Supporting Information

Styrylphenyl phthalimides as novel transrepression-selective liver X receptor (LXR) modulators

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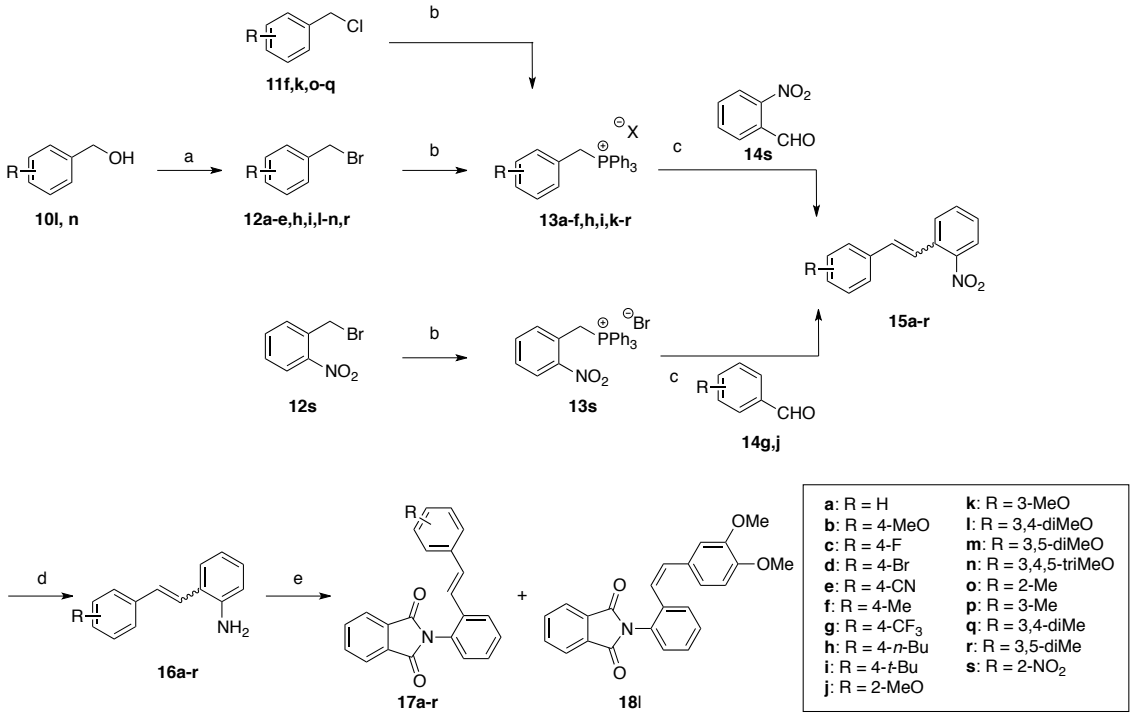
Purity of compounds

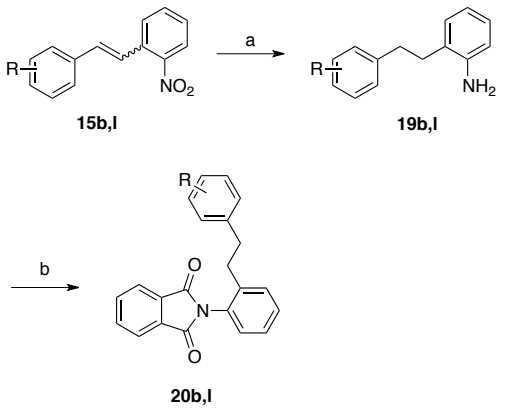
Synthesis

Styrylphenylphthalimide analogs were synthesized as shown in Scheme S1. Bromination of alcohols **10l,n** yielded benzyl bromides **12l,n**. Various benzyl chlorides **11** and bromides **12** were treated with PPh_3 to generate phosphonium ylides **13a-r**. Wittig reaction of ylides **13a-f,h,i,k-r** and aldehyde **14s**, and ylide **13s** and aldehydes **14g,j** afforded stilbenes **15a-r**. The nitro group was reduced with $SnCl_2 \cdot 2H_2O$, and then cyclization with phthalic anhydride and separation of *EZ* isomers gave the *E*-styrylphenylphthalimides **17a-r**. The *Z*-isomer **18l** was synthesized from the corresponding *Z*-aniline derivative.



Scheme S1. Reagents and conditions: (a) PBr_3, DCM, 0 ºC; (b) PPh_3, CH_3CN, reflux; (c) **14**, 18-crown-6, K_2CO_3, DCM, reflux; (d) $SnCl_2 \cdot 2H_2O$, AcOEt, reflux; (e) phthalic anhydride, AcOH, reflux, then column chromatography.

Synthesis of phenethylphenylphthalimide analogs is outlined in Scheme S2. Hydrogenation of nitrostilbenes **15b,l** gave phenethylanilines **19b,l**. Cyclization with phthalic anhydride with **19** gave **20b,l**.



Scheme S2. Reagents and conditions: (a) Pd/C, AcOEt, rt; (b) phthalic anhydride, AcOH, reflux, or 200 ºC.

Figures S1-S3

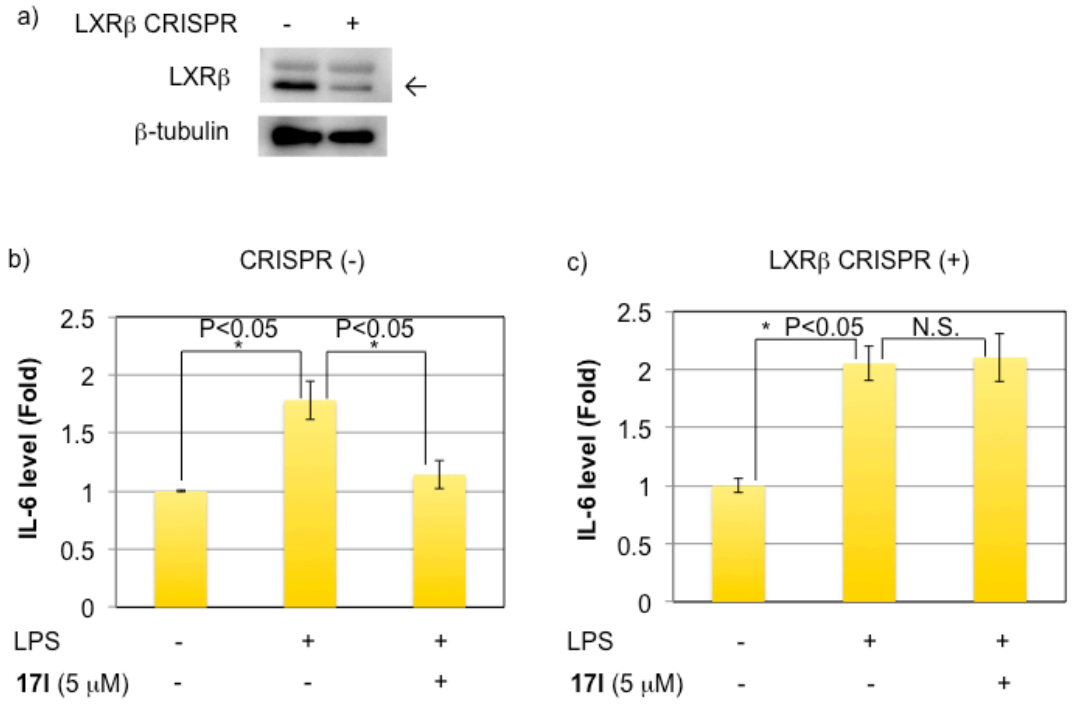


Figure S1. LXRβ-dependence of reduction in IL-6 level by **17l**. (a) Western blot analysis of LXRβ level in differentiated THP-1 cells with or without transfection of LXRβ CRISPR knockout plasmid. (b) IL-6 level without transfection (c) IL-6 level with transfection of LXRβ CRISPR knockout plasmid.

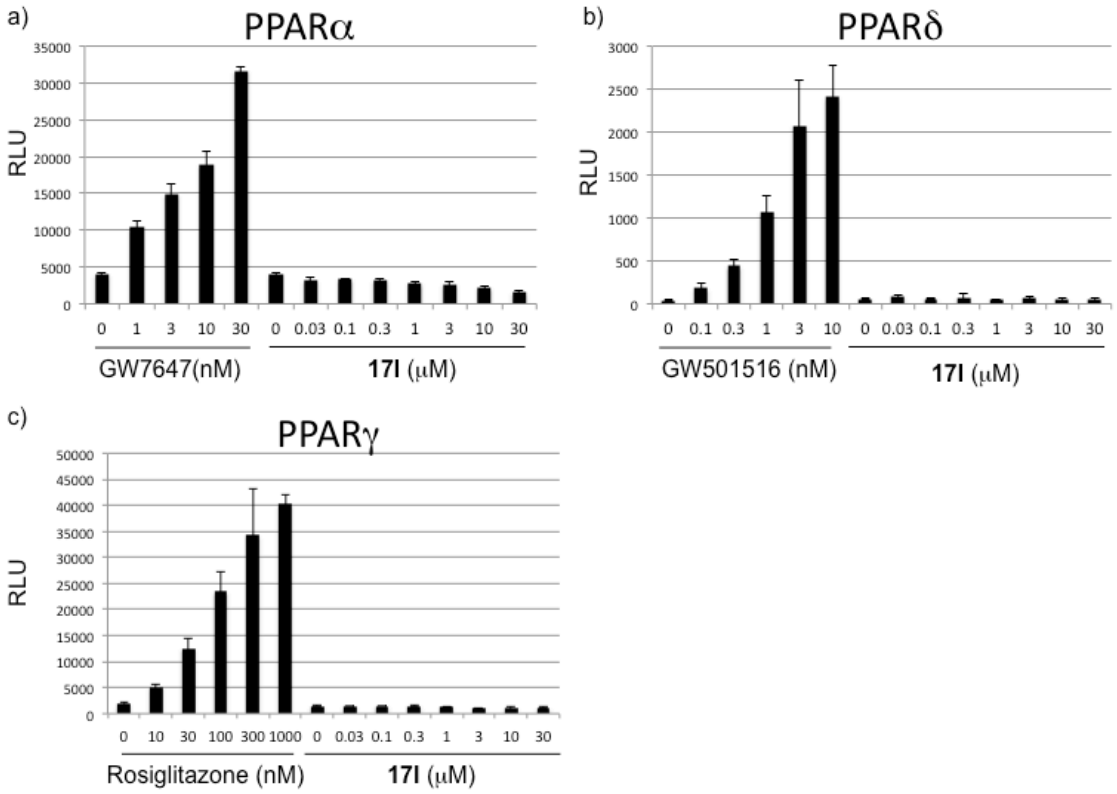


Figure S2. PPARs luciferase assay. GW7647, GW501516 and rosiglitazone (BRL49653) were used as PPAR selective agonists for α, δ and γ, respectively. RLU: relative luciferase unit.

