Supporting Information

Discovery of a potent retinoid X receptor antagonist structurally closely related to RXR agonist NEt-3IB

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Contents:

2. Chemistry

Melting points were determined with a Yanagimoto hot-stage melting point apparatus and are uncorrected. IR spectra were recorded on JASCO FT/IR-350 (KBr). ^{1}H-NMR spectra were recorded on a JEOL JNM-AL300 FT-NMR system (300 MHz) spectrometer. Elemental analysis was carried out with a Yanagimoto MT-5 CHN recorder elemental analyzer. FAB-MS was carried out with a JEOL JMS-700 MStation.

General Procedure for hydrolysis of 13a, 13b, 13g (GP-1).

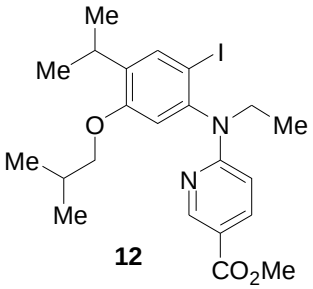
To a solution of each methyl ester intermediate (1.0 mmol) in MeOH (10 mL) was added 2 N NaOH (5.0 mL). The reaction mixture was stirred at 60°C for 1 hr, then neutralized with 2 N HCl. The mixture was extracted with EtOAc (3 × 30 mL). The organic layer was washed with water and brine, and dried over $MgSO_4$. The solution was evaporated under reduced pressure. The residue was purified by flash column chromatography to afford the desired product.

General Procedure for hydrolysis of 13e, 13f (GP-2).

To a solution of each methyl ester intermediate (1.0 mmol) in MeOH (10 mL) were added 2 N NaOH (5.0 mL) and THF (3.0 mL). The reaction mixture was stirred at 60°C for 1 hr, then neutralized with 2 N HCl. The mixture was extracted with EtOAc (3 × 30 mL). The organic layer was washed with water and brine, and dried over $MgSO_4$. The solution was evaporated under reduced pressure. The residue was purified by flash column chromatography or re-crystallized to provide the desired product.

Methyl 6-[*N*-ethyl-*N*-(3-isobutoxy-4-isopropylphenyl)amino]nicotinate (11).

This compound was prepared according to reference 1.



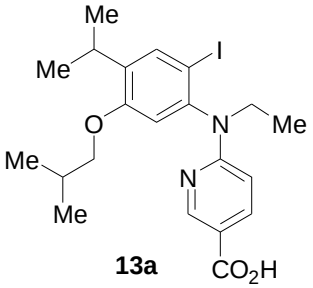
12

Methyl 6-[*N*-ethyl-*N*-(2-iodo-5-isobutoxy-4-isopropylphenyl)amino]nicotinate (12).

To a solution of **11** (200 mg, 0.54 mmol) in MeOH (4.0 mL) were added Ag_2SO_4 (170 mg, 0.54 mmol) and I_2 (160 mg, 0.62 mmol). The mixture was stirred at r.t. for 11 h, then *sat.* $Na_2S_2O_3$ (4.0 mL) was added, and the whole was filtered through Celite. The filtrate was extracted with EtOAc (30 mL × 3). The organic layer was washed with H_2O (50 mL × 2) and brine (50 mL),

then dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the resulting crude material was purified by flash column chromatography (15:1 *n*-hexane:EtOAc) to provide 190 mg (72%) of **12** as a yellow oil.

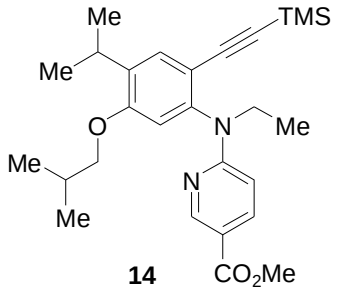
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.89 (d, *J* = 2.0 Hz, 1H), 7.86 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.68 (s, 1H), 6.65 (s, 1H), 5.98 (d, *J* = 9.0 Hz, 1H), 4.32 (dq, *J* = 14.0, 7.0 Hz, 1H), 3.86 (s, 3H), 3.67-3.59 (m, 3H), 3.31 (sep, *J* = 7.0 Hz, 1H), 2.21 (sep, *J* = 6.5 Hz, 1H), 1.27 (d, *J* = 7.0 Hz, 6H), 1.25 (t, *J* = 6.5 Hz, 3H), 1.05 (d, *J* = 6.5 Hz, 6H).



6-[*N*-Ethyl-*N*-(2-iodo-5-isobutoxy-4-isopropylphenyl)amino]nicotinic acid (13a).

Hydrolysis of **12** was performed according to GP-1. The resulting crude material was purified by flash column chromatography (1:1 *n*-hexane:EtOAc) to provide 41 mg (85%) of **13a** as a white solid.

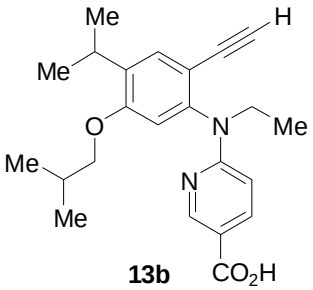
M.p. 228.0-231.5 ºC; HPLC (50 mM $AcONH_4$ aq:MeOH = 10:90): 11.14 min. 100% purity; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.97 (d, *J* = 2.0 Hz, 1H), 7.71 (s, 1H), 7.70 (dd, *J* = 9.0, 2.0 Hz, 1H), 6.66 (s, 1H), 6.00 (d, *J* = 9.0 Hz, 1H), 4.35 (dq, *J* = 14.0, 7.0 Hz, 1H), 3.68-3.59 (m, 3H), 3.31 (sep, *J* = 7.0 Hz, 1H), 2.12 (sep, *J* = 6.5 Hz, 1H), 1.27 (d, *J* = 7.0 Hz, 6H), 1.26 (t, *J* = 7.0 Hz, 3H), 1.05 (d, *J* = 6.5 Hz, 6H); FAB-MS: *m/z*: 483 $[M + H]^+$.



Methyl 6-[*N*-ethyl-*N*-(5-isobutoxy-4-isopropyl-2-trimethylsilanylethynylphenyl)amino]nicotinate (14).

