物 **21** と，**15a–j** に対応する種々のアルキルハライドを反応させることで **22a–j** を得，加水分解によって目的化合物 **15a–j** を合成した．

General methods.

Melting points were determined with a Yanagimoto hot-stage melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a VarianVXR-300 (300 MHz), JEOL JMN-AL-300 (300 MHz) or VarianVXR-500 (500 MHz) spectrometer. Elemental analysis was carried out with a Yanagimoto MT-5 CHN recorder elemental analyzer and results were within ±0.4% of the theoretical. FAB-MS was carried out with a VG70-SE.

HPLC conditions

The HPLC system used in this study was a Shimadzu liquid chromatographic system (Kyoto, Japan) consisting of an LC-10AD pump, SPD-10AV UV-Vis spectrophotometric detector, CTO-10AS column oven and C-R5A Chromatopac. The samples (each 20 μL) were injected. The chromatographic analyses were carried out on an Inertsil ODS-3 (4.6 i.d. x 250 mm, 5 μm, GL Sciences, Tokyo, Japan) with a guard column of Inertsil ODS-3 (4.0 i.d. x 10 mm, 5 μm, GL Sciences) kept at 40°C, using methanol : 25 mM ammonium acetate (adjusted with acetic acid to pH 5.0) (80:20 or 70:30, v/v) as a mobile phase. The flow rate was 0.7 mL/min and the absorbance at 278 nm was monitored.

2-Isopropyl-4-nitrophenol (**17**).

Conc. HNO_3 (3.4 mL, 45 mmol) was added to a solution of 2-isopropylphenol (6.1 mL, 45 mmol) in EtOAc (200 mL) in an ice bath. The reaction mixture was placed in an ultrasonic reactor, and $ZnCl_2$ (6.1 g, 45 mmol) was added, then stirred for 0.8 h. The reaction mixture was washed with H_2O (2 × 100 mL) and brine (100 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash chromatography to yield **17** (4.9 g, 60%) as brown solid. 1H NMR (300 MHz, $CDCl_3$) δ : 8.13 (d, J = 2.8 Hz, 1H), 8.00 (dd, J = 9.0, 2.8 Hz, 1H), 6.83 (d, J = 9.0 Hz, 1H), 6.04 (s, 1H), 3.26 (sep, J = 7.0 Hz, 1H), 1.30 (d, J = 7.0 Hz, 6H).

Isopropoxy-2-isopropyl-4-nitrobenzene (**18**).

2-Bromopropane (990 μL, 11 mmol), K_2CO_3 (2.6 g, 19 mmol) and KI (800 mg, 4.8 mmol) were added to a solution of **17** (1.7 g, 9.6 mmol) in dry DMF (4.8 mL). The reaction mixture was stirred at 70°C under Ar atmosphere for 3 h. Then the reaction mixture was poured into 2N HCl (70 mL) and extracted with EtOAc (3 × 80 mL). The organic layer was collected, washed with H_2O (2 × 80 mL) and brine (80 mL), and dried over $MgSO_4$. The solvent was

evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **18** (1.7 g, 80%) as orange oil. 1H NMR (300 MHz, $CDCl_3$) δ : 8.13 (d, *J* = 2.5 Hz, 1H), 8.07 (dd, *J* = 9.0, 3.0 Hz, 1H), 6.86 (d, *J* = 9.0 Hz, 1H), 4.70 (sep, *J* = 6.0 Hz, 1H), 3.31 (sep, *J* = 7.0 Hz, 1H), 1.40 (d, *J* = 6.0 Hz, 6H), 1.24 (d, *J* = 7.0 Hz, 6H).

Methyl 6-[(4-isopropoxy-3-isopropylphenyl)amino]pyridine-3-carboxylate (19).

10% activated Pd-C (catalytic amount) was added to a solution of **18** (4.7 g, 21 mmol) in MeOH (30 mL). The reaction mixture was stirred under H_2 atmosphere at room temperature for 5.5 h. The reaction mixture was filtered through celite, and the celite cake was washed with EtOAc. The solvent was evaporated under reduced pressure. 6-Chloronicotinic acid (3.7 g, 23 mmol) and $MeSO_3H$ (1.5 mL, 23 mmol) was added to a solution of the residue in dry dioxane (30 mL). The reaction mixture was stirred at 120°C under Ar atmosphere for 17 h. The reaction mixture was evaporated under reduced pressure. Conc. H_2SO_4 (2.0 mL) was added to a solution of the residue in dry MeOH (20 mL). The reaction mixture was stirred at 80°C under Ar atmosphere for 5.7 h. The reaction mixture was evaporated under reduced pressure, and neutralized with 2N NaOH, then extracted with EtOAc (3 × 80 mL). The organic layer was collected, washed with H_2O (2 × 100 mL) and brine (100 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **19** (5.6 g, 73%) as brown solid. 1H NMR (300 MHz, $CDCl_3$) δ : 8.79 (d, *J* = 2.2 Hz, 1H), 8.00 (dd, *J* = 8.8, 2.2 Hz, 1H), 7.12–7.09 (m, 2H), 6.90 (s, 1H), 6.85 (d, *J* = 9.2 Hz, 1H), 6.64 (d, *J* = 8.8 Hz, 1H), 4.54 (sep, *J* = 6.0 Hz, 1H), 3.88 (s, 3H), 3.33 (sep, *J* = 7.0 Hz, 1H), 1.36 (d, *J* = 6.0 Hz, 6H), 1.20 (d, *J* = 7.0 Hz, 6H).

Methyl 6-[ethyl-(4-isopropoxy-3-isopropylphenyl) amino]pyridine-3-carboxylate (20).

Compound **19** (1.2 g, 3.6 mmol) was added to a suspension of NaH (200 mg, 5.0 mmol, 60% dispersion in oil) in dry DMF (6.0 mL) at rt under Ar atmosphere. After stirring for 5 min, iodoethane (400 μL, 5.0 mmol) was added, and then it was stirred for 0.3 h. The reaction mixture was poured into ice and extracted with EtOAc (3 × 40 mL). The organic layer was collected, washed with H_2O (2 × 50 mL) and brine (30 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **20** (960 mg, 75%) as brown oil. 1H NMR (300 MHz, $CDCl_3$) δ :

8.83 (d, J = 2.5 Hz, 1H), 7.77 (dd, J = 9.0, 2.5 Hz, 1H), 7.01 (d, J = 2.5 Hz, 1H), 6.95 (dd, J = 8.5, 2.5 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.15 (d, J = 9.0 Hz, 1H), 4.58 (sep, J = 6.0 Hz, 1H), 4.00 (q, J = 7.0 Hz, 2H), 3.85 (s, 3H), 3.32 (sep, J = 7.0 Hz, 1H), 1.38 (d, J = 6.0 Hz, 6H), 1.22 (t, J = 7.0 Hz, 3H), 1.19 (d, J = 7.0 Hz, 6H).

Methyl 6-[ethyl-(4-hydroxy-3-isopropylphenyl)amino]pyridine-3-carboxylate (21).

$AlCl_3$ (1.2 g, 9.0 mmol) was added to a solution of **19** (960 mg, 2.7 mmol) in dry CH_2Cl_2 (14 mL). The reaction mixture was stirred at rt under Ar atmosphere for 3.5 h. Then the reaction mixture was poured into H_2O (100 mL) and extracted with EtOAc (3 × 50 mL). The organic layer was collected, washed with H_2O (2 × 50 mL) and brine (50 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **20** (820 mg, 97%) as pale yellow oil. 1H NMR (300 MHz, $CDCl_3$) δ : 8.83 (d, J = 2.5 Hz, 1H), 7.78 (dd, J = 9.0, 2.5 Hz, 1H), 7.02 (d, J = 2.5 Hz, 1H), 6.90 (dd, J = 8.5, 2.5 Hz, 1H), 6.82 (d, J = 8.5 Hz, 1H), 6.15 (d, J = 9.0 Hz, 1H), 5.06 (s, 1H), 4.00 (q, J = 7.0 Hz, 2H), 3.86 (s, 3H), 3.22 (sep, J = 7.0 Hz, 1H), 1.25 (d, J = 7.0 Hz, 6H), 1.24 (t, J = 7.0 Hz, 3H).

Methyl 6-[ethyl-(4-isobutoxy-3-isopropylphenyl)amino]pyridine-3-carboxylate (22a).