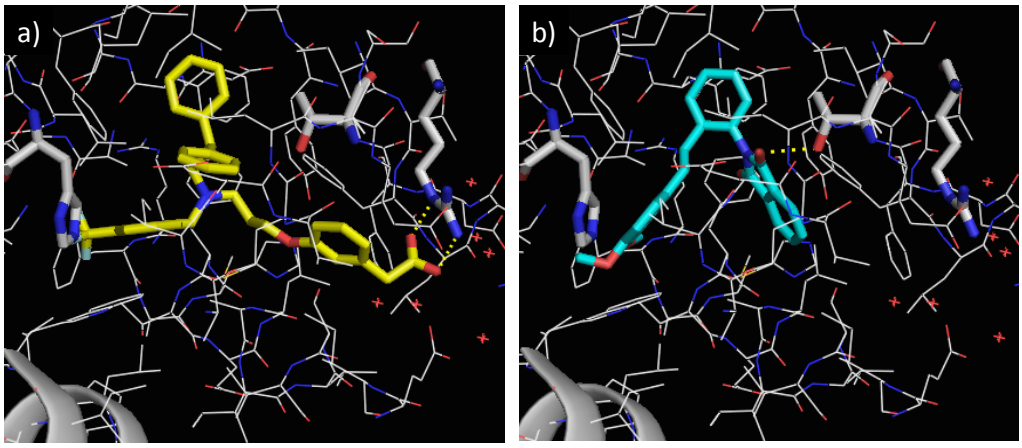


Figure S3. (a) X-ray crystal structure of the complex of **4** (yellow) with LXRα LBD (white). Hydrogen bonds between Arg305 and the carboxyl group were observed; b) docking simulation of LXRα LBD and **17l** (cyan). His421, Thr302 and Arg305 of LXRα are presented as stick models, and H12 is presented as a cartoon. Important hydrogen bonds are indicated as yellow dotted lines.

Experimental Section

Chemistry.

General Methods.

^{1}H NMR spectra were recorded on a JEOL JNM-ECA500 (500 MHz) spectrometer, and ^{13}C NMR spectra were recorded on a JEOL JNM-ECA500 (125 MHz) spectrometer. Chemical shifts (δ) are expressed in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal reference, with coupling constants in Hz. The abbreviations s, d, t, q, br, and m signify singlet, doublet, triplet, quartet, broad, and multiplet, respectively. Fast atom bombardment mass spectra (FAB-MS) were recorded on a JEOL JMS-HX110 spectrometer with m-nitrobenzyl alcohol. Electrospray ionization mass spectra (ESI-MS) were recorded on Bruker micrOTOF II spectrometer. Analytical and preparative thin-layer chromatographies (TLC and PTLC) were performed on Merck 60 F254 pre-coated silica gel plates. Flash column chromatography was performed on silica gel 60 Kanto Kagaku (40−50 μm).

General procedure A (GP-A) (Triphenylphosphonium salt)

To a solution of substituted benzyl halide in acetonitrile was added PPh_3 (1.5 eq). The mixture was heated to reflux for 4.5 h and then concentrated. The residue was taken up in toluene, and the product was collected by filtration.

(4-Bromobenzyl)triphenylphosphonium bromide (13a)

Compound **13a** (2.31 g, 5.33 mmol) was prepared from benzyl bromide by means of GP-A in 90% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.76-7.70 (9H, m), 7.64-7.61 (6H, m), 7.21-7.07 (5H, m), 5.41 (2H, d, J = 14.3 Hz).

(4-Methoxybenzyl)triphenylphosphonium chloride (13b)

Compound **13b** was prepared from 4-methoxybenzyl chloride by means of GP-A in

90% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.78-7.74 (9H, m), 7.65-7.61 (6H, m), 7.01 (2H, d, *J* = 8.7, Hz), 6.66 (2H, d, *J* = 8.7 Hz), 5.45 (2H, d, *J* = 13.7 Hz), 3.73 (3H, s).

(4-Fluorobenzyl)triphenylphosphonium bromide (13c)

Compound **13c** was prepared from 4-fluorobenzyl bromide by means of GP-A in 100% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.79-7.75 (9H, m), 7.65-7.63 (6H, m), 7.17-7.13 (2H, m), 6.83-81 (2H, m), 5.51 (2H, d, *J* = 13.7 Hz).

(4-Bromobenzyl)triphenylphosphonium bromide (13d)

Compound **13d** (3.90 g) was prepared from 4-bromobenzyl bromide by means of GP-A in 100% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.79-7.77 (9H, m), 7.65-7.63 (6H, m), 7.25 (2H, d, *J* = 8.6 Hz), 7.05 (2H, dd, *J* = 8.6, 2.3 Hz), 5.53 (2H, d, *J* = 14.9 Hz).

(4-Cyanobenzyl)triphenylphosphonium bromide (13e)

Compound **13e** was prepared from 4-cyanobenzyl bromide by means of GP-A in 100% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.81-7.77 (9H, m), 7.65-7.61 (6H, m), 7.39-7.37 (4H, m), 5.82 (2H, d, *J* = 15.5 Hz).

(4-Methylbenzyl)triphenylphosphonium chloride (13f)

Compound **13f** was prepared from 4-methylbenzyl chloride by means of GP-A in 100% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.77-7.73 (9H, m), 7.64-7.63 (6H, m), 6.95-6.93 (4H, m), 5.38 (2H, d, *J* = 14.3 Hz), 2.25 (3H, s).

(4-Butylbenzyl)triphenylphosphonium bromide (13h)

Compound **13h** (2.86 g) was prepared from 4-butylbenzyl bromide by means of GP-A in 100% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.79-7.75 (3H, m), 7.74-7.69 (6H, m), 7.67-7.61 (6H, m), 6.97-6.94 (4H, m), 5.30-5.25 (2H, m), 2.55-2.46 (2H, m), 1.51-1.50 (2H, m), 1.29-1.28 (2H, m), 0.95-0.82 (3H, m).

(4-*tert*-Butylbenzyl)triphenylphosphonium bromide (13i)

Compound **13i** was prepared from 1-(bromomethyl)-4-(*tert*-butyl)benzene by means of GP-A in 100% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.77-7.72 (9H, m), 7.64-7.63 (6H, m), 7.18-7.14 (4H, m), 5.31 (2H, d, 13.7 Hz), 1.23 (9H, s).

(3-Methoxybenzyl)triphenylphosphonium chloride (13k)

Compound **13k** was prepared from 1-(chloromethyl)-3-methoxybenzene by means of GP-A in 81% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.78-7.76 (9H, m), 7.65-7.62 (6H, m), 7.02 (1H, dd, J = 7.3, 7.3 Hz), 6.76-6.75 (2H, m), 6.65 (1H, d, J = 7.3 Hz), 5.50 (2H, d, J = 14.3 Hz), 3.54 (3H, s).

(3,4-Dimethoxybenzyl)triphenylphosphonium bromide (13l)

To a solution of 3,4-trimethoxybenzyl alcohol (2.00 mL, 13.5 mmol) in DCM (15.0 mL) was added dropwise PBr_3 (658 μL, 7.0 mmol) at 0 ºC under an argon atmosphere. The mixture was stirred for 1 h, then poured into ice water (50 mL) and neutralized with sat.$NaHCO_3$ (50 mL). The aqueous mixture was extracted with DCM (20 mL×3). The organic layer was washed with H_2O (20 mL×2) and then dried over $MgSO_4$. The solvent was removed under reduced pressure. To a solution of the intermediate in toluene (30 mL) was added PPh_3 (3.74 g, 14.25 mmol). The mixture was refluxed for 30 min. The precipitate was collected by filtration to give **13l** (4.46 g, 80% in 2 steps) as a

white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.77-7.60 (15H, m), 6.87 (1H, d, *J* = 7.5 Hz), 6.81 (1H, d, *J* = 7.5 Hz), 6.69 (1H, s), 5.39 (2H, d, *J* = 14.4 Hz), 2.17 (3H, s) 2.00 (3H, s).

(3,5-Dimethoxybenzyl)triphenylphosphonium bromide (13m)

Compound **13m** was prepared from 1-(bromomethyl)-3,5-dimethoxybenzene by means of GP-A in 100% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.78-7.76 (9H, m), 7.66-7.63 (6H, m), 6.35 (2H, t, *J* = 2.3 Hz), 6.30 (1H, q, *J* = 2.3 Hz), 5.32 (2H, d, *J* = 14.3 Hz), 3.54 (6H, s).