According to reference 2, to a solution of **12** (450 mg, 0.90 mmol), $PdCl_2(PPh_3)_2$ (13 mg, 0.018 mmol), CuI (1.7 mg, 0.0090 mmol) in dry THF (1.8 mL) and triethylamine (900 μL) was added trimethylsilylacetylene (760 μL, 5.4 mmol). The mixture was stirred under an Ar atmosphere at

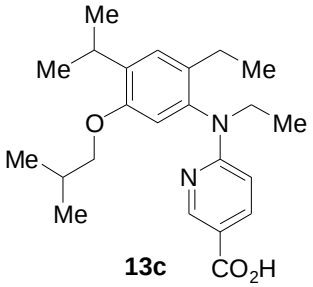
r.t. for 10 h, then poured into H_2O and extracted with EtOAc (30 mL × 3). The organic layer was washed with H_2O (30 mL × 2) and brine (30 mL), then dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the resulting crude material was purified by flash column chromatography (15:1 *n*-hexane:EtOAc) to provide 340 mg (81%) of **14** as a yellow oil.
^{1}H-NMR (300 MHz, DMSO-*d*6) δ: 8.79 (d, *J* = 2.3 Hz, 1H), 7.92 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.42 (s, 1H), 7.06 (s, 1H), 6.21 (d, *J* = 8.7 Hz, 1H), 3.91-3.87 (m, 5H), 3.38 (m, 3H), 2.14 (sep, *J* = 6.5 Hz, 1H), 1.30 (d, *J* = 7.0 Hz, 6H), 1.23 (t, *J* = 7.5 Hz, 3H), 1.09 (d, *J* = 6.5 Hz, 6H), 0.00 (s, 9H).



13b

6-[*N*-Ethyl-*N*-(2-ethynyl-5-isobutoxy-4-isopropylphenyl)amino]nicotinic acid (13b).
To a solution of **14** (130 mg, 0.27 mmol) in MeOH (2.3 mL) was added K_2CO_3 (110 mg, 0.81 mmol). The mixture was stirred at r.t. for 6 h, then poured into H_2O (70 mL) and extracted with EtOAc (30 mL × 3). The organic layer was washed with H_2O (30 mL × 2) and brine (30 mL), then dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the resulting crude material was purified by flash column chromatography (15:1 *n*-hexane:EtOAc) to provide 79 mg (74%) of a colorless oil.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.87 (d, *J* = 2.5 Hz, 1H), 7.84 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.44 (s, 1H), 6.62 (s, 1H), 6.61 (d, *J* = 9.0 Hz, 1H), 4.07 (q, *J* = 7.0 Hz, 2H), 3.86 (s, 3H), 3.86 (d, *J* = 6.5 Hz, 2H), 3.33 (sep, *J* = 7.0 Hz, 1H), 2.98 (s, 1H), 2.05 (sep, *J* = 6.5 Hz, 1H), 1.26 (d, *J* = 6.5 Hz, 6H), 1.25 (t, *J* = 7.0 Hz, 3H), 1.05 (d, *J* = 7.0 Hz, 6H).

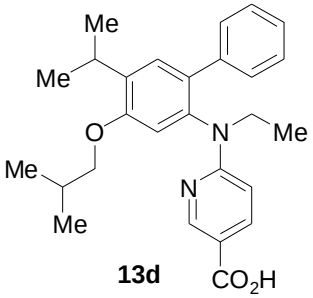
Hydrolysis of the methyl ester precursor of **13b** was performed according to GP-1. The resulting crude material was purified by flash column chromatography (1:1 to 0:1 *n*-hexane:EtOAc) to provide 62 mg (82%) of **13b** as a brown solid.
M.p. 162.8-164.0 ºC; HPLC (50 mM $AcONH_4$ aq:MeOH = 10:90): 8.73 min. 99.5% purity; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.92 (d, *J* = 2.0 Hz, 1H), 7.86 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.42 (s, 1H), 6.60 (s, 1H), 6.10 (d, *J* = 9.0 Hz, 1H), 4.11 (q, *J* = 7.0 Hz, 2H), 3.68 (d, *J* = 6.5 Hz, 2H), 3.31 (sep, *J* = 7.0 Hz, 1H), 2.11 (sep, *J* = 6.5 Hz, 1H), 1.24 (t, *J* = 7.0 Hz, 3H), 1.24 (d, *J* = 7.0 Hz, 6H), 1.03 (d, *J* = 6.5 Hz, 6H); FAB-MS: m/z: 381 $[M + H]^+$.



6-[*N*-Ethyl-*N*-(2-ethyl-5-isobutoxy-4-isopropylphenyl)amino]nicotinic acid (13c).

To a solution of **13b** (30 mg, 0.079 mmol) in MeOH (2.5 mL) was added 10% activated Pd-C (catalytic amount). The reaction mixture was stirred under an H_2 atmosphere at r.t. for 5 h, then filtered through Celite. The filtrate was washed with H_2O (40 mL × 2) and brine (40 mL), then dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the resulting crude material was purified by flash column chromatography (8:1 *n*-hexane:EtOAc) to provide 21 mg (70%) of **13c** as a white solid.

M.p. 172.8-175.3 ºC; HPLC (50 mM $AcONH_4$ aq:MeOH = 10:90): 11.99 min. 99.7% purity; ^{1}H-NMR (300 MHz, CDCl3) δ: 8.95 (d, *J* = 2.5 Hz, 1H), 7.84 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.19 (s, 1H), 6.52 (s, 1H), 6.00 (d, *J* = 9.0 Hz, 1H), 4.32 (dq, *J* = 14.0, 7.0 Hz, 1H), 3.70-3.60 (m, 3H), 3.35 (sep, *J* = 7.0 Hz, 1H), 2.40 (q, *J* = 7.5 Hz, 2H), 2.11 (sep, *J* = 6.5 Hz, 1H), 1.28 (t, *J* = 5.5 Hz, 3H), 1.27 (d, *J* = 7.0 Hz, 6H), 1.11 (t, *J* = 7.5 Hz, 3H), 1.05 (d, *J* = 6.5 Hz, 6H); FAB-MS: m/z: 385 $[M + H]^+$.



6-[*N*-Ethyl-*N*-(4-isobutoxy-5-isopropylbiphenyl-2-yl)amino]nicotinic acid (13d).

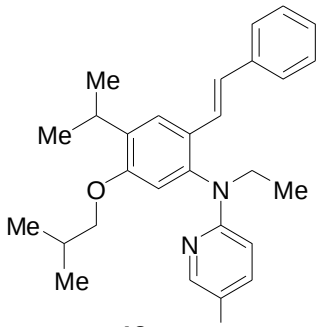
According to reference 3, to a solution of **12** (100 mg, 0.20 mmol) in EtOH (2 mL) were added phenylboronic acid (27 mg, 0.22 mmol), Na_2CO_3 (42 mg, 0.40 mmol) and 10% activated Pd-C (catalytic amount). The mixture was stirred under an Ar atmosphere at 80ºC for 5 h, then filtered through Celite. The filtrate was washed with H_2O (40 mL × 2) and brine (40 mL), then dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the resulting crude material was purified by flash column chromatography (20:1 *n*-hexane:EtOAc) to provide 71 mg (79%) of a white solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.84 (d, *J* = 2.0 Hz, 1H), 7.90 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.32 (s,

1H), 7.29-7.20 (m, 5H), 6.39 (d, *J* = 9.0 Hz, 1H), 4.03 (s, 1H), 3.87 (s, 3H), 3.69 (d, *J* = 6.5 Hz, 2H), 3.39 (sep, *J* = 7.0 Hz, 1H), 2.97 (dq, *J* = 14.0, 7.0 Hz, 1H), 2.13 (sep, *J* = 6.5 Hz, 1H), 1.29 (d, *J* = 7.0 Hz, 6H), 1.06 (t, *J* = 7.0 Hz, 3H), 1.06 (d, *J* = 6.5 Hz, 6H).