1-Bromo-2-methylpropane (370 μL, 3.4 mmol), K_2CO_3 (240 mg, 1.7 mmol) and KI (catalytic amount) were added to a solution of **21** (310 mg, 1.0 mmol) in dry DMF (10 mL). The reaction mixture was stirred at 60°C under Ar atmosphere for 18 h. Then the reaction mixture was poured into H_2O (100 mL) and extracted with EtOAc (3 × 30 mL). The organic layer was collected, washed with H_2O (2 × 50 mL) and brine (30 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **22a** (300 mg, 81%) as pale yellow oil. 1H NMR (300 MHz, $CDCl_3$) δ : 8.83 (d, J = 2.5 Hz, 1H), 7.77 (dd, J = 9.0, 2.5 Hz, 1H), 7.62 (d, J = 2.5 Hz, 1H), 6.97 (dd, J = 8.5, 2.5 Hz, 1H), 6.86 (d, J = 8.5 Hz, 1H), 6.14 (d, J = 9.0 Hz, 1H), 4.00 (q, J = 7.0 Hz, 2H), 3.85 (s, 3H), 3.77 (sep, J = 6.0 Hz,1H), 3.36 (sep, J = 7.0 Hz, 1H), 2.22–2.08 (m, 1H), 1.22 (d, J = 7.0 Hz, 6H), 1.22 (t, J = 7.0 Hz, 3H), 1.08 (d, J = 6.5 Hz, 6H).

Methyl 6-[ethyl-(4-cyclopropylmethoxy-3-isopropylphenyl)amino]pyridine-3-carboxylate (22b).

(Bromomethoxy)cyclopropane (100 μL, 1.1 mmol), K_2CO_3 (190 mg, 1.4 mmol) and

KI (60 mg, 0.35 mmol) were added to a solution of **21** (220 mg, 0.69 mmol) in dry DMF (3.0 mL). The reaction mixture was stirred at 60°C under Ar atmosphere for 4 h. Then the reaction mixture was poured into H_2O (20 mL) and acidified with 2N HCl, then extracted with EtOAc (3 × 30 mL). The organic layer was collected, washed with H_2O (2 × 30 mL) and brine (30 mL), and dried over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **22b** (240 mg, 94%) as pale yellow oil. 1H NMR (300 MHz, $CDCl_3$) δ : 8.82 (dd, *J* = 2.5, 0.5 Hz, 1H), 7.76 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.02 (d, *J* = 2.5 Hz, 1H), 6.95 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.84 (d, *J* = 8.5 Hz, 1H), 6.14 (d, *J* = 9.0, 0.5 Hz, 1H), 4.00 (q, *J* = 7.0 Hz, 2H), 3.86 (d, *J* = 7.5 Hz, 2H), 3.85 (s, 3H), 3.37 (sep, *J* = 7.0 Hz, 1H), 1.23−1.20 (m, 5H), 0.67−0.62 (m, 2H), 0.40−0.38 (m, 2H).

Methyl 6-{ethyl-[3-isopropyl-4-(3-methyl-but-2-enyloxy)phenyl]amino}pyridine-3-carboxylate (22c).

1-Bromo-3-methyl-2-butene (210 μL, 1.8 mmol), K_2CO_3 (330 mg, 2.4 mmol) and KI (catalytic amount) were added to a solution of **21** (190 mg, 0.60 mmol) in dry DMF (20 mL). The reaction mixture was stirred at 120°C under Ar atmosphere for 21 h. Then the reaction mixture was poured into H_2O (120 mL) and extracted with EtOAc (3 × 50 mL). The organic layer was collected, washed with H_2O (2 × 50 mL) and brine (50 mL), and dried over MgSO4. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **22c** (62 mg, 27%) as colorless oil. 1H NMR (300 MHz, $CDCl_3$) δ : 8.83 (dd, *J* = 2.5, 0.5 Hz, 1H), 7.78 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.02 (d, *J* = 2.5 Hz, 1H), 6.97 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.89 (d, *J* = 8.5 Hz, 1H), 6.16 (dd, *J* = 9.0, 0.5 Hz, 1H), 5.52 (t, *J* = 6.5 Hz, 1H), 4.55 (d, *J* = 6.5 Hz, 2H), 4.01 (q, *J* = 7.0 Hz, 2H), 3.85 (s, 3H), 3.35 (sep, *J* = 7.0 Hz, 1H), 1.82 (s, 3H), 1.76 (s, 3H), 1.26 (t, *J* = 7.0 Hz, 3H), 1.20 (d, *J* = 7.0 Hz, 6H).

Methyl 6-{ethyl-[3-isopropyl-4-(2-methylallyloxy)phenyl]amino}pyridine-3-carboxylate (22d).

3-Bromo-2-methylpropene (100 μL, 1.0 mmol), K_2CO_3 (69 mg, 0.50 mmol) and KI (catalytic amount) were added to a solution of **21** (80 mg, 0.25 mmol) in dry DMF (3.0 mL). The reaction mixture was stirred at 60°C under Ar atmosphere for 4 h. Then the reaction mixture was poured into H_2O (60 mL) and acidified with 2N HCl, then extracted with EtOAc (3 × 20 mL). The organic layer was collected, washed with H_2O (40 mL) and brine (40 mL), and dried

over $MgSO_4$. The solvent was evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **22d** (73 mg, 78%) as pale yellow oil. 1H NMR (300 MHz, $CDCl_3$) δ : 8.83 (dd, *J* = 2.5, 0.5 Hz, 1H), 7.77 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.03 (d, *J* = 2.5 Hz, 1H), 6.97 (dd, *J* = 9.0, 2.5 Hz, 1H), 6.88 (d, *J* = 8.5 Hz, 1H), 6.14 (dd, *J* = 9.0, 0.5 Hz, 1H), 5.15 (s, 1H), 5.02 (s, 1H), 4.47 (s, 2H), 4.01 (q, *J* = 7.0 Hz, 2H), 3.85 (s, 3H), 3.39 (sep, *J* = 7.0 Hz, 1H), 1.88 (s, 3H), 1.22 (t, *J* = 7.0 Hz, 3H), 1.22 (d, *J* = 7.0 Hz, 6H).

Methyl 6-[(4-butoxy-3-isopropylphenyl)ethylamino]pyridine-3-carboxylate (22e).

1-Bromo-butane (69 μL, 0.64 mmol), K_2CO_3 (88 mg, 0.64 mmol) and KI (catalytic amount) were added to a solution of **21** (100 mg, 0.32 mmol) in dry DMF (3.0 mL). The reaction mixture was stirred at 60°C under Ar atmosphere for 3 h. Then the reaction mixture was poured into H_2O (60 mL) and acidified with 2N HCl, then extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (40 mL) and brine (40 mL), and dried over $MgSO_4$. The solution was evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **22e** (69 mg, 59%) as yellow oil. 1H NMR (300 MHz, $CDCl_3$) δ: 8.84 (d, 1H, *J* = 2.0 Hz), 7.78 (dd, 1H, *J* = 9.0, 2.5 Hz), 7.03 (d, 1H, *J* = 2.5 Hz), 6.98 (dd, 1H, *J* = 8.5, 2.5 Hz), 6.89 (d, 1H, *J* = 9.0 Hz), 6.15 (d, 1H, *J* = 9.0 Hz), 4.01 (q, 2H, *J* = 7.0 Hz), 4.01 (t. 2H, *J* = 6.0 Hz), 3.85 (s, 3H), 3.34 (sep, 1H, *J* = 7.0 Hz), 1.87−1.78 (m, 2H), 1.62−1.51 (m, 2H), 1.22 (t, 3H, *J* = 7.0 Hz), 1.21 (d, 6H, *J* = 7.0 Hz), 1.01 (t, 3H, *J* = 7.5 Hz).

Methyl 6-[ethyl-(3-isopropyl-4-pentyloxyphenyl)amino]pyridine-3-carboxylate (22f).

n-Amyl bromide (82 μL, 0.66 mmol), K_2CO_3 (91 mg, 0.66 mmol) and KI (catalytic amount) were added to a solution of **21** (104 mg, 0.33 mmol) in dry DMF (3.0 mL). The reaction mixture was stirred at 60°C under Ar atmosphere for 6.5 h. Then the reaction mixture was poured into H2O (60 mL) and acidified with 2N HCl, then extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (40 mL) and brine (40 mL), and dried over $MgSO_4$. The solution was evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **22f** (76 mg, 60%) as yellow oil. 1H NMR (300 MHz, $CDCl_3$) δ: 8.84 (d, 1H, *J* = 2.5 Hz), 7.78 (dd, 1H, *J* = 9.0, 2.5 Hz), 7.06 (d, 1H, *J* = 2.5 Hz), 6.98 (dd, 1H, *J* = 8.5, 2.5 Hz), 6.88 (d, 1H, *J* = 8.5 Hz), 6.15 (d, 1H, *J* = 9.0 Hz), 4.01 (q, 2H, *J* = 7.0 Hz), 4.00 (t. 2H, *J* = 7.0 Hz), 3.86 (s, 3H), 3.34 (sep, 1H, *J* = 7.0 Hz), 1.89−1.80

(m, 2H), 1.55–1.41 (m, 4H), 1.22 (t, 3H, J = 6.5 Hz), 1.21 (d, 6H, J = 6.5 Hz), 1.01 (t, 3H, J = 7.0 Hz).

Methyl 6-[ethyl-(4-hexyloxy-3-isopropylphenyl)amino]pyridine-3-carboxylate (22g).