Triphenyl-(3,4,5-trimethoxybenzyl)phosphonium bromide (13n)

To a solution of 3,4,5-trimethoxybenzyl alcohol (1.98 g, 10.0 mmol) in DCM (15.0 mL) was added dropwise PBr_3 (658 μL, 7.00 mmol) at 0 ºC under an argon atmosphere. The mixture was stirred for 1 h, then poured into ice water (50 mL) and neutralized with sat.$NaHCO_3$ (50 mL). The aqueous mixture was extracted with DCM (20 mL×3). The organic layer was washed with H_2O (20 mL×2) and then dried over $MgSO_4$. The solvent was removed under reduced pressure. To a solution of the intermediate (**12n**) in toluene (30 mL) was added PPh_3 (3.74 g, 14.3 mmol). The mixture was refluxed for 30 min. The precipitate was collected by filtration to afford the product (2.01 g, 38% in 2 steps) as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.79-7.75 (9H, m), 7.63-7.62 (6H, m), 6.49 (2H, d, *J* = 2.9 Hz), 5.41 (2H, d, *J* = 14.3 Hz), 3.77 (3H, s), 3.51 (6H, s).

(2-Methylbenzyl)triphenylphosphonium chloride (13o)

Compound **13o** (4.44 g) was prepared from 1-(chloromethyl)-2-methylbenzene by means of GP-A in 100% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.79-7.77 (3H, m), 7.72-7.68 (6H, m), 7.64-7.62 (6H, m), 7.16-7.11 (2H, m), 6.99-6.97 (2H, m), 5.44 (2H, d, *J* = 14.3 Hz), 1.67 (3H, s).

(3-Methylbenzyl)triphenylphosphonium chloride (13p)

Compound **13p** (4.12 g) was prepared from 1-(chloromethyl)-3-methylbenzene by means of GP-A in 100% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.78-7.76 (9H, m), 7.65-7.60 (6H, m), 7.02-7.00 (1H, m), 6.91-6.89 (2H, m), 6.77 (1H, s), 5.47 (2H, d, *J* = 14.3 Hz), 2.14 (3H, s).

(3,4-Dimethylbenzyl)triphenylphosphonium chloride (13q)

Compound **13q** (3.98 g) was prepared from 1-(chloromethyl)-3,4-methylbenzene by means of GP-A in 67% yield as a pale brown solid.

^{1}H-NMR ($CDCl_3$) δ: 7.77-7.60 (15H, m), 6.87 (1H, d, *J* = 7.5 Hz), 6.81 (1H, d, *J* = 7.5 Hz), 6.69 (1H, s), 5.39 (2H, d, *J* = 14.4 Hz), 2.17 (3H, s) 2.00 (3H, s).

(3,5-Dimethylbenzyl)triphenylphosphonium bromide (13r)

Compound **13r** (3.61 g) was prepared from 1-(bromomethyl)-3,5-dimethylbenzene by means of GP-A in 83% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.79-7.73 (9H, m), 7.65-7.64 (6H, m), 6.84 (1H, s), 6.58 (2H, s), 5.29 (2H, d, *J* = 14.3 Hz), 2.07 (6H, s).

(2-Nitrobenzyl)triphenylphosphonium bromide (13s)

Compound **13s** (6.82 g) was prepared from 2-nitrobenzyl bromide by means of GP-A in 100% yield as a pale yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 8.16-8.13 (1H, m), 7.94 (1H, d, *J* = 8.6 Hz), 7.81-7.78 (3H, m), 7.71-7.64 (13H, m), 7.50-7.48 (1H, m), 6.15 (2H, d, *J* = 14.9 Hz).

General procedure B (GP-B) (Wittig reaction)

To a solution of aldehyde **14** in dehydrated DCM were added benzyltriphenylphosphonium salt **13** (1.0 eq), potassium carbonate (1.1 eq) and

18-crown-6 (0.18 eq). The mixture was stirred and refluxed for 6 h, then filtered. The filtrate was concentrated and the residue was purified by silica gel column chromatography (eluent: n-hexane/ethyl acetate = 10:1 to 3:1, v/v) to give the product as an *EZ* mixture.

1-Nitro-2-styrylbenzene (15a)

Compound **15a** (1.36 g, as an *EZ* mixture) was prepared from **13a** (2.07 g, 4.78 mmol) by means of GP-B in 100% yield as a yellow solid.

^{1}H-NMR ($CDCl_3$) (as a mixture: *E* : *Z* = 4 : 6) δ: 8.10-8.07 (1H, m), 7.96 (1H, dd, *J* = 8.2, 1.2 Hz), 7.77 (1H, dd, *J* = 8.2, 1.2 Hz), 7.61-7.15 (14H, m), 7.09-7.06 (3H, m), 6.89 (1H, d, *J* = 12.3 Hz), 6.77 (1H, d, J = 12.3 Hz).

1-(4-Methoxystyryl)-2-nitrobenzene (15b)

Compound **15b** (1.40 g, as an *EZ* mixture) was prepared from **13b** and 2-nitrobenzaldehyde by means of GP-B as a yellow oil. This compound was used for the next reaction without further purification.

1-(4-Fluorostyryl)-2-nitrobenzene (15c)

Compound **15c** (1.23 g, as an *EZ* mixture) was prepared from **13c** by means of GP-B in 100% yield as a yellow oil.

^{1}H-NMR ($CDCl_3$) (as an *EZ* mixture 1:1) δ: 8.10-8.08 (1H, m), 7.97 (1H, dd, *J* = 8.0, 1.1 Hz), 7.75 (1H, d, *J* = 8.0 Hz), 7.61 (1H, dd, *J* = 8.0, 8.0 Hz), 7.54-6.83 (13H, m), 7.08 (1H, d, *J* = 16.6 Hz), 6.88 (1H, d, *J* = 12.0 Hz), 6.72 (1H, d, *J* = 12.0 Hz).

1-(4-Bromostyryl)-2-nitrobenzene (15d)

Compound **15d** (1.62 g, as an *EZ* mixture) was prepared from **13d** by means of GP-B in 89% yield as a yellow solid.

^{1}H-NMR ($CDCl_3$) (as a mixture: *E* : *Z* = 3 : 7) δ: 8.12-8.09 (1H, m), 7.98 (1H, d, *J* = 8.0

Hz), 7.75 (1H, d, *J* = 8.0 Hz), 7.63-7.22 (12H, m), 7.01 (1H, d, *J* = 16.0 Hz), 6.93-6.91 (3H, m), 6.69 (1H, d, *J* = 12.0 Hz).

4-(2-Nitrostyryl)benzonitrile (15e)

Compound **15e** (1.42 g, as an *EZ* mixture) was prepared from **13e** by means of GP-B in 95% yield as a yellow solid.

^{1}H-NMR ($CDCl_3$) (as a mixture: *E* : *Z* = 2 : 3) δ: 8.15 (1H, dd, *J* = 7.2, 2.0 Hz), 8.03 (2H, dd, *J* = 8.0, 1.1 Hz), 7.78-7.61 (5H, m), 7.48-7.43 (6H, m), 7.18-7.13 (1H, m), 7.14 (2H, d, *J* = 8.0 Hz), 7.07 (1H, d, *J* = 12.3 Hz), 7.07-7.04 (1H, m), 6.77 (1H, d, *J* = 12.3 Hz).

1-(4-Methylstyryl)-2-nitrobenzene (15f)

Compound **15f** (2.18 g, as an *EZ* mixture) was prepared from **13f** (3.11g, 7.72 mmol) by means of GP-B in 100% yield as a yellow oil. For the compound characterization, a portion was purified with PTLC.

E isomer: ^{1}H-NMR ($CDCl_3$) δ: 7.93 (1H, dd, *J* = 7.8, 1.1 Hz), 7.75 (1H, dd, *J* = 7.8, 1.1 Hz), 7.59 (1H, ddd, *J* = 7.8, 7.8, 1.3 Hz), 7.55 (1H, d, *J* = 16.0 Hz), 7.44 (2H, d, *J* = 8.0 Hz), 7.40-7.37 (1H, m), 7.20 (2H, d, *J* = 8.0 Hz), 7.07 (1H, d, *J* = 16.0 Hz), 2.38 (3H, s).

Z isomer: ^{1}H-NMR ($CDCl_3$) δ: 8.09-8.04 (1H, m), 7.40-7.26 (3H, m), 6.95 (2H, d, *J* = 8.0 Hz), 6.92 (2H, d, *J* = 8.0 Hz), 6.82 (1H, d, *J* =12.0 Hz), 6.71 (1H, d, *J* = 12.0 Hz), 2.25 (3H, s).

2-Nitro-2-(4-(trifluoromethyl)styryl)benzene (15g)

Compound **15g** (1.60 g, as an *EZ* mixture) was prepared from compound **13s** and **14g** by means of GP-B in 91% yield as a yellow solid/oil mixture.

^{1}H-NMR ($CDCl_3$) (as a mixture: *E : Z* = 1 : 2) δ: 8.15-8.11 (1H, m), 8.01 (1H, dd, *J*= 7.8, 1.1 Hz), 7.76 (1H, d, *J*= 7.8 Hz), 7.69 (1H, d, *J*= 16.0 Hz), 7.64-7.61 (5H, m), 7.46 (1H, ddd, *J* = 7.8, 7.8, 1.1 Hz), 7.43-7.42 (4H, m), 7.21-7.19 (1H, m), 7.15 (2H, d, *J* =

8.0 Hz), 7.09 (1H, *J* = 16.0 Hz), 7.03 (1H, *J* = 12.0 Hz), 6.79 (1H, *J* = 12.0 Hz).

1-(4-Butylstyryl)-2-nitrobenzene (15h)

Compound **15h** (2.13g, as an *EZ* mixture) was prepared from **13h** by means of GP-B in 100% yield as a yellow oil.