To a solution of the residue (71 mg, 0.16 mmol) in MeOH (10 mL) were added THF (3 mL) and 2 N NaOH (4.2 mL). The mixture was stirred at 60ºC for 3 h, then evaporated under reduced pressure. The residue was neutralized with *sat.* NH_4Cl, and extracted with EtOAc (30 mL × 3). The organic layer was washed with H_2O (50 mL × 2) and brine (50 mL), then dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the resulting crude material was purified by flash column chromatography (2:1 *n*-hexane:EtOAc) to provide 53 mg (77%) of **13d** as a white solid.

M.p. 237.0-239.5ºC; HPLC (50 mM $AcONH_4$ aq:MeOH = 10:90): 13.17 min. 98.9% purity; ^{1}H-NMR (300 MHz, DMSO-*d*6) δ: 8.91 (d, *J* = 2.5 Hz, 1H), 7.92 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.31 (s, 1H), 7.30-7.20 (m, 6H), 6.62 (s, 1H), 6.41 (d, *J* = 9.0 Hz, 1H), 4.04 (br s, 1H), 3.69 (t, *J* = 6.4 Hz, 2H), 3.40 (sep, *J* = 7.0 Hz, 1H), 2.98 (dq, *J* = 14.0, 7.0 Hz, 1H), 2.16−2.09 (m, 1H), 1.31−1.26 (m, 6H), 1.07 (d, *J* = 6.4 Hz, 6H), 1.07 (t, *J* = 7.0 Hz, 3H); FAB-MS: m/z: 433 $[M + H]^+$.



13e

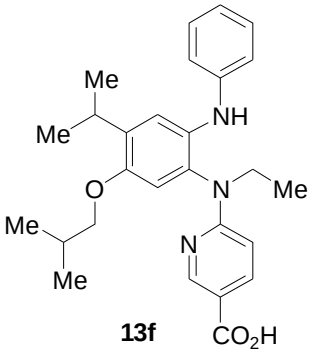
6-[*N*-Ethyl-*N*-(5-isobutoxy-4-isopropyl-2-(*E*)-styrylphenyl)amino]nicotinic acid (13e).

According to reference 4, to **12** (200 mg, 0.40 mmol) were added styrene (460 μL, 4.0 mmol), $Pd(OAc)_2$ (9.0 mg, 0.040 mmol), tri(*o*-tolyl)phosphine (24 mg, 0.080 mmol), triethylamine (280 μL, 2.0 mmol) and acetonitrile (480 μL). The mixture was stirred under an Ar atmosphere at 110ºC for 3 days, then filtered through Celite. The filtrate was washed with H_2O (50 mL × 2) and brine (50 mL), then dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the resulting crude material was purified by flash column chromatography (30:1 *n*-hexane:EtOAc) to provide 184 mg (97%) of a brown oil.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.90 (d, *J* = 2.0 Hz, 1H), 7.81 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.65 (s, 1H), 7.38-7.18 (m, 5H), 6.79 (d, *J* = 8.0 Hz, 2H), 6.57 (s, 1H), 6.07 (d, *J* = 9.0 Hz, 1H), 4.24 (dq, *J* = 14.0, 7.0 Hz, 1H), 3.86-3.84 (m, 4H), 3.69 (d, *J* = 6.7, 2H), 3.39 (sep, *J* = 7.0 Hz, 1H), 2.13

(sep, J = 6.7 Hz, 1H), 1.33 (dd, J = 7.0, 3.0 Hz, 6H), 1.26 (t, J = 7.0 Hz, 3H), 1.06 (d, J = 6.7 Hz, 6H).

Hydrolysis of the methyl ester precursor of **13e** was performed according to GP-2. The residue was re-crystallized from MeOH/H_2O to provide 16 mg (35%) of **13e** as white crystals.
M.p. 204.8-218.7ºC decomp.; HPLC (50 mM $AcONH_4$ aq:MeOH = 10:90): 17.32 min. 98.6% purity; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 12.51 (s, 1H), 8.73 (d, J = 2.0 Hz, 1H), 7.80 (dd, J = 9.0, 2.0 Hz, 1H), 7.78 (s, 1H), 7.39-7.18 (m, 6H), 6.87 (d, J = 16.5 Hz, 1H), 6.81 (s, 1H), 6.03 (d, J = 9.0 Hz, 1H), 4.09 (dq, J = 14.0, 7.0 Hz, 1H), 3.90 (dq, J = 14.0, 7.0 Hz, 1H), 3.77 (t, J = 5.0 Hz, 2H), 3.31 (sep, J = 6.5 Hz, 1H), 2.06 (sep, J = 6.5 Hz, 1H), 1.31 (d, J = 6.5 Hz, 6H), 1.19 (t, J = 7.0 Hz, 3H), 1.01 (d, J = 6.5 Hz, 6H); FAB-MS: m/z: 459 $[M + H]^+$.



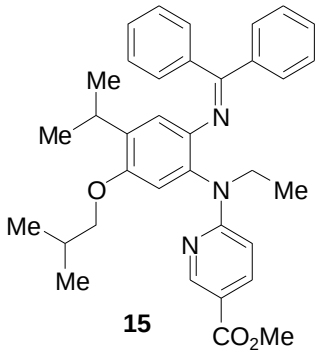
13f

6-[*N*-Ethyl-*N*-(5-isobutoxy-4-isopropyl-2-anilinophenyl)amino]nicotinic acid (13f).
According to reference 5, to **12** (100 mg, 0.20 mmol) were added Cs_2CO_3 (360 mg, 1.1 mmol), $Pd(OAc)_2$ (1.4 mg, 0.0060 mmol), (±) BINAP (4.6 mg, 0.0073 mmol) and toluene (800 μL), and then aniline (130 μL, 1.4 mmol) was added dropwise to the solution. The mixture was stirred under an Ar atmosphere at 110ºC for 3 days, then filtered through Celite. The filtrate was washed with H_2O (50 mL × 2) and brine (50 mL), then dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the resulting crude material was purified by flash column chromatography (10:1 *n*-hexane:EtOAc) 64 mg (69%) to provide a yellow solid.
^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.88 (d, J = 2.0 Hz, 1H), 7.86 (dd, J = 9.0, 2.0 Hz, 1H), 7.34 (s, 1H), 7.21 (t, J = 7.5 Hz, 2H), 6.91 (d, J = 7.5 Hz, 2H), 6.86 (t, J = 7.5 Hz, 1H), 6.61 (s, 1H), 6.22 (d, J = 9.0 Hz, 1H), 5.37 (s, 1H), 4.04 (br s, 1H), 3.87-3.82 (m, 4H), 3.65 (d, J = 6.5 Hz, 2H), 3.36 (sep, J = 7.0 Hz), 2.11 (sep, J = 6.5 Hz, 1H), 1.24 (d, J = 7.0 Hz, 6H), 1.22 (t, J = 7.0 Hz, 3H), 1.05 (d, J=6.5 Hz, 6H).

