem.*, **278**, 1131—1136 (2003).
10) Mitro N., Mak P. A., Vatgas L., Godio C., Hampton E., Molteni V., Kreusch A., Saez E., *Nature* (London), **445**, 219—223 (2007).
11) Hashimoto Y., *Curr. Med. Chem.*, **5**, 163—178 (1998).
12) Hashimoto Y., *Bioorg. Med. Chem.*, **10**, 461—479 (2002).

13) Hashimoto Y., Tanatani A., Nagasawa K., Miyachi H., *Drugs Future*, **29**, 383—391 (2004).
14) Hashimoto Y., *Mini-Rev. Med. Chem.*, **2**, 543—551 (2002).
15) Shimazawa R., Takayama H., Fujimoto Y., Komoda M., Dodo K., Yamasaki R., Shirai R., Koiso Y., Miyata K., Kato F., Kato M., Miyachi H., Hashimoto Y., *J. Enzyme Inhibit.*, **14**, 249—275 (1999).
16) Sou S., Mayumi S., Takahashi H., Yamasaki R., Kadoya S., Sodeoka M., Hashimoto Y., *Bioorg. Med. Chem. Lett.*, **10**, 1081—1084 (2000).
17) Takahashi H,, Sou S., Yamasaki R., Sodeoka M., Hashimoto Y., *Chem. Pharm. Bull.*, **48**, 1494—1499 (2000).
18) Sou S., Takahashi H., Yamasaki R., Kagechika H., Endo Y., Hashimoto Y., *Chem. Pharm. Bull.*, **49**, 791—793 (2001).
19) Shimazawa R., Takayama H., Kato F., Kato M., Hashimoto Y., *Bioorg. Med. Chem. Lett.*, **9**, 559—562 (1999).
20) Noguchi-Yachide T., Aoyama A., Makishima M., Miyachi H., Hashimoto Y., *Bioorg. Med. Chem. Lett.*, **17**, 3957—3961 (2007).
21) Makishima M., Lu T. T., Xie W., Whitfield G. K., Domoto H., Evans R. M., Haussler M. R., Mangelsdorf D. J., *Science*, **296**, 1313—1316 (2002).
22) Makishima M., Okamoto A. Y., Repa J. J., Tu H., Learned R. M., Luk A., Hull M. V., Lustig K. D., Mangelsdorf D. J., Shan B., *Science*, **284**, 1362—1365 (1999).
23) Kasuga J., Makishima M., Hashimoto Y., Miyachi H., *Bioorg. Med. Chem. Lett.*, **16**, 554—558 (2006).