^{1}H-NMR ($CDCl_3$) (as a mixture: *E : Z* = 1 :1) δ: 8.10-8.07 (1H, m), 7.95 (1H, dd, *J* = 8.0, 1.1 Hz), 7.77 (1H, dd, *J* = 8.0, 1.1 Hz), 7.59-7.20 (3H, m), 7.55 (1H, d, *J* = 16.0 Hz), 7.08 (1H, d, *J* = 16.0 Hz), 6.84 (1H, d, *J* = 12.0 Hz), 6.73 (1H, d, *J* = 12.0 Hz), 2.20-1.24 (12H, m), 0.95-0.88 (6H, m).

1-(4-*tert*-Butylstyryl)-2-nitrobenzene (15i)

Compound **15i** (1.06 g, as an *EZ* mixture) was prepared from **13i** by means of GP-B as a yellow oil. It was used for the next reaction without further purification.

1-(2-Methoxystyryl)-2-nitrobenzene (15j)

Compound **15j** (1.46 g, as an *EZ* mixture) was prepared from **13s** and **14j** by means of GP-B in 95% yield as a yellow oil. It was used for the next reaction without further purification.

1-(3-Methoxystyryl)-2-nitrobenzene (15k)

Compound **15k** (1.71 g, as an *EZ* mixture) was prepared from **13k** by means of GP-B in 100% yield as a yellow oil.

^{1}H-NMR ($CDCl_3$) (as a mixture: *E* : *Z* = 1:1) δ: 8.10-8.08 (1H, m), 7.98-7.96 (1H, m), 7.78-7.76 (1H, m), 7.61-7.58 (2H, m), 7.43-7.38 (3H, m), 7.31-7.30 (2H,m), 7.14-7.04 (4H, m), 6.90 (1H, d, *J* = 12.0 Hz), 6.89-6.87 (1H, m), 6.74 (1H, d, *J* = 12.0 Hz), 6.73-6.71 (1H, m), 6.65-6.64 (1H, m), 6.57 (1H, s), 3.86 (3H, s), 3.61 (3H, s).

1,2-Dimethoxy-4-(2-nitrostyryl)benzene (15l)

Compound **15l** (2.81 g, as an *EZ* mixture) was prepared from **13l** by means of GP-B in 94% yield as a yellow oil.

^{1}H-NMR ($CDCl_3$) (as a mixture: E : Z = 1:1) δ: 8.08 (1H, dd, *J* = 8.0, 1.7 Hz), 7.95 (1H, dd, *J* = 8.2, 1.3 Hz), 7.76 (1H, dd, *J* = 8.2, 1.3 Hz), 7.61-7.57 (1H, m), 7.47 (1H, d, *J* = 16.0 Hz), 7.41-7.36 (4H, m), 7.10-7.09 (2H, m), 7.05 (1H, d, *J* = 16.0 Hz) 6.89-6.87 (1H, m), 6.81 (1H, d, *J* = 12.0 Hz),6.71-6.66 (3H, m), 6.50 (1H, d, *J* = 1.7 Hz), 3.95 (3H, s), 3.92 (3H, s) 3.83 (3H, s), 3.54 (3H, s).

1,3-Dimethoxy-5-(2-nitrostyryl)benzene (15m)

Compound **15m** (1.83 g, as an *EZ* mixture) was prepared from **13m** by means of GP-B as a yellow oil. It was used for the next reaction without further purification.

1,2,3-Trimethoxy-5-(2-nitrostyryl)benzene (15n)

Compound **15n** (1.42 g, as an *EZ* mixture) was prepared from **13n** by means of GP-C in 75% yield as a yellow solid.

^{1}H-NMR ($CDCl_3$) (as a mixture: *E* : *Z* = 1 : 1) δ: 8.07 (1H, d, *J* = 9.0 Hz), 7.97 (1H, d, *J* = 8.0 Hz), 7.75 (1H, d, *J* = 8.0 Hz), 7.61 (1H, dd, *J* = 8.0, 8.0 Hz), 7.49 (1H, d, *J* = 16.0 Hz), 7.41 (1H, dd, *J* = 8.0 Hz), 7.45(1H, d, *J* = 7.45), 7.40 (1H, dd, *J* = 7.45), 7.33 (1H, d, *J* = 7.45), 7.02 (1H, d, *J* = 16.0 Hz), 6.86 (1H, d, *J* = 12.0 Hz), 6.66 (1H, d, *J* = 12.0 Hz), 6.23 (2H, s), 6.75 (2H, s), 3.91 (6H, s), 3.87 (3H, s), 3.78 (3H, s), 3.58 (6H, s).

1-(2-Methylstyryl)-2-nitrobenzene (15o)

Compound **15o** (1.39 g, as an *EZ* mixture) was prepared from **13o** by means of GP-C as a yellow oil. It was used for the next reaction without further purification.

1-(3-Methylstyryl)-2-nitrobenzene (15p)

Compound **15p** (1.86 g, as an *EZ* mixture) was prepared from **13p** by means of GP-C as a yellow oil. It was used for the next reaction without further purification.

3,4-Dimethyl-1-(2-nitrostyryl)benzene (15q)

Compound **15q** (907 mg, as an *EZ* mixture) was prepared from **13q** by means of GP-C as a yellow oil. It was used for the next reaction without further purification.

3,5-Dimethyl-1-(2-nitrostyryl)benzene (15r)

Compound **15r** (as an *EZ* mixture) was prepared from **13r** by means of GP-C as a yellow oil. It was used for the next reaction without further purification.

General procedure C (GP-C) (Reduction by $SnCl_2.2H_2O$)

To a solution of substituted nitrobenzene **15** in AcOEt was added $SnCl_2.2H_2O$ (5.0 eq). The mixture was refluxed for 4 h, and then the reaction was terminated by the addition of sat.$NaHCO_3$. The resulting mixture was filtered through a pad of Celite and extracted with AcOEt. The organic layer was washed with H_2O and brine, and then dried over $MgSO_4$. The solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (eluent: n-hexane/ethyl acetate = 10:1 to 3:1, v/v) to give the target compound **16** as an *EZ* mixture.

2-Styrylaniline (16a)

Compound **16a** (640 mg, as a *EZ* mixture) was prepared from **15a** (870 mg, 3.86 mmol) by means of GP-C in 85% yield as a red oil. For the compound characterization, a portion was purified with PTLC.

E isomer: ^{1}H-NMR ($CDCl_3$) δ: 7.51 (2H, d, *J* = 7.4 Hz), 7.40 (1H, dd, *J* = 7.7, 1.4 Hz), 7.36 (2H, d, *J* = 7.4 Hz), 7.28-7.23 (1H, m), 7.16 (1H, d, *J* = 16.0 Hz), 7.11 (1H, dd, *J* = 7.4, 7.4 Hz), 6.99 (1H, d, *J* = 16.0 Hz), 6.81 (1H, dd, *J* = 7.4, 7.4 Hz), 6.72 (1H, d, *J* = 7.4 Hz), 3.82 (3H, s).

Z isomer: a red solid: ^{1}H-NMR ($CDCl_3$) δ: 7.27-7.16 (5H. m) 7.07 (2H, d, *J* = 7.5 Hz), 6.69 (2H, d, *J* = 7.5 Hz), 6.64 (1H, d, *J* = 12.0 Hz), 6.50 (1H, d, *J* = 12.0 Hz), 3.71 (3H,

s).

2-(4-Methoxystyryl)aniline (16b)

Compound **16b** (as an *EZ* mixture) was prepared from **15b** by means of GP-C in 87% yield (2 steps) as a red solid/oil mixture.

^{1}H-NMR ($CDCl_3$) (as a mixture: *E* : *Z* = 6 : 4) δ: 7.45 (2H, d, *J* = 8.6 Hz), 7.38 (1H, dd, J = 7.7, 1.4 Hz), 7.16 (2H, d, *J* = 7.3 Hz), 7.10-7.07 (3H, m), 7.02 (1H, d, J = 16.3 Hz), 6.93 (1H, d, *J* = 16.3 Hz), 6.92-6.67 (8H, m), 6.59 (1H, d, *J* = 12.0 Hz), 6.40 (1H, d, *J* = 12.0 Hz), 3.83 (3H, s), 3.76 (3H, s).

2-(4-Fluorostyryl)aniline (16c)

Compound **16c** (560 mg, as an *EZ* mixture) was prepared from **15c** by means of GP-C in 79% yield as a yellow oil. For the compound characterization, a portion was purified with PTLC.

E isomer: ^{1}H-NMR ($CDCl_3$) δ: 7.48-7.46 (2H, m), 7.38 (1H, dd, *J* = 7.8, 1.2 Hz), 7.10-7.05 (4H, m), 6.95 (1H, d, *J* = 16.0 Hz), 6.81 (1H, ddd, *J* = 7.8, 7.8, 1.2 Hz), 6.73 (1H, dd, *J* = 7.8, 1.2 Hz).

Z isomer: ^{1}H-NMR ($CDCl_3$) δ: 7.19-7.18 (2H, m), 7.09 (1H, dd, *J* = 7.1 Hz), 7.05 (1H, d, *J* = 7.1 Hz), 6.88-6.86 (2H, m), 6.70-6.68 (2H, m), 6.61 (1H, d, *J* = 12.0 Hz), 6.49 (1H, d, *J* = 12.0 Hz).

2-(4-Bromostyryl)aniline (16d)

Compound **16d** (240 mg, as a *EZ* mixture) was prepared from **13d** by means of GP-C as a yellow solid. It was used for the next reaction without further purification.