Hydrolysis of the methyl ester precursor of **13f** was performed according to GP-2. The resulting crude material was purified by flash column chromatography (2:1 *n*-hexane:EtOAc) to provide

37 mg (q.y.) of **13f** as a white solid.

M.p. 233.0-234.9ºC; HPLC (50 mM $AcONH_4$ aq:MeOH = 10:90): 12.03 min. 99.7% purity; ^{1}H-NMR (300 MHz, DMSO-*d*6) δ: 12.46 (br s, 1H), 8.66 (d, *J* = 2.0 Hz, 1H), 7.79 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.21 (s, 1H), 7.10 (t, *J* = 7.6 Hz, 2H), 6.86 (d, *J* = 7.6 Hz, 2H), 6.72 (s, 1H), 6.68 (t, *J* = 7.6 Hz, 1H), 6.24 (d, *J* = 9.0 Hz, 1H), 4.13 (br s, 1H), 3.68 (d, *J* = 6.5 Hz, 2H), 3.49 (br s, 1H), 3.29 (m, 1H), 2.02 (sep, *J* = 6.5 Hz, 1H), 1.19 (d, *J* = 7.0 Hz, 6H), 1.11 (t, *J* = 7.0 Hz, 3H), 1.00 (d, *J* = 6.5 Hz, 6H); FAB-MS: m/z: 448 $[M + H]^+$.

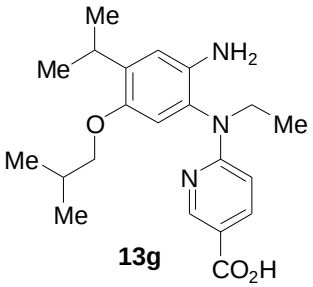


15

Methyl 6-{*N*-[2-(Benzhydrylideneamino)-5-isobutoxy-4-isopropylphenyl]-*N*-ethylamino}nicotinate (15).

According to reference 5, to **12** (100 mg, 0.20 mmol) were added Cs_2CO_3 (180 μg, 0.56 mmol), $Pd(OAc)_2$ (4.5 mg, 0.020 mmol), (±) BINAP (19 mg, 0.030 mmol) and toluene (800 μL), and then benzophenoneimine (81 mL, 0.48 mmol) was added dropwise to the solution. The mixture was stirred under an Ar atmosphere at 100ºC for 2 days, then filtered through Celite. The filtrate was washed with H_2O (30 mL × 2) and brine (40 mL), then dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the resulting crude material was purified by flash column chromatography (15:1 *n*-hexane:EtOAc) to provide 48 mg (44%) of **15** as a yellow oil.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.77 (d, *J* = 2.0 Hz, 1H), 7.65 (dd, *J* = 9.0, 2.0 Hz, 1H), 7.59 (d, *J* = 8.0 Hz, 2H), 7.43–7.16 (m, 6H), 7.02 (d, *J* = 8.5, 1.5 Hz, 2H), 6.69 (s, 1H), 6.50 (s, 1H), 5.86 (d, *J* = 9.0 Hz, 1H), 3.86 (s, 3H), 3.57 (d, *J* = 6.5 Hz, 2H), 3.24 (sep, *J* = 7.0 Hz, 1H), 2.06 (sep, *J* = 6.5 Hz, 1H), 1.23 (t, *J* = 7.0 Hz, 3H), 1.07 (d, *J* = 7.0 Hz, 6H), 1.01 (d, *J* = 6.5 Hz, 6H).



13g

6-[*N*-(2-Amino-5-isobutoxy-4-isopropylphenyl)-*N*-ethylamino]nicotinic acid (13g).

To a solution of **15** (67 mg, 0.12 mmol) in THF (400 μL) was added 2 N HCl (80 μL). The mixture was stirred at r.t. for 4 h, then poured into H_2O, alkalinized with 2 N NaOH and extracted with EtOAc (30 mL × 3). The organic layer was washed with H_2O (20 mL × 2) and brine (20 mL), then dried over $MgSO_4$. The solvent was evaporated under reduced pressure and the resulting crude material was purified by flash column chromatography (3:1 *n*-hexane:EtOAc) to provide 45 mg (96%) of a yellow solid.

^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.87 (d, *J* = 2.5 Hz, 1H), 7.85 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.74 (s, 1H), 6.50 (s, 1H), 6.15 (d, *J* = 9.0 Hz, 1H), 4.11 (dq, *J* = 14.0, 7.0 Hz, 1H), 3.87 (dq, *J* = 14.0, 7.0 Hz, 1H), 3.87 (s, 3H), 3.60 (d, *J* = 6.5 Hz, 2H), 3.40 (br s, 2H), 3.32 (sep, *J* = 7.0 Hz, 1H), 2.08 (sep, *J* = 6.5 Hz, 1H), 1.25 (d, *J* = 7.0 Hz, 6H), 1.23 (t, *J* = 7.0 Hz, 3H), 1.03 (d, *J* = 6.5 Hz, 6H).

Hydrolysis of the methyl ester precursor of **13g** was performed according to GP-1. The resulting crude material was purified by flash column chromatography (30:1 CH_2Cl_2:MeOH) to provide 14 mg (60%) of **13g** as a yellow solid.

M.p. 197.5-200.5ºC; HPLC (25 mM $AcONH_4$ aq:MeOH = 20:80): 13.41 min. 98.6% purity; ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 8.92 (d, *J* = 2.3 Hz, 1H), 7.88 (dd, *J* = 9.2, 2.3 Hz, 1H), 6.74 (s, 1H), 6.50 (s, 1H), 6.16 (d, *J* = 9.2 Hz, 1H), 4.13 (dq, *J* = 14.0, 7.0 Hz, 1H), 3.89 (dq, *J* = 14.0, 7.0 Hz, 1H), 3.60 (d, *J* = 6.5 Hz, 2H), 3.32 (sep, *J* = 7.0 Hz, 1H), 2.08 (sep, *J* = 6.5 Hz, 1H), 1.30-1.22 (m, 9H), 1.08 (d, *J* = 6.5 Hz, 6H); FAB-MS: m/z: 372 $[M + H]^+$.