1-Iodohexane (97 μL, 0.66 mmol), K_2CO_3 (91 mg, 0.66 mmol) and KI (catalytic amount) were added to a solution of **21** (105 mg, 0.33 mmol) in dry DMF (3.0 mL). The reaction mixture was stirred at 60°C under Ar atmosphere for 3.7 h. Then the reaction mixture was poured into H_2O (60 mL) and acidified with 2N HCl, then extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (40 mL) and brine (40 mL), and dried over $MgSO_4$. The solution was evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **22g** (110 mg, 82%) as yellow oil. 1H NMR (300 MHz, $CDCl_3$) δ: 8.84 (d, 1H, J = 2.0 Hz), 7.78 (dd, 1H, J = 9.0, 2.5 Hz), 7.02 (d, 1H, J = 2.5 Hz), 6.98 (dd, 1H, J = 8.5, 2.5 Hz), 6.88 (d, 1H, J = 8.5 Hz), 6.15 (d, 1H, J = 9.0 Hz), 4.01 (q, 2H, J = 6.5 Hz), 4.00 (t, 2H, J = 6.5 Hz), 3.85 (s, 3H), 3.34 (sep, 1H, J = 7.0 Hz), 1.88–1.79 (m, 2H), 1.57–1.52 (m, 2H), 1.40–1.35 (m, 4H), 1.22 (t, 3H, J = 7.0 Hz), 1.22 (d, 6H, J = 6.5 Hz), 0.92 (t, 3H, J = 7.0 Hz).

Methyl 6-[(4-benzyloxy-3-isopropylphenyl)ethylamino]pyridine-3-carboxylate (22h).

Benzyl bromide (48 μL, 0.40 mmol), K_2CO_3 (69 mg, 0.40 mmol) and KI (catalytic amount) were added to a solution of **21** (106 mg, 0.34 mmol) in dry DMF (3.0 mL). The reaction mixture was stirred at 60°C under Ar atmosphere for 2.5 h. Then the reaction mixture was poured into H_2O (60 mL) and extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (2 × 40 mL) and brine (30 mL), and dried over $MgSO_4$. The solution was evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **18h** (76 mg, 55%) as colorless oil. 1H NMR (300 MHz, $CDCl_3$) δ: 8.83 (d, 1H, J = 2.5 Hz), 7.78 (dd, 1H, J = 9.0, 2.5 Hz), 7.48–7.35 (m, 5H), 7.06–6.97 (m, 3H), 6.16 (d, 1H, J = 9.0 Hz), 5.12 (s, 2H), 4.01 (q, 2H, J = 7.0 Hz), 3.85 (s, 3H), 3.42 (sep, 1H, J = 7.0 Hz), 1.23 (d, 6H, J = 7.0 Hz), 1.22 (t, 3H, J = 7.0 Hz).

Methyl 6-[ethyl-(3-isopropyl-4-phenethyloxyphenyl)lamino]pyridine-3-carboxylate (22i).

(2-Bromoethyl)benzene (190 μL, 1.4 mmol), K_2CO_3 (170 mg, 1.2 mmol) and KI (catalytic amount) were added to a solution of **22** (220 mg, 0.70 mmol) in dry DMF (4.0 mL). The reaction mixture was stirred at 80°C under Ar atmosphere for 16 h. Then the reaction mixture was poured into H_2O (100 mL) and extracted with EtOAc (3 × 30 mL).

The organic layer was washed with water (2 × 40 mL) and brine (30 mL), and dried over $MgSO_4$. The solution was evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **22i** (46 mg, 16%) as colorless oil. 1H NMR (500 MHz, $CDCl_3$) δ: 8.82 (d, 1H, *J* = 2.0 Hz), 7.76 (dd, 1H, *J* = 9.0, 2.0 Hz), 7.33–7.32 (m, 5H), 7.00 (d, 1H, *J* = 2.5 Hz), 6.95 (dd, 1H, *J* = 8.5, 2.5 Hz), 6.86 (d, 1H, *J* = 8.5 Hz), 6.13 (d, 1H, *J* = 9.0 Hz), 4.21 (t, 2H, *J* = 6.5 Hz), 4.00 (q, 2H, *J* = 7.0 Hz), 3.85 (s, 3H), 3.30 (sep, 1H, *J* = 7.0 Hz), 3.15 (t, 2H, *J* = 6.5 Hz), 1.21 (t, 3H, *J* = 7.0 Hz), 1.16 (d, 6H, *J* = 7.0 Hz).

Methyl 6-{ethyl-[(3-isopropyl-4-phenylpropoxy)phenyl]amino}pyridine-3-carboxylate (22j).

3-Phenylpropyl bromide (54 μL, 0.36 mmol), K_2CO_3 (83 mg, 0.60 mmol) and KI (17 mg, 0.10 mmol) were added to a solution of **21** (75 mg, 0.24 mmol) in dry DMF (3.0 mL). The reaction mixture was stirred at 90°C under Ar atmosphere for 14 h. Then the reaction mixture was poured into H_2O (100 mL) and extracted with EtOAc (3 × 30 mL). The organic layer was washed with water (2 × 40 mL) and brine (30 mL), and dried over $MgSO_4$. The solution was evaporated under reduced pressure. The residue was purified by flash column chromatography to yield **22j** (76 mg, 73%) as white oil. 1H NMR (300 MHz, $CDCl_3$) δ: 8.83 (d, 1H, *J* = 2.5 Hz), 7.78 (d, 1H, *J* = 9.0, 2.5 Hz), 7.35–7.22 (m, 5H), 7.03 (d, 1H, *J* = 2.5 Hz), 6.96 (dd, 1H, *J* = 8.5, 2.5 Hz), 6.84 (d, 1H, *J* = 8.5 Hz), 6.15 (d, 1H, *J* = 9.0 Hz), 4.01 (t, 2H, *J* = 6.0 Hz), 4.01 (q, 2H, *J* = 7.5 Hz), 3.85 (s, 3H), 3.37 (sep, 1H, *J* = 7.0 Hz), 2.87 (t, 2H, *J* = 7.0 Hz), 2.22-2.12 (m, 2H), 1.24 (d, 6H, *J* = 7.0 Hz), 1.22 (t, 3H, *J* = 7.5 Hz).

6-[Ethyl-(4-isobutoxy-3-isopropylphenyl)amino]pyridine-3-carboxylic acid (15a).

To a solution of **22a** (300 mg, 0.81 mmol) in MeOH (10 mL) were added 2 N NaOH (4.0 mL) and THF (3.0 mL). The reaction mixture was stirred at 60 °C for 2 hr. The reaction mixture was evaporated under reduced pressure, then neutralized with 2N HCl. The mixture was extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (2 × 40 mL) and brine (30 mL), and dried over $MgSO_4$. The solution was evaporated under reduced pressure and the residue was recrystallized with MeOH to yield **15a** (140 mg, 49%) as white needles. Mp 162.0–164.0 °C. IR (KBr) cm^{-1}: 1677 (CO). 1H NMR (300 MHz, $CDCl_3$) δ: 8.90 (d, 1H, *J* = 2.5 Hz), 7.81 (dd, 1H, *J* = 9.0, 2.5 Hz), 7.03 (d, 1H, *J* = 2.5 Hz), 6.98 (dd, 1H, *J* = 8.5, 2.5 Hz), 6.88 (d, 1H, *J* = 8.5 Hz), 6.16 (d, 1H, *J* = 9.0 Hz), 4.00 (q, 2H, *J* = 7.0 Hz), 3.77 (d, 2H, *J* = 6.0 Hz), 3.49 (s, 1H), 3.36 (sep, 1H, *J* = 7.0 Hz), 1.23 (t, 3H, *J* = 7.0 Hz),

1.22 (d, 6H, J = 7.0 Hz), 1.08 (d, 6H, J = 6.5 Hz). FAB-MS m/z: 357 [M + H]+. Anal. Calcd for $C_{21}H_{28}N_2O_3$: C, 70.76; H, 7.92; N, 7.86. Found: C, 70.49; H, 7.64; N, 7.73.

6-[Ethyl-(4-cyclopropylmethoxy-3-isopropylphenyl)amino]pyridine-3-carboxylic acid (15b).