4-(2-Aminostyryl)benzonitrile (16e)

Compound **16e** (600 mg, as an *EZ* mixture) was prepared from **15e** by means of GP-C in 81% yield as a yellow solid.

^{1}H-NMR ($CDCl_3$) (as a mixture: *E* : *Z* = 1 : 2) δ: 7.63 (2H, d, *J* = 7.7 Hz), 7.57 (2H, d, *J* = 7.7 Hz), 7.46 (2H, d, *J* = 7.5 Hz), 7.41 (1H, d, *J* = 7.4 Hz), 7.31 (1H, d, *J* = 8.0 Hz), 7.27 (2H, d, *J* = 7.5 Hz), 7.27-7.26 (4H, m), 6.99 (1H, dd, *J* = 7.7, 7.7 Hz), 6.82 (1H, dd, *J* = 7.4, 7.4 Hz), 6.75-6.67 (3H, m), 6.4 (1H, *J* = 12.0 Hz), 3.85 (2H, s), 3.72 (2H, s).

2-(4-Methylstyryl)aniline (16f)

Compound **16f** (240 mg, as an *EZ* mixture) was prepared from **15f** (304 mg, 1.00 mmol) by means of GP-C as an orange solid (*EZ* mixture). It was used for the next reaction without further purification.

2-(4-(Trifluoromethyl)styryl)aniline (16g)

Compound **16g** (307 mg, as an *EZ* mixture) was prepared from **15g** by means of GP-C in 33% yield. For the compound characterization, a portion was purified with PTLC.

E isomer: Yellow solid. ^{1}H-NMR ($CDCl_3$) δ: 7.62-7.58 (4H, m), 7.41 (1H, dd, *J* = 7.8, 1.2 Hz), 7.26 (1H, d, *J* = 16.0 Hz), 7.14 (1H, dd, *J* = 7.8, 7.8, 1.2 Hz), 7.01 (1H, d, *J* = 16.0 Hz), 6.83 (1H, dd, *J* = 7.8, 7.8 Hz), 6.74 (1H, dd, *J* = 7.8, 1.2 Hz), 3.83 (2H, s).

Z isomer: yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 7.43 (2H, d, *J* = 8.0 Hz), 7.31 (2H, d, *J* = 8.0 Hz), 7.10 (1H, dd, *J* = 7.4, 7.4Hz), 7.01 (1H, d, *J* = 7.4 Hz), 6.72-6.64 (4H, m), 3.71 (2H, s).

2-(4-Butylstyryl)aniline (16h)

Compound **16h** (170 mg, as an *EZ* mixture) was prepared from **15h** by means of GP-C as a yellow paste. It was used for the next reaction without further purification.

2-(4-*tert*-Butylstyryl)aniline (16i)

Compound **16i** (680 mg, as an *EZ* mixture) was prepared from **16g** by means of GP-C as an orange solid. It was used for the next reaction without purification.

2-(2-Methoxystyryl)aniline (16j)

Compound **16j** (760 mg, as an *EZ* mixture) was prepared from **15j** by means of GP-C. It was used for the next reaction without further purification.

2-(3-Methoxystyryl)aniline (16k)

Compound **16k** (501 mg, as an *EZ* mixture) was prepared from **15k** by means of GP-C. It was used for the next reaction without further purification.

2-(3,4-Dimethoxystyryl)aniline (16l)

Compound **16l** was prepared from **15l** by means of GP-C. Purification with silica gel column chromatography gave **16n** (*E* isomer: 763 mg, *Z* isomer: 459 mg) in 85% yield.

E isomer: ^{1}H-NMR ($CDCl_3$) δ: 7.38 (1H, dd, *J* = 8.0, 1.1 Hz), 7.10 (1H, ddd, *J* = 7.7, 7.7, 1.2 Hz), 7.07-7.04 (2H, m), 7.03 (1H, d, *J* = 16.0 Hz), 6.93 (1H, d, *J* = 16.0 Hz) 6.87 (1H, d, *J* = 7.7 Hz), 6.81 (1H, ddd, *J* = 7.7, 7.7, 1.2 Hz), 6.73 (1H, d, *J* = 7.7 Hz), 3.94 (3H, s), 3.91 (3H, s).

Z isomer: ^{1}H-NMR ($CDCl_3$) δ: 7.12 (1H, d, *J* = 7.4 Hz), 7.07 (1H, ddd, *J* = 7.7, 7.7, 1.3 Hz), 6.80 (1H, ddd, *J* = 8.3, 8.3, 2.3 Hz), 6.74-6.69 (3H, m), 6.59 (1H, d, *J* = 12.0 Hz), 6.44 (1H, d, *J* = 12.0 Hz), 3.51 (3H, s), 3.83 (3H, s).

2-(3,5-Dimethoxystyryl)aniline (16m)

Compound **16m** (907 mg, as an *EZ* mixture) was prepared from **15m** by means of GP-C in 62% yield (2 steps) as an orange paste.

^{1}H-NMR ($CDCl_3$) (as a mixture: *E* : *Z* = 4 : 6) δ: 7.38-7.37 (1H, m), 7.14 (1H, d, *J* = 16.0 Hz), 7.10-7.17 (3H, m), 6.92 (1H, d, *J* = 16.0 Hz), 6.82-6.79 (1H, m), 7.72-6.65 (5H, m), 6.59 (1H, d, *J* = 12.0 Hz), 6.54 (1H, d, *J* = 12.0 Hz), 6.40-6.39 (3H, m), 6.29-6.28(1H, m), 3.83 (6H, s), 3.58 (6H, s)

2-(3,4,5-Trimethoxystyryl)aniline (16n)

Compound **16n** was prepared from **15n** by means of GP-C. Purification with silica gel column chromatography gave **16n** (*E* isomer: 305 mg, *Z* isomer: 560 mg) in 95% yield.
E isomer: a yellow paste. ^{1}H-NMR ($CDCl_3$) δ: 7.38 (1H, dd, *J* = 7.6, 1.3 Hz), 7.11 (1H, ddd, *J* = 7.6, 7.6, 1.3 Hz), 7.07 (1H, d, *J* = 16.0 Hz), 6.91 (1H, d, *J* = 16.0 Hz), 6.82 (1H, dd, *J* = 7.6, 7.6 Hz), 6.74-6.72 (3H, m), 3.91 (6H, s), 3.87 (3H, s).
Z isomer: a red oil. ^{1}H-NMR ($CDCl_3$) δ: 7.11 (1H, d, *J* = 7.4 Hz), 7.07 (1H, ddd, *J* = 7.4, 7.4, 1.3 Hz), 6.72-6.70 (2H, m), 6.55 (1H, d, *J* = 12.0 Hz), 6.49 (1H, d, *J* = 12.0 Hz), 6.46 (2H, s), 3.80 (3H, s), 3.60 (6H, s).

2-(2-Methylstyryl)aniline (16o)

Compound **16o** (809 mg, as an *EZ* mixture) was prepared from **15o** by means of GP-C. It was used for the next reaction without purification.

2-(3-Methylstyryl)aniline (16p)

Compound **16p** (601 mg, as an *EZ* mixture) was prepared from **15p** by means of GP-C. It was used for the next reaction without purification.

2-(3,4-Dimethylstyryl)aniline (16q)

Compound **16q** (1.11 g, as an *EZ* mixture) was prepared from **15q** by means of GP-C as an orange oil. It was used for the next reaction without further purification.

2-(3,5-Dimethylstyryl)aniline (16r)

Compound **16r** (653 mg, as an *EZ* mixture) was prepared from **15r** by means of GP-C as an orange oil. It was used for the next reaction without further purification.

General procedure D (GP-D) (Formation of phthalimide)

To a solution of substituted aniline in AcOH was added phthalic anhydride (1.5 eq). The mixture was refluxed for 3 h, and then the reaction was terminated by the addition of sat.

$NaHCO_3$. The resulting mixture was extracted with $CHCl_3$. The organic layer was dried over $MgSO_4$. The solvent was removed under reduced pressure, and the residue was purified by PTLC to afford the *E*-isomer.

(*E*)-2-(2-Styrylphenyl)isoindoline-1,3-dione (17a)

Compound **6c** (12.0 mg) was prepared from **16a** by means of GP-D in 5% yield as a yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 8.00-7.98 (2H, m), 7.86-7.80 (3H, m), 7.50 (1H, dd, *J* = 7.6, 7.6 Hz), 7.42 (1H, ddd, *J* = 7.6, 7.6, 1.5 Hz), 7.38-7.36 (2H, m), 7.29 (1H, ddd, *J* = 7.6, 7.6, 1.5 Hz), 7.26-7.26 (2H, m), 7.24-7.22 (1H, m), 7.12 (1H, d, *J* = 16.0 Hz), 6.91 (1H, d, *J* = 16.0 Hz).

HRMS (ESI) calcd for $C_{22}H_{15}NO_2Na$ 348.0995; found 348.0987 $(M + Na)^+$.

(*E*)-2-(2-(4-Methoxystyryl)phenyl)isoindoline-1,3-dione (17b)

Compound **17b** (14.7 mg) was prepared from **16b** by means of GP-D in 7% yield as a pale yellow paste.

^{1}H-NMR ($CDCl_3$) δ: 7.99-7.98 (2H, m), 7.83-7.80 (3H,m), 7.47 (1H, dd, *J* = 8.1, 8.1 Hz), 7.38 (1H, ddd, *J* = 8.1, 8.1, 1.3 Hz), 7.30 (2H, d, *J* = 8.6 Hz), 7.23 (1H, dd, *J* = 8.1, 1.3 Hz), 7.06 (1H, d, *J* = 16.3 Hz), 6.80 (2H, d, *J* = 8.6 Hz), 6.77 (1H, d, *J* = 16.3 Hz), 3.77 (3H, s).