3. Combustion analysis data

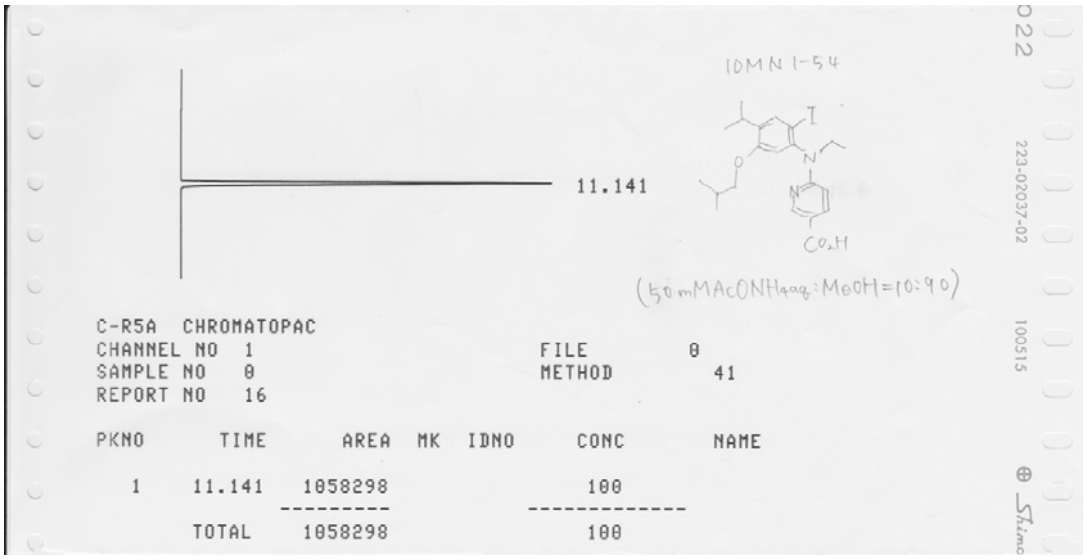
Table S1. Combustion analysis data for compounds **13a–13g**

compound	Formula	Calculated			Found		
		C	H	N	C	H	N
13a	$C_{21}H_{27}IN_2O_3$	52.29	5.64	5.81	52.00	5.48	5.72
13b	$C_{23}H_{28}N_2O_3$	72.60	7.42	7.36	72.32	7.42	7.36
13c	$C_{23}H_{32}N_2O_3 \cdot 1/8\ H_2O$	71.43	8.40	7.24	71.37	8.16	7.16
13d	$C_{27}H_{32}N_2O_3$	74.97	7.46	6.48	74.63	7.20	6.27
13e	$C_{29}H_{34}N_2O_3 \cdot 1/4\ H_2O$	75.21	7.51	6.05	75.37	7.45	6.08
13f	$C_{27}H_{33}N_3O_3$	72.46	7.43	9.39	72.23	7.26	9.24
13g	$C_{21}H_{29}N_3O_3$	67.90	7.87	11.31	67.69	7.77	11.19

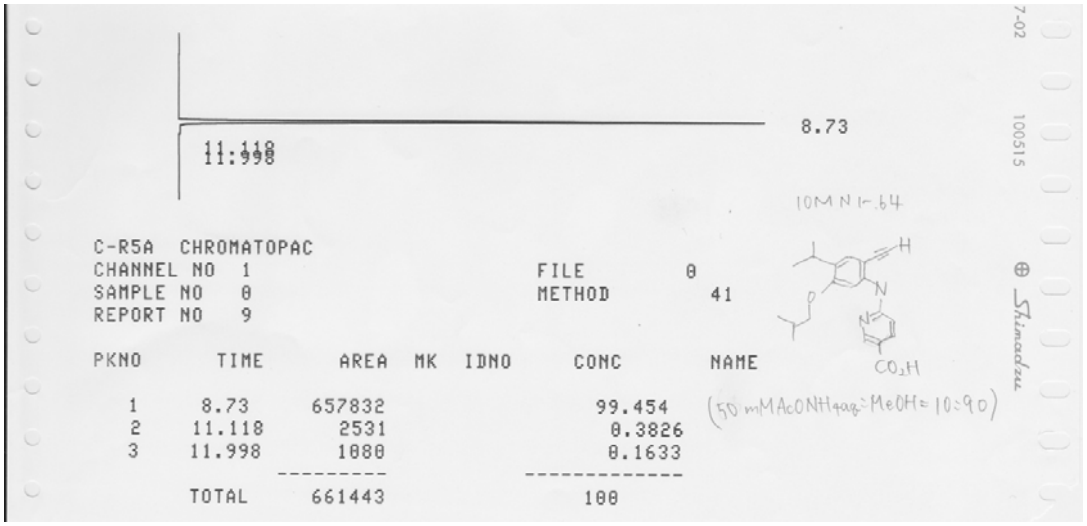
4. HPLC Charts

The HPLC system used was a Shimadzu liquid chromatographic system (Kyoto, Japan) consisting of a LC-10AD pump, SPD-10AV UV-Vis spectrophotometric detector, CTO-10AS column oven and C-R5A Chromatopac. The chromatographic analyses were carried out on an Inertsil ODS-3 column (4.6 i.d. x 250 mm, 5 μm, GL Sciences, Tokyo, Japan) with a guard column of Inertsil ODS-3 (4.6 i.d. x 10 mm, 5 μm, GL Sciences) kept at 40°C, using methanol : 50 mM ammonium acetate (adjusted with acetic acid to pH 5.0) (90:10 v/v) (**13a-f**) or methanol : 25 mM ammonium acetate (adjusted with acetic acid to pH 5.0) (80:20 v/v) (**13g**) as the mobile phase. The flow rate was 0.7 mL/min and the absorbance at 280 nm was monitored.