To a solution of **22b** (130 mg, 0.35 mmol) in MeOH (8.0 mL) were added 2 N NaOH (5.0 mL) and THF (2.0 mL). The reaction mixture was stirred at 60 °C for 1.5 hr. The reaction mixture was evaporated under reduced pressure, then poured into sat.NH_4Cl. The mixture was extracted with EtOAc (3 × 30 mL). The organic layer was washed with water (2 × 40 mL) and brine (40 mL), and dried over $MgSO_4$. The solution was evaporated under reduced pressure and the residue was recrystallized with MeOH to yield **15b** (120 mg, 93%) as white needles. Mp 194.0–196.0 °C. 1H NMR (300 MHz, DMSO-d6) δ : 8.65 (dd, J = 2.5, 0.8 Hz, 1H), 7.76 (dd, J = 9.0, 2.5 Hz, 1H), 7.05–7.01 (m, 3H), 6.13 (d, J = 9.0, 0.8 Hz, 1H), 3.94 (q, J = 7.0 Hz, 2H), 3.88 (q, J = 7.0 Hz, 2H), 1.14 (d, J = 7.0 Hz, 6H), 1.12 (t, J = 7.0 Hz, 3H), 0.62–0.56 (m, 2H), 0.38–0.33 (m, 2H). FAB-MS m/z: 355 [M + H]+. Anal. Calcd for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90. Found: C, 70.99; H, 7.25; N, 7.81.

6-{Ethyl-[3-isopropyl-4-(3-methyl-but-2-enyloxy)phenyl]amino}pyridine-3-carboxylic acid (15c).

To a solution of **22c** (62 mg, 0.16 mmol) in MeOH (3.0 mL) were added 2 N NaOH (3.0 mL) and THF (2.0 mL). The reaction mixture was stirred at 60 °C for 0.75 hr. The reaction mixture was evaporated under reduced pressure, then poured into sat.NH_4Cl (40 mL). The mixture was extracted with EtOAc (3 × 30 mL). The organic layer was washed with water (2 × 30 mL) and brine (30 mL), and dried over MgSO4. The solution was evaporated under reduced pressure and the residue was recrystallized with MeOH to yield **15c** (7.2 mg, 12%) as white needles. Mp 139.5–141.0 °C. IR (KBr) cm^{-1}: .1671 (CO). 1H NMR (300 MHz, DMSO-d6) δ : 8.66 (d, J = 2.5 Hz, 1H), 7.76 (dd, J = 9.0, 2.5 Hz, 1H), 7.06–7.05 (m, 3H), 6.14 (d, J = 9.0 Hz, 1H), 5.48 (t, J = 6.5 Hz, 1H), 4.58 (d, J = 6.5 Hz, 2H), 3.94 (q, J = 7.0 Hz, 2H), 1.78 (s, 3H), 1.73 (s, 3H), 1.15 (d, J = 7.0 Hz, 6H), 1.12 (t, J = 7.0 Hz, 3H). FAB-MS m/z: 369 [M + H]+. Anal. Calcd for $C_{22}H_{28}N_2O_3$: C, 71.71; H, 7.66; N, 7.60. Found: C, 71.77; H, 7.62; N, 7.57.

6-{Ethyl-[3-isopropyl-4-(2-methylallyloxy)phenyl]amino}pyridine-3-carboxylic acid (15d).

To a solution of **22d** (73 mg, 0.20 mmol) in MeOH (3.0 mL) was added 2 N NaOH (3.0 mL). The reaction mixture was stirred at 60 °C for 2 hr. The reaction mixture was

evaporated under reduced pressure, then neutralized with 2N HCl. The mixture was extracted with EtOAc (3 × 30 mL). The organic layer was washed with water (40 mL) and brine (40 mL), and dried over $MgSO_4$. The solution was evaporated under reduced pressure and the residue was recrystallized with MeOH to yield **15d** (26 mg, 37%) as white needles. Mp 162.0−163.5 °C. IR (KBr) cm^{-1}: 1677 (CO). 1H NMR (300 MHz, CDCl3) δ : 8.91 (d, *J* = 2.0 Hz, 1H), 7.82 (dd, *J* = 9.0, 2.5 Hz, 1H), 7.05 (d, *J* = 2.5 Hz, 1H), 6.99 (dd, *J* = 8.5, 2.5 Hz, 1H), 6.90 (d, *J* = 9.0 Hz, 1H), 6.17 (d, *J* = 9.0 Hz, 1H), 5.15 (s, 1H), 5.02 (s, 1H), 4.47 (s, 2H), 4.03 (q, *J* = 7.0 Hz, 2H), 3.39 (sep, *J* = 7.0 Hz, 1H), 1.88 (s, 3H), 1.23 (t, *J* = 7.0 Hz, 3H), 1.23 (d, *J* = 7.0 Hz, 6H). FAB-MS m/z: 355 [M + H]+. Anal. Calcd for $C_{21}H_{26}N_2O_3$: C, 71.16; H, 7.39; N, 7.90. Found: C, 71.54; H, 7.52; N, 7.95.

6-[(4-Butoxy-3-isopropylphenyl)ethylamino]pyridine-3-carboxylic acid (15e).

To a solution of **22e** (69 mg, 0.19 mmol) in MeOH (3.0 mL) were added 2 N NaOH (3.0 mL). The reaction mixture was stirred at 60 °C for 4 hr. The reaction mixture was evaporated under reduced pressure, then neutralized with 2N HCl. The mixture was extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (40 mL) and brine (40 mL), and dried over $MgSO_4$. The solution was evaporated under reduced pressure and the residue was recrystallized with MeOH to yield **15e** (29 mg, 44%) as white needles. Mp 161.0-163.5 °C. IR (KBr) cm^{-1}: 1613 (CO). 1H NMR (300 MHz, DMSO-d6) δ: 8.66 (d, 1H, *J* = 2.0 Hz), 7.76 (dd, 1H, *J* = 9.0, 2.5 Hz), 7.09−7.00 (m, 3H), 6.13 (d, 1H, *J* = 9.0 Hz), 4.01 (t, 2H, *J* = 6.0 Hz), 3.93 (q, 2H, *J* = 7.0 Hz), 3.24 (sep, 1H, *J* = 7.0 Hz), 1.79−1.70 (m, 2H), 1.55−1.43 (m, 2H), 1.16 (d, 6H, *J* = 7.0 Hz), 1.11 (t, 3H, *J* = 7.0 Hz), 0.95 (t, 3H, *J* = 7.5 Hz). FAB-MS m/z: 357 [M + H]+. Anal. Calcd for $C_{21}H_{28}N_2O_3$: C, 70.76; H, 7.92; N, 7.86. Found: C, 70.70; H, 7.73; N, 7.86.

6-[Ethyl-(3-isopropyl-4-pentyloxyphenyl)amino]pyridine-3-carboxylic acid (15f).

To a solution of **22f** (76 mg, 0.20 mmol) in MeOH (6.0 mL) were added 2 N NaOH (3.0 mL) and THF (1.5 mL). The reaction mixture was stirred at 60 °C for 3 hr. The reaction mixture was evaporated under reduced pressure, then neutralized with 2N HCl. The mixture was extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (40 mL) and brine (40 mL), and dried over $MgSO_4$. The solution was evaporated under reduced pressure and the residue was recrystallized with MeOH to yield **15f** (47 mg, 64%) as pale yellow needles. Mp 154.5−156.0 °C. IR (KBr) cm^{-1}: 1691 (CO).

1H NMR (300 MHz, DMSO-d6) δ: 8.67 (d, 1H, *J* = 2.5 Hz), 7.77 (dd, 1H, *J* = 9.0, 2.5 Hz), 7.10–7.01 (m, 3H), 6.14 (d, 1H, *J* = 9.0 Hz), 4.02 (t, 2H, *J* = 6.0 Hz), 3.94 (q, 2H, *J* = 7.0 Hz), 3.26 (sep, 1H, *J* = 7.0 Hz), 1.82–1.73 (m, 2H), 1.49–1.37 (m, 4H), 1.17 (d, 6H, *J* = 6.5 Hz), 1.12 (t, 3H, *J* = 7.0 Hz), 0.92 (t, 3H, *J* = 7.0 Hz). FAB-MS m/z: 371 [M + H]+. Anal. Calcd for $C_{22}H_{30}N_2O_3$: C, 71.32; H, 8.16; N, 7.56. Found: C, 71.16; H, 7.98; N, 7.29.

6-[Ethyl-(4-hexyloxy-3-isopropylphenyl)amino]pyridine-3-carboxylic acid (15g).

To a solution of **22g** (109 mg, 0.27 mmol) in MeOH (6.0 mL) were added 2 N NaOH (3.0 mL) and THF (1.5 mL). The reaction mixture was stirred at 60 °C for 2.3 hr. The reaction mixture was evaporated under reduced pressure, then neutralized with 2N HCl. The mixture was extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (40 mL) and brine (40 mL), and dried over $MgSO_4$. The solution was evaporated under reduced pressure and the residue was recrystallized with MeOH to yield **15g** (64 mg, 61%) as white needles. Mp 167.0–169.0 °C. IR (KBr) cm^{-1}: 1654 (CO). 1H NMR (300 MHz, DMSO-d6) δ: 8.67 (d, 1H, *J* = 2.5 Hz), 7.77 (dd, 1H, *J* = 9.0, 2.5 Hz), 7.10-7.01 (m, 3H), 6.14 (d, 1H, *J* = 9.0 Hz), 4.02 (t, 2H, *J* = 6.0 Hz), 3.94 (q, 2H, *J* = 7.0 Hz), 3.26 (sep, 1H, *J* = 7.0 Hz), 1.81–1.72 (m, 2H), 1.50–1.44 (m, 2H), 1.37–1.30 (m, 4H), 1.17 (d, 6H, *J* = 7.0 Hz), 1.12 (t, 3H, *J* = 7.0 Hz), 0.89 (t, 3H, *J* = 7.0 Hz). FAB-MS m/z: 385 [M + H]+. Anal. Calcd for $C_{23}H_{32}N_2O_3$: C, 71.84; H, 8.39; N, 7.29. Found: C, 71.63; H, 8.38; N, 7.37.