HRMS (ESI) calcd for $C_{23}H_{17}NO_3Na$ 378.1101; found 378.1074 $(M + Na)^+$.

(*E*)-2-(2-(4-Fluorostyryl)phenyl)isoindoline-1,3-dione (17c)

Compound **17c** (11.0 mg) was prepared from **16c** by means of GP-D in 5% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 8.00-7.98 (2H, m), 7.85-7.80 (3H, m), 7.49 (1H, dd, *J* = 7.7, 7.7, 1.3 Hz), 7.42 (1H, dd, *J* = 7.7, 7.7, 1.3 Hz) 7.34-7.32 (2H, m), 7.25 (1H, dd, *J* = 7.7, 1.3 Hz), 7.07 (1H, d, *J* = 16.0 Hz), 6.96 (2H, dd, *J* = 7.7, 7.7 Hz), 6.41 (1H, d, *J* = 16.0 Hz).

HRMS (ESI) calcd for $C_{22}H_{14}FNO_2Na$ 366.0901; found 366.0920 $(M + Na)^+$.

(*E*)-2-(2-(4-Bromostyryl)phenyl)isoindoline-1,3-dione (17d)

Compound **17d** (40.0 mg) was prepared from **16d** by means of GP-D in 15% yield (2 steps) as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.98-7.83 (2H, ddd, *J* = 6.3, 6.3, 3.3 Hz), 7.83 (2H, ddd, *J* = 6.3, 6.3, 3.3 Hz), 7.80 (1H, d, *J* = 1.2 Hz), 7.49 (1H, ddd, *J* = 7.8, 7.8, 1.2 Hz), 7.43 (1H, ddd, *J* = 7.8, 7.8, 1.2 Hz), 7.39 (2H, d, *J* = 7.8 Hz), 7.24-7.25 (1H, m) 7.22 (2H, d, *J* = 7.8 Hz), 7.04 (1H, d, *J* = 16.0 Hz), 6.88 (1H, d, *J* = 16.0 Hz).

HRMS (ESI) calcd for $C_{22}H_{14}BrNO_2K$ 443.9822; found 443.9824 $(M + K)^+$.

(*E*)-4-(2-(1,3-Dioxoisoindolin-2-yl)styryl)benzonitrile (17e)

Compound **17e** (20.8 mg) was prepared from **16e** (100 mg, 0.454 mmol) by means of GP-D in 13% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 8.01-7.98 (2H, m), 7.85-7.83 (3H, m), 7.56 (2H, d, *J* = 7.5 Hz), 7.54-7.46 (2H, m), 7.44 (2H, d, *J* = 7.5 Hz), 7.28 (1H, d, *J* = 7.4 Hz), 7.11 (1H, d, *J* = 16.3 Hz), 7.01 (1H, d, *J* = 16.3 Hz). HRMS (ESI) calcd for $C_{23}H_{14}N_2O_2Na$ 373.0947; found 373.0948 $(M + Na)^+$.

(*E*)-2-(2-(4-Methylstyryl)phenyl)isoindoline-1,3-dione (17f)

Compound **17f** (10.0 mg) was prepared from **16f** by means of GP-D in 25% yield (2 steps) as a yellow oil.

H^1-NMR ($CDCl_3$) δ: 8.00-7.96 (2H, m), 7.83-7.81 (3H, m), 7.48 (1H, dd, *J* = 7.6, 7.6Hz), 7.40 (1H, ddd, *J* = 7.6, 7.6, 1.3 Hz), 7.27-7.23 (3H, m), 7.10-7.07 (2H,m), 7.08 (1H, d, *J* = 16.0 Hz), 6.85 (1H, d, *J* = 16.0 Hz), 2.31 (3H, s). HRMS (ESI) calcd for $C_{23}H_{17}NO_2Na$ 362.1151; found 362.1147 $(M + Na)^+$.

(*E*)-2-(2-(4-(Trifluoromethyl)styryl)phenyl)isoindoline-1,3-dione (17g)

Compound **17g** (4.9 mg) was prepared from **16g** by means of GP-D in 3.8% yield as a white solid.

^{1}H-NMR ($CDCl_3$) d: 8.00-7.97 (2H, m), 7.85-7.82 (3H, m), 7.51-7.47 (6H, m), 7.27 (1H, dd, *J* = 8.0, 1.1 Hz), 7.13 (1H, d, *J* = 16.3 Hz), 6.99 (1H, d, *J* = 16.3 Hz).

HRMS (ESI) calcd for $C_{23}H_{14}F_3NO_2Na$ 416.0869; found 416.0864 $(M + Na)^+$.

(*E*)-2-(2-(4-Butylstyryl)phenyl)isoindoline-1,3-dione (17h)

Compound **17h** (24.0 mg) was prepared from **16h** by means of GP-D in 0.5% yield (2 steps) as a yellow oil.

^{1}H-NMR ($CDCl_3$) δ: 7.99-7.98 (2H, m), 7.83-7.81 (3H, m), 7.47 (1H, dd, *J* = 7.8, 7.8 Hz), 7.39 (1H, dd, *J* = 7.8, 7.8 Hz), 7.27-7.21 (3H, m), 7.09-7.07 (3H, m), 6.86 (1H, d, *J* = 16.6 Hz), 2.56 (2H, t, *J* = 7.7 Hz), 1.55-1.53 (2H, m), 1.36-1.28 (2H, m), 0.90 (3H, t, *J* = 7.2 Hz).

HRMS (ESI) calcd for $C_{26}H_{23}NO_2Na$ 404.1621; found 404.1625 $(M + Na)^+$.

(*E*)-2-(2-(4-*tert*-Butylstyryl)phenyl)isoindoline-1,3-dione (17i)

Compound **17i** (47.7 mg) was prepared from **16i** by means of GP-D in 16% yield (3 steps) as white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.99-7.97 (2H, m), 7.83-7.81 (3H, m), 7.48 (1H, d, *J* = 7.6 Hz), 7.40 (1H, dd, *J* = 7.6, 1.3 Hz), 7.32-7.29 (4H, m), 7.24 (1H, dd, *J* = 7.6, 1.3 Hz), 7.09 (1H, d, *J* = 16.0 Hz), 6.87 (1H, d, *J* = 16.0 Hz), 1.28 (9H, s).

HRMS (ESI) calcd for $C_{26}H_{23}NO_2$ 404.1621; found 404.1628 $(M + Na)^+$.

(*E*)-2-(2-(2-Methoxystyryl)phenyl)isoindoline-1,3-dione (17j)

Compound **17j** (14.5 mg) was prepared from **16j** by means of GP-D in 12% yield (3 steps) as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.98 (2H, dd, *J* = 5.2, 2.5 Hz), 7.87 (1H, d, *J* = 8.0 Hz), 7.81 (2H, dd, *J* = 5.2, 2.5 Hz), 7.49 (1H, d, *J* = 8.0 Hz), 7.43 (1H, d, *J* = 16.0 Hz), 7.39-7.37 (2H,

m), 7.24 (1H, d, *J* = 7.5 Hz), 7.19 (1H, dd, *J* = 7.5, 7.5 Hz), 6.95 (1H, d, *J* = 16.0 Hz), 6.85 (1H, d, *J* = 7.5 Hz), 6.82 (1H, d, *J* = 7.5 Hz), 3.72 (3H, s).

HRMS (ESI) calcd for $C_{23}H_{17}NO_3Na$ 387.1101; found 387.1111 $(M + Na)^+$.

(*E*)-2-(2-(3-Methoxystyryl)phenyl)isoindoline-1,3-dione (17k)

Compound **17k** (18.0 mg) was prepared from **16k** by means of GP-D in 8.8% yield (2 steps) as a yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 7.98 (2H, dd, *J* = 5.7, 3.0 Hz), 7.82-7.81 (3H, m), 7.49 (1H, dd, *J* = 7.7, 1.3 Hz), 7.45-7.40 (1H, m), 7.24 (1H, dd, *J* = 7.7, 1.3 Hz), 7.19 (1H, dd, *J* = 7.7, 7.7 Hz), 7.07 (1H, d, *J* = 16.0 Hz), 6.97 (1H, dd, *J* = 7.4 Hz), 6.89 (1H, d, *J* = 16.0 Hz), 6.89 (1H, s), 6.78-6.79 (1H, m), 3.75 (3H, s).

HRMS (ESI) calcd for $C_{23}H_{17}NO_3$ 378.1101; found 378.1081 $(M + Na)^+$.

(*E*)-2-(2-(3,4-Dimethoxystyryl)phenyl)isoindoline-1,3-dione (17l)

Compound **17l** was prepared from the *E*-isomer of **16l** by means of GP-D in 94% yield as a yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 7.98 (2H, dd, *J* = 8.0, 3.5 Hz), 7.82 (2H, dd, *J* = 8.0, 3.5 Hz), 7.79 (1H, d, *J* = 8.0, 1.3 Hz), 7.48 (1H, ddd, *J* = 8.0, 8.0, 1.3 Hz), 7.40 (1H, d, *J* = 8.0, 1.3 Hz), 7.24 (1H, dd, *J* = 8.3, 1.6 Hz), 7.04 (1H, d, *J* = 16.0 Hz), 6.94 (1H, dd, *J* = 8.3, 1.6 Hz), 6.86 (1H, d, *J* = 1.6 Hz), 6.78 (1H, d, *J* = 8.6 Hz), 6.76 (1H, d, *J* = 16.0 Hz), 3.85 (3H, s), 3.80 (3H, s).