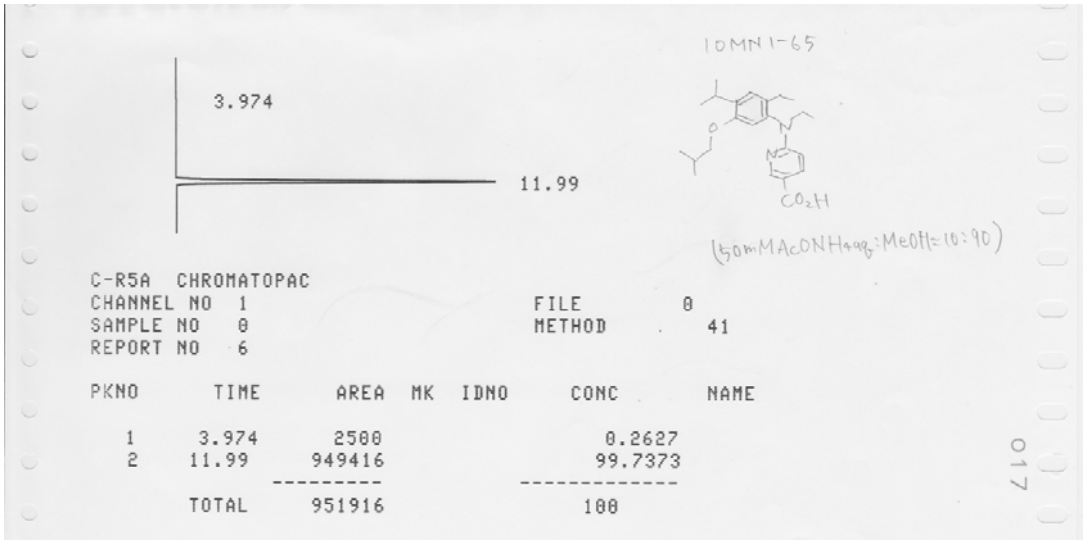
13a: 50 mM $AcONH_4$ aq:MeOH = 10:90



13b: 50 mM $AcONH_4$ aq:MeOH = 10:90



13c: 50 mM $AcONH_4$ aq:MeOH = 10:90



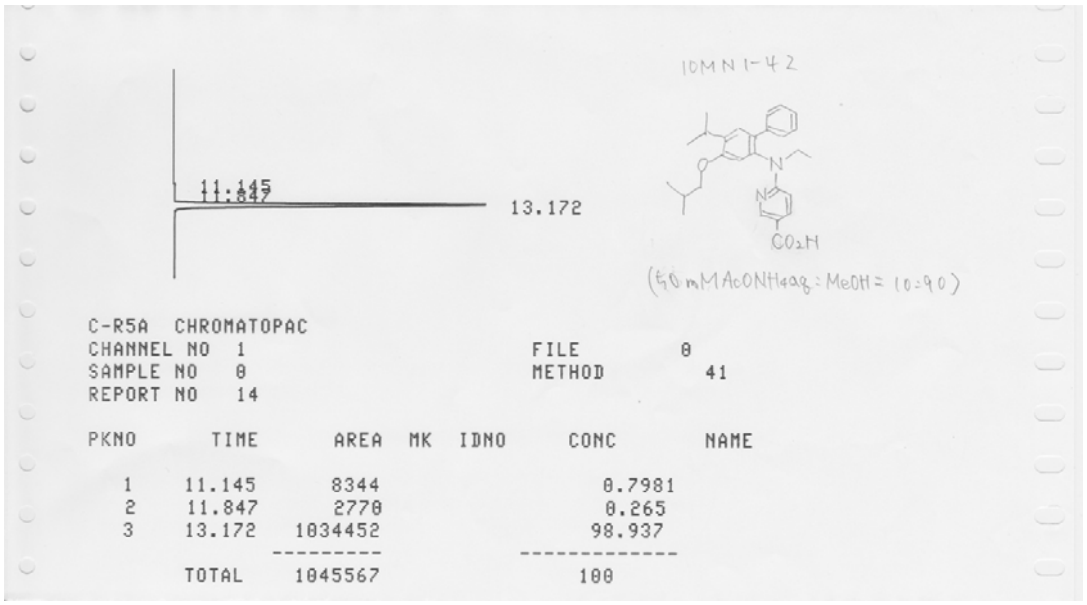
C-R5A CHROMATOPAC

CHANNEL NO	1			FILE	0	
SAMPLE NO	0			METHOD	41	
REPORT NO	6					

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.974	2500			0.2627	
2	11.99	949416			99.7373	
	TOTAL	951916			100	

017

13d: 50 mM $AcONH_4$ aq:MeOH = 10:90

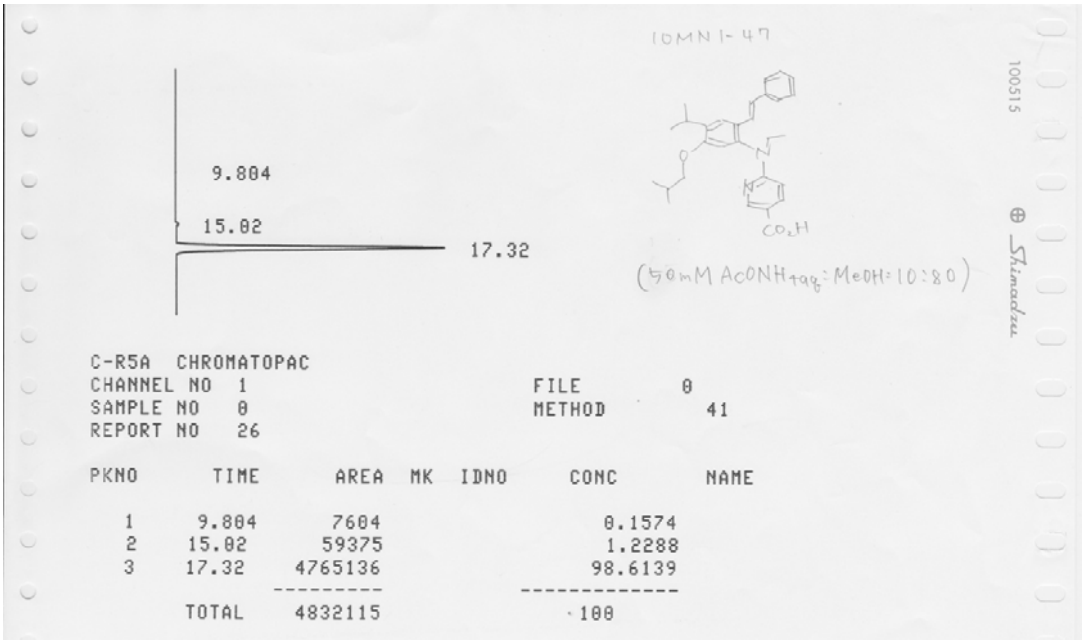


C-R5A CHROMATOPAC

CHANNEL NO	1			FILE	0	
SAMPLE NO	0			METHOD	41	
REPORT NO	14					

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	11.145	8344			0.7981	
2	11.847	2770			0.265	
3	13.172	1034452			98.937	
	TOTAL	1045567			100	