6-[(4-benzyloxy-3-isopropylphenyl)ethylamino]pyridine-3-carboxylic acid (15h).

To a solution of **22h** (76 mg, 0.19 mmol) in MeOH (8.0 mL) were added 2 N NaOH (3.0 mL) and THF (2.0 mL). The reaction mixture was stirred at 60 °C for 2 hr. The reaction mixture was evaporated under reduced pressure, then neutralized with 2N HCl. The mixture was extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (2 × 40 mL) and brine (30 mL), and dried over $MgSO_4$. The solution was evaporated under reduced pressure and the residue was recrystallized with MeOH to yield **15h** (38 mg, 52%) as colorless cube. Mp 183.0–184.5 °C. IR (KBr) cm^{-1}: 1674 (CO). 1H NMR (300 MHz, DMSO-d6) δ: 12.36 (br s, 1H), 8.65 (d, 1H, *J* = 2.5 Hz), 7.76 (dd, 1H, *J* = 9.0, 2.5 Hz), 7.51–7.35 (m, 5H), 7.16–7.04 (m, 3H), 6.14 (d, 1H, *J* = 9.0 Hz), 5.15(s, 2H), 3.95 (q, 2H, *J* = 7.0 Hz), 1.19 (d, 6H, *J* = 7.0 Hz), 1.13 (t, 3H, *J* = 7.0 Hz). FAB-MS m/z: 391 [M + H]+. Anal. Calcd for $C_{24}H_{26}N_2O_3$: C, 73.82; H, 6.71; N, 7.17. Found: C, 73.58; H, 6.93; N, 7.14.

6-[Ethyl-(3-isopropyl-4-phenethyloxyphen

yl)lamino]pyridine-3-carboxylic acid (15i).

To a solution of **22i** (46 mg, 0.11 mmol) in MeOH (6.0 mL) were added 2 N NaOH (2.0 mL) and THF (2.0 mL). The reaction mixture was stirred at 60 °C for 4.3 hr. The reaction mixture was evaporated under reduced pressure, then neutralized with 2N HCl. The mixture was extracted with EtOAc (3 × 20 mL). The organic layer was washed with water (2 × 30 mL) and brine (30 mL), and dried over $MgSO_4$. The solution was evaporated under reduced pressure and the residue was purified by flash column chromatography, then was recrystallized with MeOH to yield **15i** (25 mg, 58%) as colorless cube. Mp 167.5–169.0 °C. IR (KBr) cm^{-1}: 1693 (CO). 1H NMR (300 MHz, DMSO-d6) δ: 8.63 (d, 1H, J = 2.5 Hz), 7.72 (dd, 1H, J = 9.0, 2.5 Hz), 7.36–7.22 (m, 5H), 7.03–6.99 (m, 3H) 6.10 (d, 1H, J = 9.0 Hz), 4.23 (t, 2H, J = 6.5 Hz), 3.93 (q, 2H, J = 7.0 Hz), 3.19 (sep, 1H, J = 7.0 Hz), 3.08 (t, 2H, J = 6.5 Hz), 1.12 (t, 3H, J = 7.0 Hz), 1.09 (d, 6H, J = 7.0 Hz). FAB-MS m/z: 405 [M + H]+. Anal. Calcd for $C_{25}H_{28}N_2O_3$: C, 74.23; H, 6.98; N, 6.93. Found: C, 74.33; H, 6.88; N, 7.06.

6-{Ethyl-[(3-isopropyl-4-phenylpropoxy)phenyl]amino}pyridine-3-carboxylic acid (15j).

To a solution of **22j** (76 mg, 0.18 mmol) in MeOH (7.0 mL) were added 2 N NaOH (2.0 mL) and THF (3.0 mL). The reaction mixture was stirred at 60 °C for 2 hr. The reaction mixture was evaporated under reduced pressure, then poured into sat.NH4Cl. The mixture was extracted with EtOAc (3 × 30 mL). The organic layer was washed with water (2 × 30 mL) and brine (30 mL), and dried over $MgSO_4$. The solution was evaporated under reduced pressure and recrystallized with CH_2Cl_2/*n*-Hexane to yield **15j** (56 mg, 75%) as colorless plate. Mp 171.5–176.0 °C. IR (KBr) cm^{-1}: 1660 (CO). 1H NMR (300 MHz, DMSO-d6) δ: 8.65 (d, 1H, J = 2.0 Hz), 7.75 (d, 1H, J = 9.0, 2.5 Hz), 7.33–7.17 (m, 5H), 7.05–7.01 (m, 3H), 6.13 (d, 1H, J = 9.0 Hz), 4.01 (t, 2H, J = 6.0 Hz), 3.94 (q, 2H, J = 7.0 Hz), 2.81 (t, 2H, J = 8.0 Hz), 2.13–2.04 (m, 2H), 1.20 (d, 6H, J = 7.0

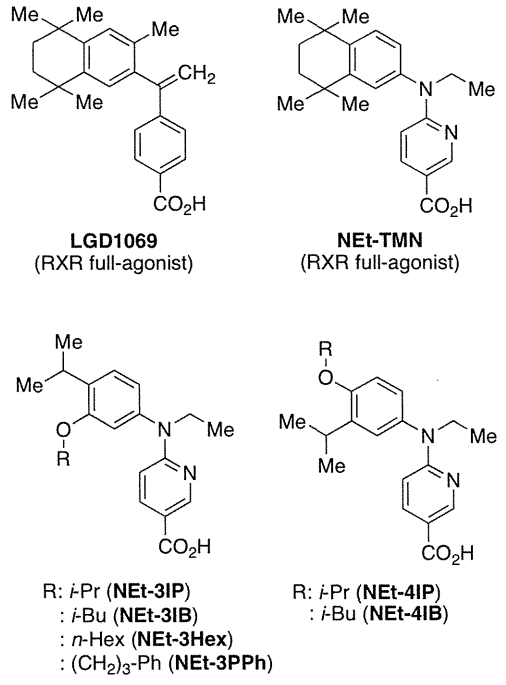


Figure 11. Chemical structures of RXR agonists.

Hz), 1.13 (t, 3H, J = 7.0 Hz). FAB-MS m/z: 419 [M + H]+. Anal. Calcd for $C_{26}H_{30}N_2O_3$: C, 74.61; H, 7.22; N, 6.69. Found: C, 74.61; H, 7.22; N, 6.69.

LGD1069.

This compound was prepared according to reference 21.

NEt-3IB.

This compound was prepared according to reference 8.

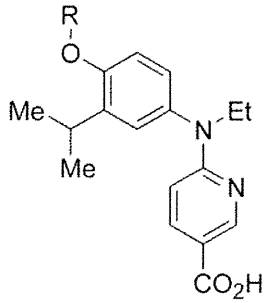
NEt-3nHex, NEt-3PPh.

These compounds were prepared according to reference 7.

【参考文献】

21. *J. Med. Chem.*, 1994, *37*, 2930–2941.

Table 10. Luciferase reporter gene assay data for compounds **15a-j**



		RXRa[a]			
		Efficacy (%)			
Compound	R	1 μM	10 μM	EC_{50} (nM)[b]	E_{max} (%)
15a	*i*-Bu	53 ± 0	54 ± 3	169 ± 3	55 ± 2
15b	CH_2*c*-Pr	52 ± 6	64 ± 0	324 ± 62	62 ± 2
15c	$CH_2CH=C(CH_3)_2$	77 ± 2	84 ± 2	268 ± 32	84 ± 3
15d	$CH_2C(CH_3)=CH_2$	78 ± 6	83 ± 6	218 ± 55	83 ± 3
15e	*n*-Bu	53 ± 3	75 ± 3	567 ± 57	73 ± 3
15f	*n*-Pen	37 ± 2	58 ± 3	772 ± 80	57 ± 4
15g	*n*-Hex	28 ± 1	51 ± 1	918 ± 53	52 ± 1
15h	CH_2-Ph	52 ± 1	75 ± 1	540 ± 4	73 ± 1
15i	$(CH_2)_2$-Ph	15 ± 3	49 ± 2	1130 ± 60	45 ± 1
15j	$(CH_2)_3$-Ph	20 ± 2	44 ± 2	1030 ± 80	42 ± 2

a) Luciferase activity of LGD1069 at 1 μM was defined as 100%.

b) EC_{50} values were determined from full dose-response curves in COS-1 cells.