HRMS (ESI) calcd for $C_{24}H_{19}NO_4Na$ 408.1206; found 408.1235 $(M + Na)^+$.

(*Z*)-2-(2-(3,4-Dimethoxystyryl)phenyl)isoindoline-1,3-dione (18l)

Compound **18l** was prepared from *Z*-isomer of **16l** by means of GP-D in 99% yield as a yellow paste.

^{1}H-NMR ($CDCl_3$) δ: 7.89 (2H, dd, *J* = 5.2, 2.9 Hz), 7.76 (2H, dd, *J* = 5.2, 2.9 Hz), 7.39-7.35 (1H, m), 7.33-7.28 (3H, m), 6.67 (2H, dd, *J* = 9.5 Hz), 6.61 (1H, d, *J* = 1.1

Hz), 6.50 (1H, d, *J* = 12.0 Hz), 6.37 (1H, d, *J* = 12.0 Hz), 3.83 (3H, s), 3.53 (3H, s).

HRMS (ESI) calcd for $C_{24}H_{19}NO_4Na$ 408.1206; found 408.1208 $(M + Na)^+$.

(*E*)-2-(2-(3,5-Dimethoxystyryl)phenyl)isoindoline-1,3-dione (17m)

Compound **17m** was prepared from **16m** by means of GP-D in 12% yield as a pale yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 8.00-7.96 (2H, m), 7.84-7.80 (3H, m), 7.49 (1H, ddd, *J* = 7.7, 7.7, 1.3 Hz), 7.42 (1H,ddd, *J* = 7.7, 7.7, 1.3 Hz), 7.25 (1H, dd, *J* = 7.7, 1.3 Hz), 7.03 (1H, d, *J* = 16.0 Hz), 6.87 (1H, d, *J* = 16.0 Hz), 6.52 (2H, d, *J* = 2.3 Hz), 6.35 (1H, d, *J* = 2.3 Hz), 3.76 (6H, s).

HRMS (ESI) calcd for $C_{24}H_{19}NO_4Na$ 408.1206; found 408.1208 $(M + Na)^+$.

(*E*)-2-(2-(3,4,5-Trimethoxystyryl)phenyl)isoindoline-1,3-dione (17n)

Compound **17n** was prepared from **16n** (50.0 mg, 0.158 mmol) by means of GP-D in 20% yield as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.98 (2H, dd, *J* = 5.3, 3.0 Hz), 7.82 (2H, dd, *J* = 5.3, 3.0 Hz), 7.78 (1H, d, *J* = 7.7 Hz), 7.49 (1H, dd, *J* = 7.7, 7.7 Hz), 7.42 (1H, ddd, *J* = 7.7, 7.7, 1.3 Hz), 7.25 (1H, d, *J* = 1.3 Hz), 7.01 (1H, d, *J* = 16.0 Hz), 6.79 (1H, d, *J* = 16.0 Hz), 6.57 (2H, s), 3.81 (3H, s), 3.78 (6H, s).

HRMS (ESI) calcd for $C_{25}H_{21}NO_5Na$ 438.1312; found 438.1325 $(M + Na)^+$.

(*E*)-2-(2-(2-Methylstyryl)phenyl)isoindoline-1,3-dione (17o)

Compound **17o** (12.4 mg) was prepared from **16o** by means of GP-D in 9.5% yield (3 steps) as a white solid.

^{1}H-NMR ($CDCl_3$) δ: 7.97 (2H, dd, *J* = 5.7, 3.0 Hz), 7.83 (1H, s), 7.80 (2H, dd, *J* = 5.7, 3.0 Hz), 7.50 (1H, d, *J* = 8.0 Hz), 7.42 (1H, dd, *J* = 8.0, 1.5 Hz), 7.36 (1H, d, *J* = 7.5 Hz), 7.31 (1H, d, *J* = 16.0 Hz), 7.26-7.24 (1H, m), 7.11-7.08 (3H, m), 6.79 (1H, d, *J* = 16.0 Hz), 2.35 (3H, s).

HRMS (ESI) calcd for $C_{23}H_{17}NO_2Na$ 362.1151; found 362.1148 $(M + Na)^+$.

(*E*)-2-(2-(3-Methylstyryl)phenyl) isoindoline-1,3-dione (17p)

Compound **17p** (9.80 mg) was prepared from **16p** by means of GP-D in 18% yield (3 steps) as a pale yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 7.98 (2H, dd, J = 5.0, 3.0 Hz), 7.83-7.81 (1H, s), 7.82 (2H, dd, J = 5.0, 3.0 Hz), 7.49 (1H, d, *J* = 7.7, 7.7 Hz), 7.42 (1H, ddd, *J* = 7.7, 7.7, 1.5 Hz), 7.24 (1H, dd, *J* = 7.7, 1.5 Hz), 7.17-7.16 (3H, m), 7.08 (1H, d, *J* = 16.0 Hz), 7.04-7.02 (1H, m), 6.88 (1H, d, *J* = 16.0 Hz), 2.30 (3H, s).

HRMS (ESI) calcd for $C_{23}H_{17}NO_2Na$ 362.1151; found 362.1147 $(M + Na)^+$.

(*E*)-2-(2-(3,4-Dimethylstyryl)phenyl)isoindoline-1,3-dione (17q)

Compound **17q** (7.20 mg) was prepared from **16q** by means of GP-D in 4.5% yield (3 steps) as a yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 7.98 (2H, dd, *J* = 5.5, 2.9 Hz), 7.82-7.80 (1H, m), 7.81 (2H, dd, *J* = 5.5, 2.9 Hz), 7.48 (1H, dd, J = 7.7, 7.7 Hz), 7.40 (1H, dd, *J* = 7.7, 1.5 Hz), 7.23 (1H, dd, *J* = 7.7, 1.5 Hz), 7.12-7.02 (2H, m), 7.07-7.02 (2H, m), 6.84 (1H, d, *J* = 16.0 Hz), 2.21 (3H, s), 2.20 (3H, s).

HRMS (ESI) calcd for $C_{24}H_{19}NO_2Na$ 376.1308; found 376.1310 $(M + Na)^+$.

(*E*)-2-(2-(3,5-Dimethylstyryl)phenyl)isoindoline-1,3-dione (17r)

Compound **17r** (9.36 mg) was prepared from **16r** by means of GP-D in 18% yield (3 steps) as a yellow solid.

^{1}H-NMR ($CDCl_3$) δ: 7.98 (2H, dd, *J* = 5.5, 3.0 Hz), 7.82 (2H, dd, *J* = 5.5, 3.0 Hz), 7.79 (1H, d, *J* = 7.7 Hz), 7.48 (1H, dd, *J* = 7.7, 7.7 Hz), 7.40 (1H, ddd, *J* = 7.7, 7.7, 1.1 Hz), 7.24 (1H, dd, *J* = 7.7, 1.1 Hz), 7.04 (1H, d, *J* = 16.0 Hz), 6.98 (2H, s), 6.87 (1H, d, *J* = 16.0 Hz), 6.85 (br s), 2.25 (6H, s).

HRMS (ESI) calcd for $C_{24}H_{19}NO_2Na$ 376.1308; found 376.1309 $(M + Na)^+$.

General procedure E (GP-E) (Reduction by Pd/C)

Substituted nitrobenzene was dissolved in AcOEt and hydrogenated with 10% Pd/C (catalytic amount). The reaction mixture was stirred at room temperature and filtered through a pad of Celite. The filtrate was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (eluent: n-hexane/ethyl acetate = 10:1 to 3:1, v/v).

2-(4-Methoxyphenethyl)aniline (19b)

Compound **19b** (1.01 g, 4.44 mmol) was prepared from **15b** (1.40 g, 5.48 mmol) by means of GP-E in 66% yield as a pale brown solid.

^{1}H-NMR ($CDCl_3$) δ: 7.11 (2H, d, *J* = 8.9 Hz), 7.05-7.03 (2H, m), 6.83 (2H, d, *J* = 8.9 Hz), 6.77- 6.60 (2H, m), 3.79 (3H, s), 2.94-2.68 (4H, m).

2-(3,4-Dimethoxyphenethyl)aniline (19l)

Compound **19l** was prepared from **15l** by means of GP-E in 92% yield as a colorless oil.

^{1}H NMR (CDCl3) δ: 7.06–7.02 (m, 2H), 6.80 (d, 1H, *J* = 7.9 Hz), 6.76–6.73 (m, 2H), 6.67 (d, 1H, *J* = 7.9 Hz), 6.63 (d, 1H, *J* = 1.8 Hz), 3.86 (s, 3H), 3.81 (s, 3H), 3.47 (br s, 2H), 2.90–2.86 (m, 2H), 2.79–2.76 (m, 2H).

MS (FAB) *m/z*: 257 $(M)^+$, 258 $(M + H)^+$.

2-(2-(4-Methoxyphenethyl)phenyl)isoindoline-1,3-dione (20b)

Compound **20b** (86.0 mg) was prepared from **19b** by means of GP-D in 49% yield as a white solid.

^{1}H-NMR (CDCl3) δ: 7.96 (2H, dd, *J* = 5.4, 3.2 Hz), 7.80 (2H, dd, *J* = 5.4, 3.2 Hz), 7.42-7.33 (3H, m), 7.19 (1H, d, *J* = 8.0 Hz), 6.97 (2H, d, *J* = 8.6 Hz), 6.73 (2H, d, *J* = 8.6 Hz), 3.74 (3H, s), 2.82-2.74 (4H, m).

HRMS (ESI) calcd for $C_{23}H_{19}NO_3Na$ 380.1275; found 382.1253 $(M + Na)^+$.