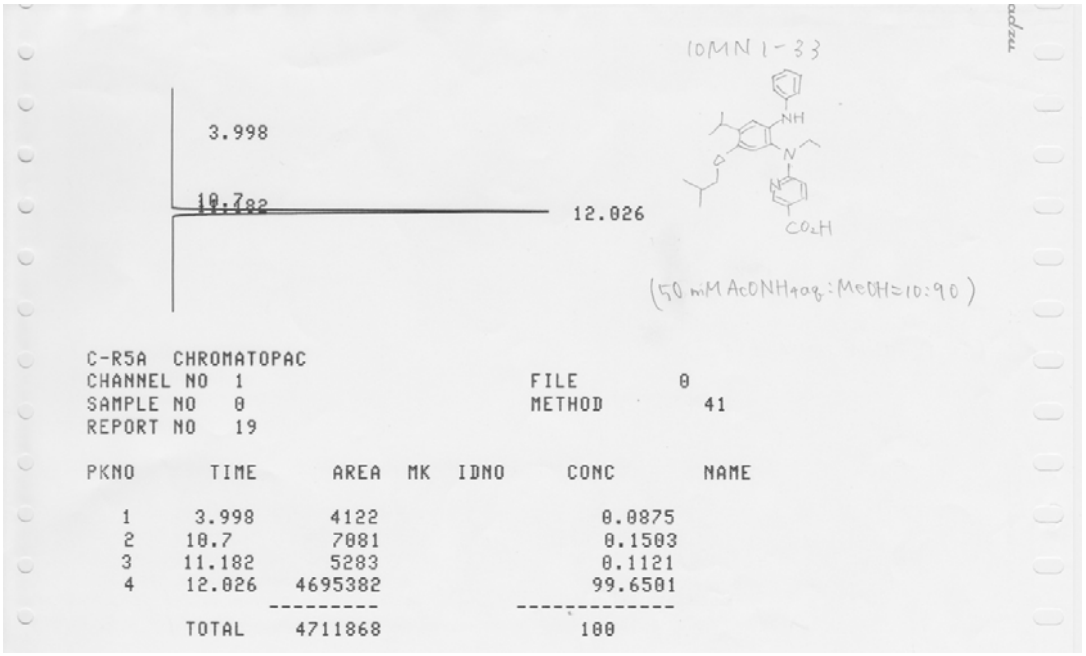
13e: 50 mM $AcONH_4$ aq:MeOH = 10:90



C-R5A CHROMATOPAC
CHANNEL NO 1 FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 26

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	9.804	7604			0.1574	
2	15.02	59375			1.2288	
3	17.32	4765136			98.6139	
	TOTAL	4832115			100	

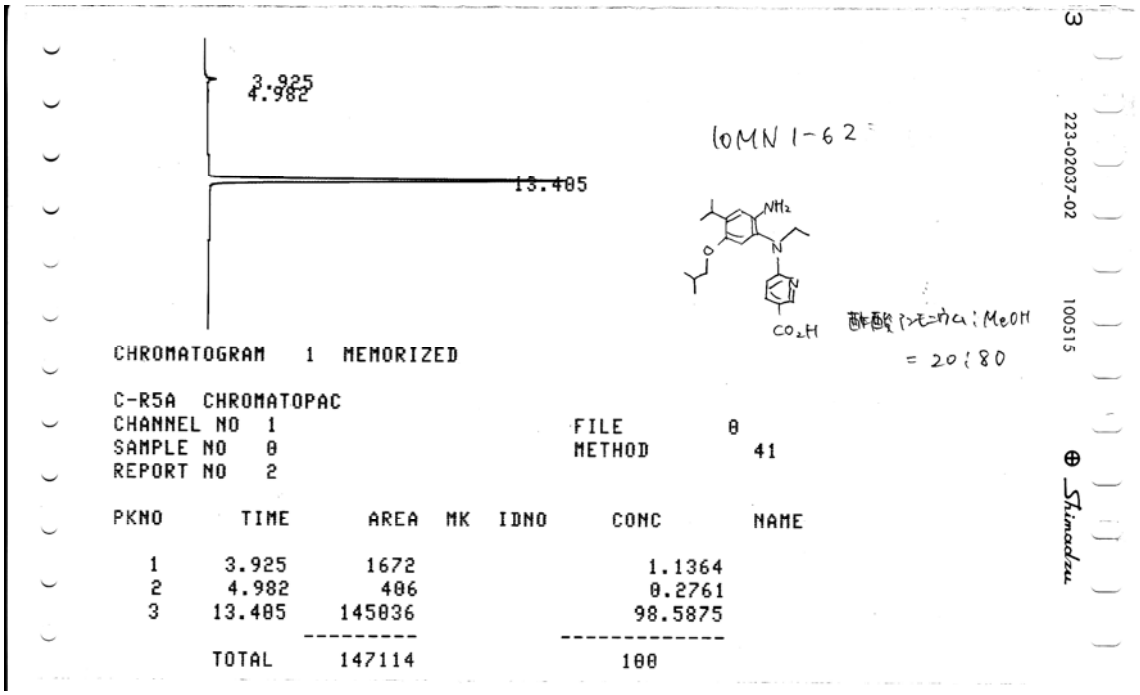
13f: 50 mM $AcONH_4$ aq:MeOH = 10:90



C-R5A CHROMATOPAC
CHANNEL NO 1 FILE 0
SAMPLE NO 0 METHOD 41
REPORT NO 19

PKNO	TIME	AREA	MK	IDNO	CONC	NAME
1	3.998	4122			0.0875	
2	10.7	7081			0.1503	
3	11.182	5283			0.1121	
4	12.026	4695382			99.6501	
	TOTAL	4711868			100	

13g: 25 mM $AcONH_4$ aq:MeOH = 20:80



5. Luciferase Reporter Gene Assay

Culture of COS-1 cells. COS-1 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% FBS in a humidified atmosphere of 5% CO_2 in air at 37ºC.

Luciferase reporter gene assay. Luciferase reporter gene assays were performed using COS-1 cells transfected with three kinds of vectors: receptor subtype (1.0 eq.), a luciferase reporter gene (4.0 eq.) under the control of the appropriate RXR response element, and secreted alkaline phosphatase (SEAP) gene (1.0 eq.) as a background. A tk-CRBPII-Luc reporter plasmid for RXR was purified with a QIA filter Plasmid Midi kit. COS-1 cells were transfected with QIA Effectene Transfection reagent according to the supplier's protocol. Test compound solutions whose DMSO concentrations were below 1% were added to transfected cells seeded at about 4 $\times$ 10^4 cells/mL in 96-well white plates. For vehicle and positive controls, the same volume of DMSO and LGD1069 (**1**) solution in DMSO were added, respectively. After incubation in a humidified atmosphere of 5% CO_2 in air at 37ºC for 18 h, some of the medium was used for SEAP and the remaining cells were used for luciferase reporter gene assays with a Steady-Glo Luciferase Assay system (Promega) according to the supplier's protocol. The luciferase activities were normalized using secreted SEAP[6] activities. Assays were carried out in triplicate three times.