7－2）創出化合物の転写活性化能

（1）RXR 活性化能

レポータージーンアッセイの結果を，Table 10 に掲載した．なお，記載データは既知の RXR フルアゴニスト LGD1069 1 μM での転写活性化能を 100%とした相対値で掲載している．

評価した全ての化合物が RXR パーシャルアゴニスト活性を示した．新規に合成した全ての化合物が RXR パーシャルアゴニスト活性を示したが，特に著者は EC_{50} の最も小さかった化合物 **15a** (以下 NEt-4IB とする) に着目した（Table 1）．

NEt-4IB (**15a**) が RXR パーシャルアゴニストであることを証明するため，RXR フルアゴニストである LGD1069 との競合試験を行った．NEt-4IB (**15a**) が RXR パーシャルアゴニストであれば，RXR フルアゴニスト存在下において NEt-4IB (**15a**) を加えると，NEt-4IB (**15a**)がパーシャルアンタゴニストとして機能すると考えられる．その結果，LGD1069 1 μM 存在下において，RXR フルアゴニストである NEt-3IB の濃度を増やしても RXR アゴニスト活性はほとんど変化しないのに対し，RXR パーシャルアゴニストである NEt-4IB (**15a**) は，その濃度上昇に応じて RXR アゴニスト活性の減弱が見られた（Figure 12）．即ち，NEt-4IB (**15a**) はその濃度上昇に応じて LGD1069 との置換が生じ，その結果 RXR アゴニスト活性の減弱が起こると考えられる．以上より，NEt-4IB (**15a**) は RXR フルアゴニスト存在下において RXR パーシャルアンタゴニストとして機能することからも，NEt-4IB (**15a**) が RXR パーシャルアゴニストであることが証明された．

（2）RXR ヘテロダイマー活性

前項で示したように，RXR パーシャルアゴニスト NEt-4IB (**15a**) の創出に成功した．RXR アゴニストは RXR ホモダイ

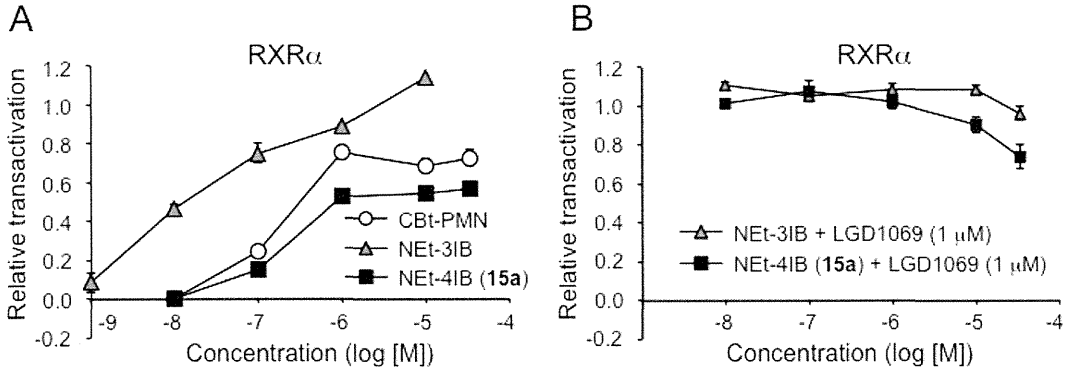


Figure 12. A) Relative transactivation data of CBt-PMN, NEt-3IB, and NEt-4IB (**15a**) towards RXRα. B) Relative transactivation data of NEt-3IB with LGD1069 (RXR full agonist), or NEt-4IB (**15a**) with LGD1069 towards RXRα. Luciferase activity of LGD1069 at 1 μM was defined as 1.

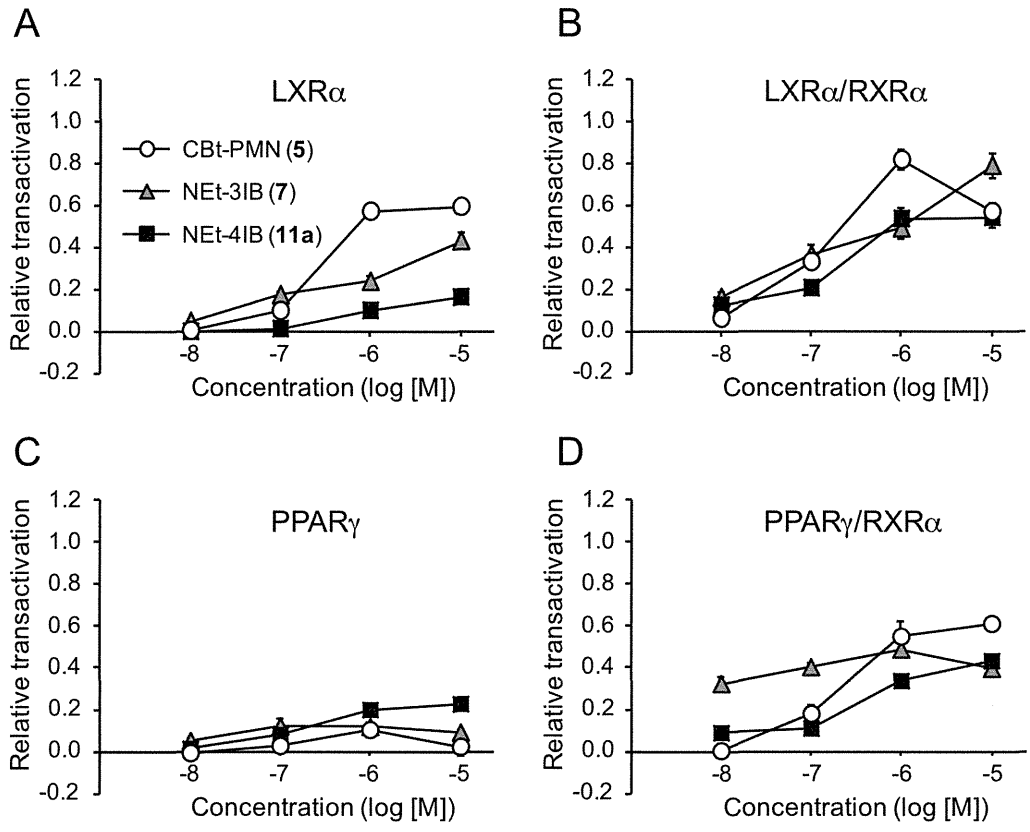


Figure 13. Relative transactivation data of CBt-PMN, NEt-3IB, and NEt-4IB (**15a**) towards LXRα, LXRα/RXRα, PPARγ, PPARγ/RXRα. Luciferase activity of TIPP-703 (RRAR-pan agonist) or carba-T0901317 (LXR-pan agonist) at 1 μM were defined as 1 for LXRα or LXRα/RXRα, PPARγ or PPARγ/RXRα, respectively.

マーの活性化によってのみならず，パーミッシブ機構を介した LXR/RXR や PPAR/RXR の活性化によって薬効や副作用を発現すると考えられる．そこで，in vivo 実験に先立ち，NEt-4IB (**15a**) の LXR/RXR，PPAR/RXR ヘテロダイマー活性化能の評価を行った．

その結果，NEt-4IB (**15a**) は LXRαホモダイマー，PPARγホモダイマーについてはほとんど活性化していなかった (Figure 13)．一方で，パーミッシブ機構を介した LXRα/RXRα，PPARγ/RXRαの活性化が見られたため，これらの活性化に基づく薬効発現が十分期待できた．

8．NEt-4IB の RXR パーシャルアゴニスト活性発現メカニズムの考察

NEt-4IB (**15a**) が RXR パーシャルアゴニスト活性を発現する理由を考察すべく，AutoDock4.2 を用いたドッキングシミュレーションを行い，RXR の LBP における NEt-4IB (**15a**) ならびに RXR フルアゴニスト NEt-3IB の立体配置について検討し

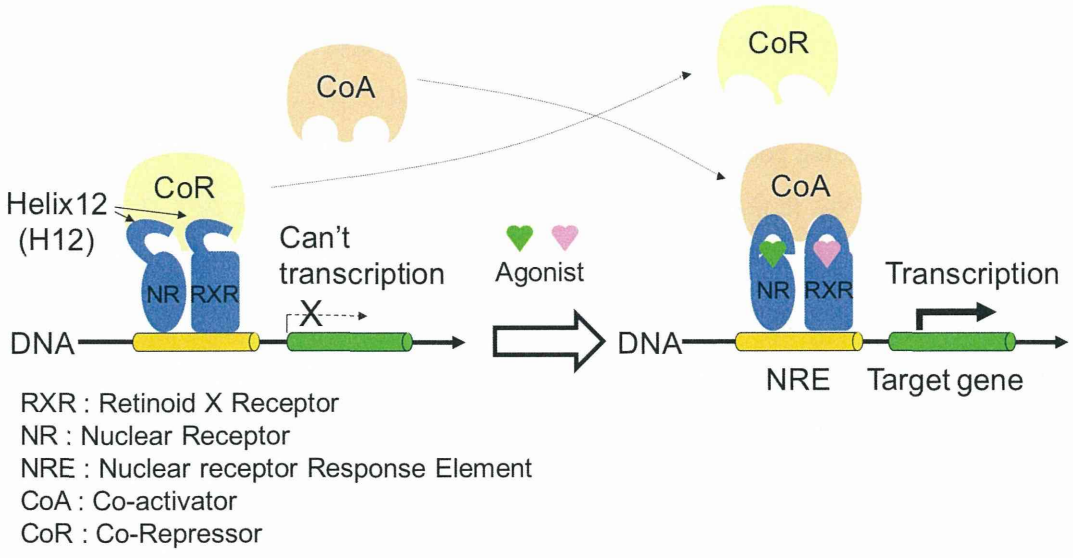


Figure 14. DNA transcriptional mechanism via nuclear receptor.