2-(2-(3,4-Dimethoxyphenethyl)phenyl)isoindoline-1,3-dione (20l)

A mixture of phthalic anhydride (87 mg, 0.59 mmol) and **19l** (151 mg, 0.59 mmol) was heated at 200 ºC for 1 h. After the reaction was completed, the residue was purified by silica gel column chromatography to give the product (201 mg) in 88% yield as a white solid.

^{1}H NMR ($CDCl_3$) δ: 7.95 (dd, 2H, *J* = 5.5, 3.1 Hz), 7.80 (dd, 2H, *J* = 5.5, 3.1 Hz), 7.41–7.37 (m, 1H), 7.37–7.33 (m, 1H), 7.31 (dd, 1H, *J* = 7.3, 1.8 Hz), 7.20 (dd, 1H, *J* = 7.3, 1.8 Hz), 6.69 (d, 1H, *J* = 7.9 Hz), 6.59 (dd, 1H, *J* = 7.9, 1.8 Hz), 6.50 (d, 1H, *J* = 1.8 Hz), 3.80 (s, 3H), 3.68 (s, 3H), 2.79 (s, 4H).

MS (FAB) *m/z* 387 $(M)^+$, 388 $(M + H)^+$.

Anal. Calcd for $C_{24}H_{21}NO_4$: C, 74.40; H, 5.46; N, 3.62. Found: C, 74.34; H, 5.59; N, 3.55.

Docking simulation.

In silico molecular docking simulation was done with AutoDock (version 4.2). The target in this study was the X-ray structure of compound **4** bound to LXRα LBD (PDB ID: 3IPQ). The grid covered the binding site of **4**. The Lamarckian genetic algorithm was used for the molecular docking simulation. Dockings were automatically ranked by AutoDock according to the lowest calculated binding energies (kcal/mol).

Biology.

Cell Culture Conditions.

Human acute monocytic leukemia THP-1 cells were cultured in RPMI 1640 containing 10% fetal bovine serum (FBS), penicillin, and streptomycin mixture at 37 °C in a humidified atmosphere of 5% CO_2 in air. Human embryonic kidney (HEK) 293 cells were cultured in D-MEM containing 5% FBS, penicillin, and streptomycin mixture at 37 °C in a humidified atmosphere of 5% CO_2 in air.

Transient Transfection Assays.

HEK293 cells were plated at a density corresponding to 20% confluence in a 96-well plate 24 h prior to transfection. Cells were cotransfected with 15 ng of CMX-Gal4N-hLXRα LBD, CMX-Gal4N-hLXRβ LBD, CMX-Gal4N-hPPARα, CMX-Gal4N-hPPARγ, or CMX-Gal4N-hPPARδ expression plasmid, 50 ng of a tk-MH100 X 4-luc reporter, and 10 ng of CMX-β-galactosidase expression vector. Transfections were performed by the calcium phosphate coprecipitation method. After 24 h, transfected cells were treated with test compound or dimethyl sulfoxide (DMSO) for 16 h. Agonistic activity was measured at a concentration of 0.1−30 μM test compound, and antagonistic activity was measured under the same conditions in the presence of **3** (LXRα: 0.3 μM, LXRβ: 0.1 μM). Treated cells were assayed for luciferase activity in a luminometer. The luciferase activity of each sample was normalized by the level of β-galactosidase activity. Each transfection assay was carried out twice in triplicate. GW7647, GW501516 and rosiglitazone (BRL49653) were used as PPAR selective agonists for α, δ and γ, respectively. Each PPAR transfection assay was carried out once in triplicate.

Measurement of IL-6.

THP-1 cells were plated at a density of 4 x 10^6 cells/ 10 cm dish, and stimulated with 10 nM TPA for 48 h. The differentiated cells were plated at a density of 1.5 x 10^5 cells/well

(96-well plate) and stimulated with or without samples 6 h prior to treatment with or without LPS (100 ng/mL). After 18 h, the concentration of IL-6 in the culture supernatant was determined with an ELISA kit (Thermo) according to the manufacturer's protocol. Each assay was carried out twice in duplicate.

CRISPR/Cas9 LXRβ Knockout Assay

THP-1 cells were plated at a density corresponding to 10% confluence in a 6-well plate. A mixture of 1 μg of CRISPR/Cas9 LXRβ knockout Plasmid (Santa Cruz), 10 μL of LTX lipofectamine reagent and 200 μL Opti-MEM was incubated at rt for 5 min and added to the cells. For the control experiment, 10 μL of LTX lipofectamine reagent and 200 μL Opti-MEM was incubated at rt for 5 min and added to the cells. After 72 h, treated cells were stimulated with 10 nM TPA for 48 h. The differentiated cells were plated at a density of 1.5 x 10^5 cells/well (96-well plate) and stimulated with or without samples 6 h prior to treatment with or without LPS (100 ng/mL). After 18 h, the concentration of IL-6 in the culture supernatant was determined with an ELISA kit (Thermo) according to the manufacturer's protocol. Each assay was carried out twice in duplicate. Error bars represent SD. Statistics were calculated using the unpaired, two-sided Student's t-test.

Immunoblotting

The cells treated with or without transfection of CRISPR/Cas9 LXRβ knockout Plasmid (without LPS stimulation) were lysed. Protein concentrations of the lysates were determined using a BCA protein assay. Equivalent amounts of protein from each lysate were separated on SuperSep™ Ace 10-20% (Wako), and transferred onto PVDF membranes. After blocking with TBS containing 5% skim milk, the transblotted membranes were probed with anti-LXRβ antibody (Perseus) (1: 500 dilution) in 5% skim milk. The membrane was washed with TBS-T and probed by HRP conjugated anti-mouse IgG antibody (Millipore) (1:1000 dilution) in 5% skim milk. After probing,

the membrane was washed with TBS-T. The immunoblots were visualized by enhanced chemiluminescence with Immobilon^{TM} Western Chemiluminescent HRP Substrate (Millipore). And the antibodies were stripped by Restore plus Western blot stripping buffer (Thermo). The membrane was reproved by anti-β-tubulin antibody (Boehringer Mannheim) (1:200 dilution) in 5% skim milk, and HRP conjugated anti-mouse IgG antibody (Millipore) (1:1000 dilution).

LXRβ Competitive Binding Assay.

LanthaScreen TR-FRET LXRβ competitive binding assay kit (Invitrogen, Carlsbad, CA) was used as specified by the supplier documentation. In brief, 4×test compound and 0.4 μM control solutions (T0901317) were made up in TR-FRET LXRβ assay buffer (1% DMSO). They were pre-mixed at a ratio of 1:1 and an aliquot of 10 μL was added to a microwell plate (Corning). A mixture of 4×fluorescein-labeled coactivator peptide, 4×terbium (Tb)-labeled anti-GST antibody, and 8×LXRβ LBD was made up in TR-FRET LXRβ assay buffer and incubated for 30 min in the dark at room temperature. After compound addition, an aliquot of 10 μL was added to each test well. Plates were stored in the dark at room temperature for 2 h prior to evaluation with an EnVision reader (PerkinElmer). The TR-FRET ratio was calculated by dividing the emission signal at 520 nm by the emission signal at 492 nm. Data points represent duplicate averages. Error bars represent SD.

Real-Time Quantitative RT-PCR Analysis.

Total RNA from samples was prepared using NucleoSpin RNA (Macherey-Nagel). RNA was reverse-transcribed into complementary DNA (cDNA) using a Prime script RT-PCR kit (Takara). The reaction mixture included $MgCl_2$, reverse transcriptase buffer, diethyl pyrocarbonate-treated water, Oligo dT, AMV, inhibitor, and sample RNA.

Sequence-specific primers of *ABCA1*, *SREBP-1c*, and *GAPDH* (glyceraldehyde-3-phosphate dehydrogenase) were used for PCR (Table 1), and the

amplifications were accomplished under the following conditions: 42 °C for 30 min, 95 °C for 5 min, and 4 °C to the end. PCR products were electrophoresed on 2 % agarose gels and stained with ethidium bromide.

Real-time PCR was performed on a TP800 Thermal cycler Dice Real time (Takara) using SYBR Green PCR Master Mix (Takara). All samples were run in duplicate using the primers shown in Table S1. The mRNA values were normalized to the expression level of *GAPDH* mRNA. Error bars represent SD. Statistics were calculated using the unpaired, two-sided Student's t-test.

Table S1. Primer sequences

AAT CCT GAC CGG GTT GTT CCC	ABCA1
CCG CCT TCA CGT GCT TCT CA	ABCA1
ACT TCG CTC AGA CAC CAT GG	GAPDH
GTA GTT GAG GTC AAT GAA GGG	GAPDH
GCG CCT TGA CAG GTG AAG TC	SREBP1C
GCC AGG GAA GTC ACT GTC TTG	SREBP1C

Table S2. Purity of the synthesized compounds

compound	method	purity	compound	method	purity
7	A	100	**17j**	B	99.6
8	A	99.4	**17k**	B	99.3
9	B	98.2	**17l**	B	100
17a	B	98.4	**17m**	B	100
17b	B	99.0	**17n**	B	99.9
17c	B	99.4	**17o**	B	99.8
17d	B	99.0	**17p**	B	99.5
17e	B	96.7	**17q**	B	99.1
17f	B	97.2	**17r**	B	99.2
17g	B	99.4	**18l**	B	96.6
17h	B	100	**20b**	B	98.6
17i	B	98.8	**20l**	B	99.5

Column: Inertsil® ODS-4, 5 μm, 4.6 x 150 mm. Mobile phase: Method A: CH_3CN/water = 80/20. Method B: CH_3CN/water = 70/30. Temperature: 40 ºC. Flow rate: 1.0 mL/min. Detection: 285–305 nm.