Assay for antagonist activity. RXR antagonist activity was determined by measuring the agonistic activity of NEt-TMN (**2**)[7] (10^{-8} to 10^{-5} M) in the presence of a test compound at 0.1 to 1 μM.

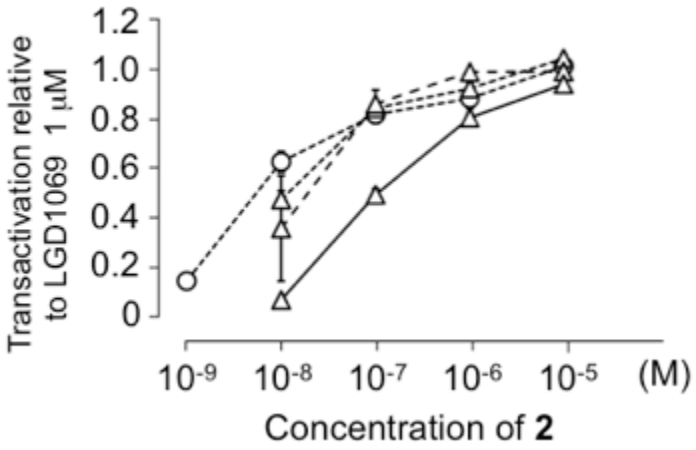


Figure. Dose-dependent RXRα agonistic activity of **2** in the absence or presence of compound **9**. Open circles indicate **2** in the absence of **9**. Open triangles indicate **2** in the presence of **9**.

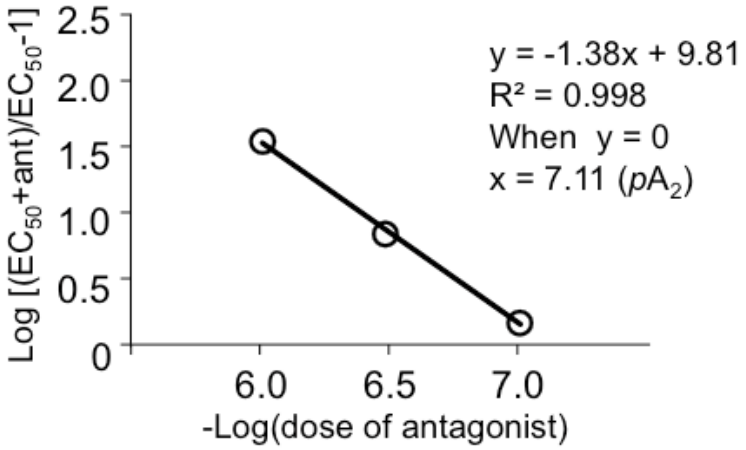
Dotted line, broken line, normal line indicate **2** in the presence of **9** at 0.1, 0.33, or 1 μM, respectively. Relative luciferase transactivation activity was calculated based on the activity of 1 μM **1**, taken as 1.0.

6. Schild Plot for pA_2 determination[8]

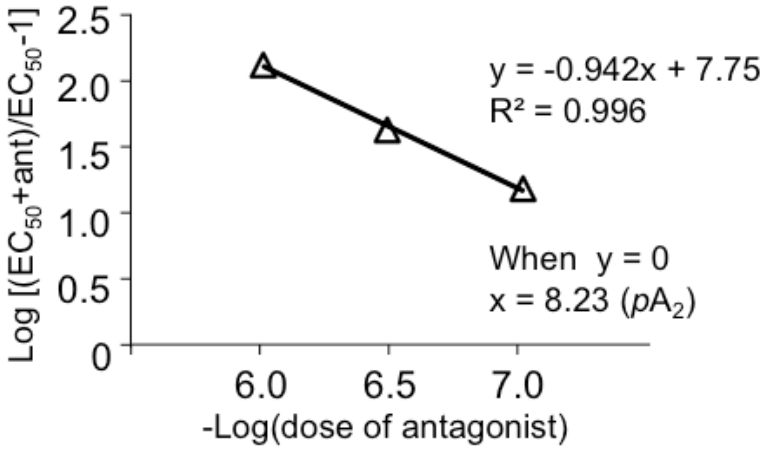
COS-1 cells were incubated with increasing concentrations of NEt-TMN or NEt-3IB (10^{-8} to 10^{-5} M) and three different concentrations of test compounds. The pA_2 values, as defined by Arunlakshana and Schild,[8] were obtained from the plot of (DR-1) vs negative log of the antagonist concentration.

DR (dose-ratio); EC_{50} in the presence of antagonist/EC_{50} in the absence of antagonist

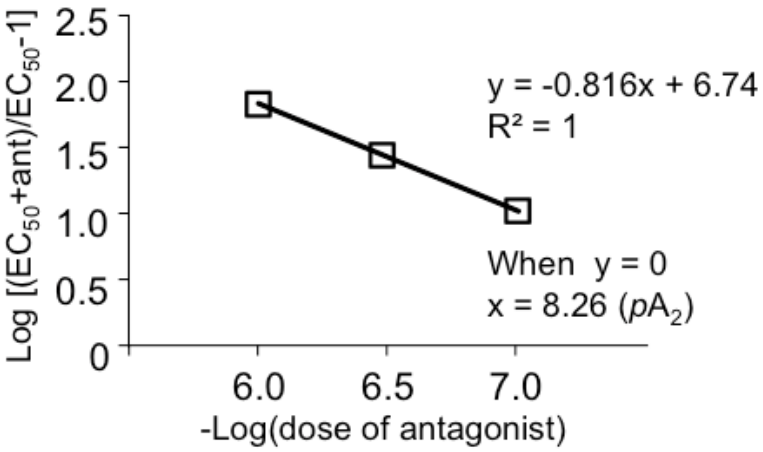
Compound 9 (vs NEt-TMN)



Compound 13e (vs NEt-TMN)



Compound 13e (vs NEt-3IB)



7. Molecular docking

The crystal structures of human RXRα ligand-binding domain were retrieved from the Brookhaven Protein Data Bank: http://www.rcsb.org/pdb/Welcome.do. (2P1V,[9] 3A9E [10]). Polar hydrogen atoms were added to both the protein and the ligand. United atom Kollman charges were assigned for the protein. The 3D structures of ligands used for the docking study were constructed by using Winmostar.[11] These ligands were energetically minimized by using Molecular Mechanics (MM). The AutoDock4.0 molecular docking program[12] was employed by using a genetic algorithm with local search (GALS). One hundred individual GA runs, 150 chromosomes, a crossover ratio of 0.80, a rate of gene mutation of 0.02, and an elitism ratio of 0.10 were used for each ligand. Molfeat [FiatLux Co., Tokyo, Japan] was used for molecular modeling.

8. REFERENCES

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