た.

まず，RXR アゴニストの活性発現メカニズムを Figure 14 に示す. RXR はアゴニスト非存在下においては主に，転写抑制因子であるコリプレッサー (CoR) が結合しており，転写が制御されている. RXR アゴニストと RXR が結合すると RXR の部分構造である H12 が折りたたみ，それによって CoR の解離ならびに転写促進因子であるコアクチベーター (CoA) の結合が生じ，標的遺伝子の転写が開始される.[21] つまり，結合によって RXR の H12 の折りたたまり，RXR に CoA を結合させるリガンドがアゴニストであり，RXR に結合するが H12 が折りたたまらず CoR の結合を維持させるリガンドをアンタゴニストということができる. 一方で，パーシャルアゴニストは結合によってアゴニスト構造もアンタゴニスト構造もとれる，言わば平衡状態にあるようなものである. RXR がリガンドの結合によってアゴニスト構造をとるかアンタゴニスト構造をとるかは H12 の折りたたみが重要である.

AutoDock4.2 を用いたドッキングシミュレーションを行った結果，RXR パーシャルアゴニストである NEt-4IB (**15a**) の 4' 位イソブトキシ基が H11 上の His435 に近接していることが示唆された（Figure 15). NEt-4IB (**15a**) の 4'位イソブトキシ基と H11 上の His435 との立体障害により，間接的に H12 に影響を与えることで，完全に CoA と RXR を結合させることができず，NEt-4IB (**15a**) が RXR に結合しても CoA が結合している RXR と，CoR が結合している RXR の両者が存在するため RXR パーシャルアゴニスト活性を示したのではないかと考えられる（Figure 15B).

化合物 **15e–g** もしくは **15h–j** のように

アルコキシ基の伸長を行うと，それに伴って EC_{50} は大きくなった．これは，化合物中の4'位アルコキシ基に対するRXRタンパク質の ligand binding pocket (LBP) に空間的余裕がないため，化合物の RXR に対する結合能が減弱したためだと考えられる．また，アルコキシ基の伸長に伴って E_{max} の減弱が見られたが，これは4'位アルコキシ基の伸長によって H11 との立体障害がより強くなり，RXR がより CoR が結合したままの構造を取りやすくなったためと考えられる．なお，当グループの大澤らは CBt-PMN の RXR パーシャルアゴニスト活性発現機構について考察しており，CBt-PMN の 5-カルボキシベンズトリアゾールの2位に存在する窒素原子のローンペアと，H4 上の Asn306 が有する末端アミドのカルボニル酸素原子との電子反発によって，CBt-PMN が RXR パーシャルアゴニスト活性を示したとしている (Figure 15A)．よって，NEt-4IB (**15a**) の RXR パーシャルアゴニスト活性発現機構は CBt-PMN とは異なると考えられる．

【参考文献】

21. *Mol. Pharmacol.*, 2001, *59*, 170–176.

9．NEt-4IB の経口投与時の血中移行性

NEt-4IB (**15a**) の副作用発現評価に先立ち，化合物の経口投与による血中移行性を調べた．また，副作用や薬効発現を

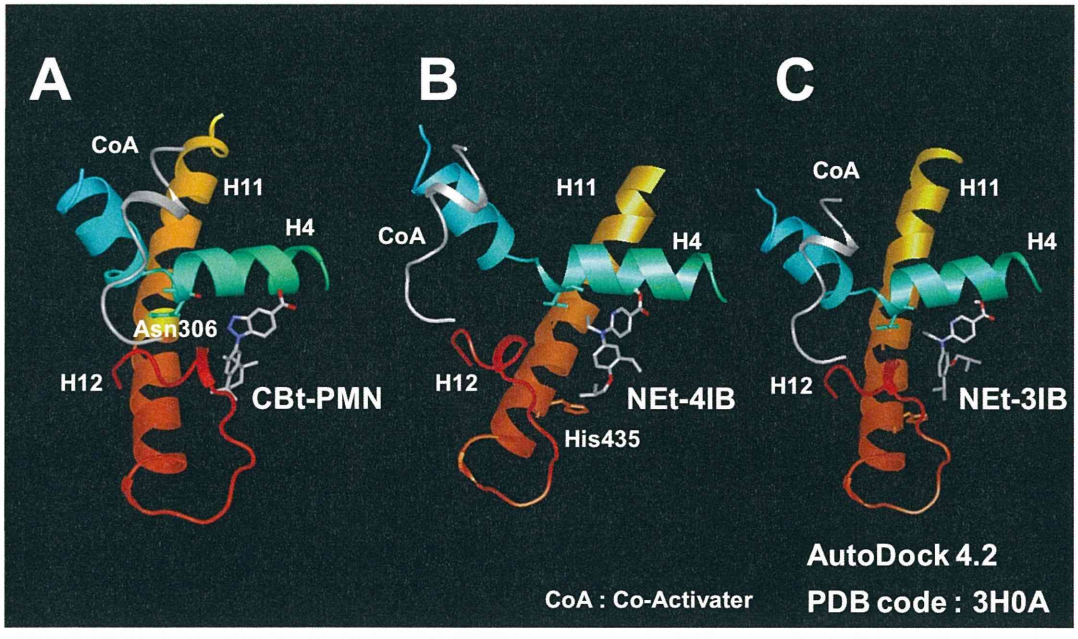


Figure 15. Structures of RXRα LBP in complex with agonists. Crystal structure of RXRα LBD in complex with LGD1069 (pdb code : 3H0A). Docking model using AutoDock4.2. (A), (B) and (C) is modeled CBt-PMN, NEt-4IB (**15a**) and NEt-3IB.

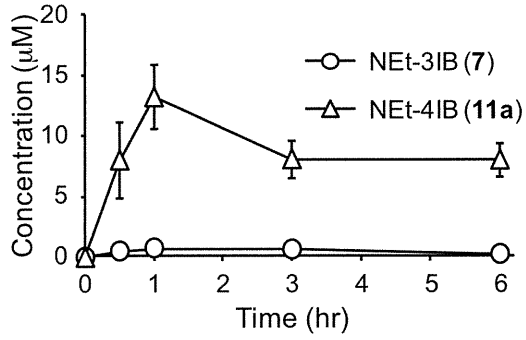


Figure 16. Plasma concentration of NEt-3IB and NEt-4IB (**15a**) in male ICR mice (single dose at 30 mg/kg by oral administration). The horizontal scale is elapsed time in hours and the vertical scale is normalized by the micromolar concentration. Data shown are the average (n = 4-7) ± SEM.

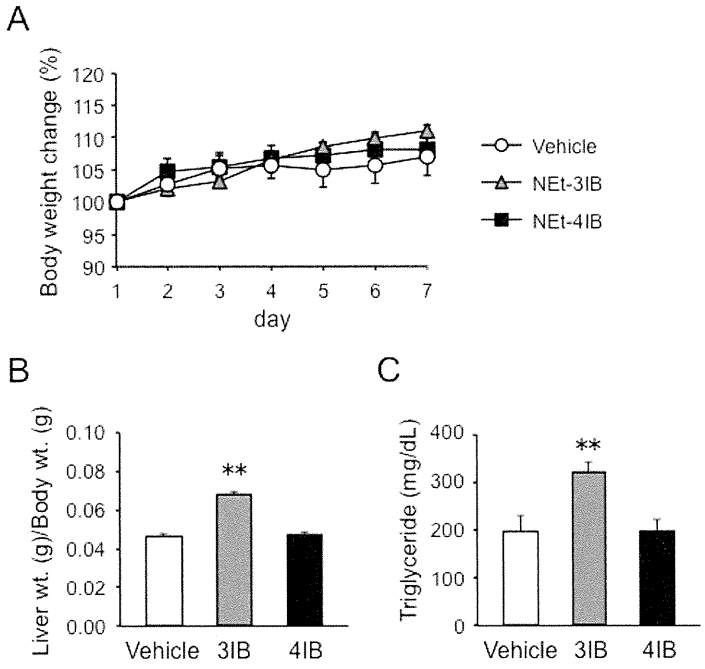


Figure 17. Repeated oral administration (30 mg/kg/day) of NEt-3IB and NEt-4IB (**15a**) to male ICR mice for 7 days. A) Time-dependent change of body weight for 7 days. B) Liver weight (g) / Body weight (g). C) Triglyceride (mg/dL). Data shown are the average (n = 8) ± SEM and analyzed by one-way ANOVA followed by Bonferroni test. ** : $p < 0.01$ vs. vehicle respectively.

RXR フルアゴニスト NEt-3IB と比較するため, NEt-3IB についても血中移行性を調べた.

その結果, NEt-4IB (**15a**) は経口投与 1 時間後に約 13 μM 程度の良好な血中移行性を有し, NEt-4IB (**15a**) の E_{max} を与える血中濃度まで達していることが分かった (Figure 16). 一方で, NEt-3IB は経口投

与 1 時間後に約 0.75 μM 程度の血中移行性であったが, NEt-3IB は低濃度においても十分 RXR を活性化できるため, 30 mg/kg の投与量においても NEt-3IB の E_{max} を与える血中濃度まで達していることが分かった. NEt-3IB と NEt-4IB (**15a**) の血中移行性がこれほどまでに異なる原因については分かっていないが, 両化合物とも 30 mg/kg の投与量で E_{max} を与える血中濃度まで達していると判断したため, この投与量で副作用発現を調べた.

１０. NEt-3IB, NEt-4IB の ICR マウス 7 日間反復経口投与による副作用発現の比較

RXR フルアゴニストは主に, 体重増加, 肝肥大, および血中 TG 値上昇といった副作用が報告されている. そこで, RXR フルアゴニストである NEt-3IB と, RXR パーシャルアゴニストである NEt-4IB (**15a**) を ICR マウスに 30 mg/kg/day の投与量で 7 日間反復経口投与を行い, 体重変化を調べた.

その結果, NEt-3IB 投与群では化合物非投与群 (vehicle) に比べ, 体重の増加傾向, 顕著な肝肥大, 血中 TG 値の増加が見られ

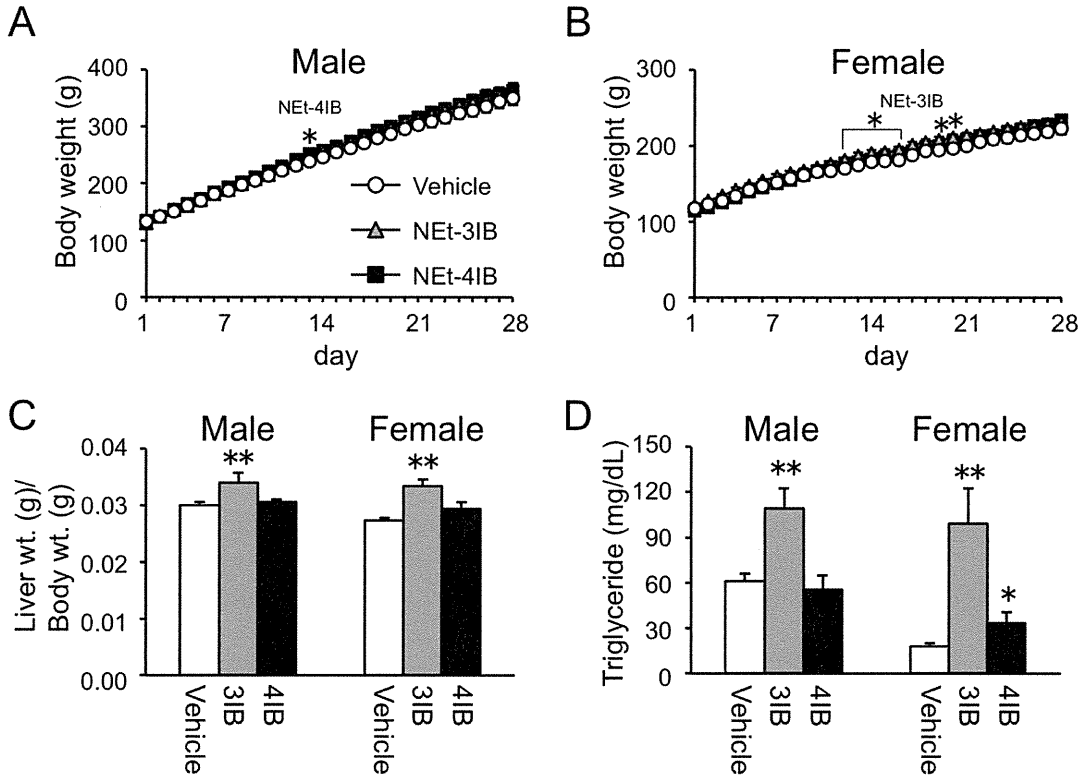


Figure 18. Repeated oral administration (30 mg/kg/day) of NEt-3IB and NEt-4IB (**15a**) to male and female SD rats for 28 days. A) Time-dependent change of male SD rat's body weight for 28 days. B) Time-dependent change of female SD rat's body weight for 28 days. C) Liver weight (g) / Body weight (g). D) Triglyceride (mg/dL). Data shown are the average (n = 6) ± SEM and analyzed by

た．一方で NEt-4IB (**15a**) 投与群については vehicle 群と同様な体重変動を示し，肝肥大，血中 TG 値の増加は見られなかった（Figure 17）．以上より，RXR フルアゴニストである NEt-3IB の投与によって認められる副作用が，RXR パーシャルアゴニストである NEt-4IB (**15a**) では生じないことが分かった．

１１．NEt-3IB，NEt-4IB のラット 28 日間反復経口投与による副作用発現の比較

NEt-3IB と NEt-4IB (**15a**) を 30 mg/kg/day の投与量で SD ラットに 28 日間反復経口投与を行い，体重変化などを調べた．最終日である 29 日目に解剖を行い，血液や臓器を採取し，血中 TG 値の測定や肝臓重量の測定を行った．

その結果，NEt-3IB，NEt-4IB (**15a**) どちらの投与群も体重増加はほとんど見られなかった（Figure 18）．一方で NEt-3IB 投与群では雄，雌ともに顕著な肝肥大や血中 TG 値の上昇が見られたが，NEt-4IB (**15a**) 投与群ではそれらが見られなかった．雌の血中 TG 値については，NEt-4IB (**15a**) 投与によって有意に上昇させてはいるが，NEt-3IB の投与による TG 値の上昇よりもかなり低減されており，値をみても問題にならない程度であると判断できる．以上のことから，NEt-4IB (**15a**) は長期投与においても体重増加，肝肥大，

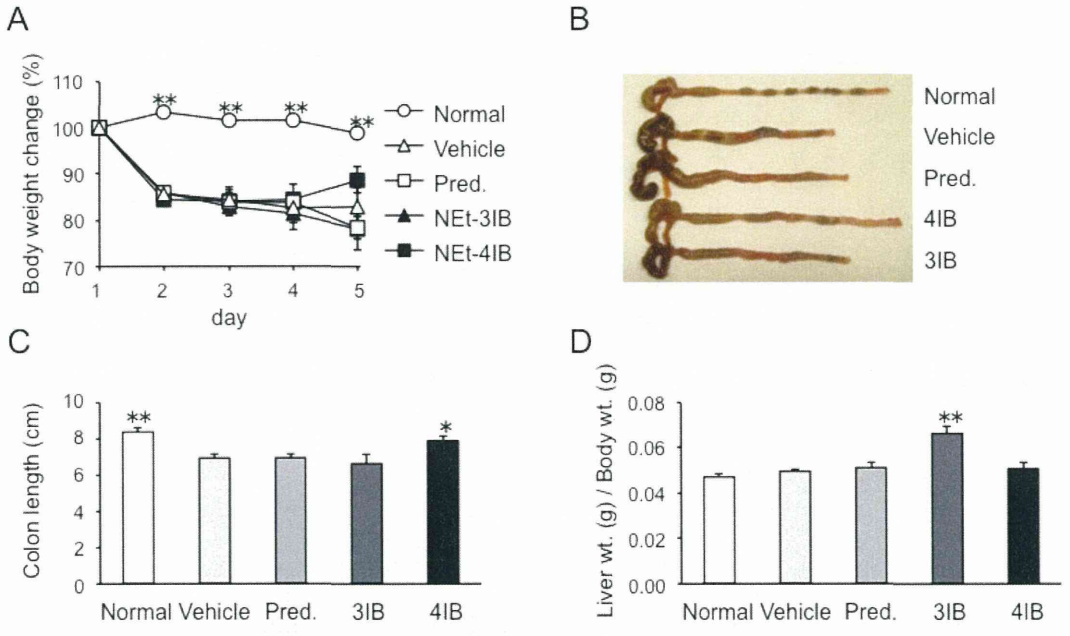


Figure 19. Anti-inflammatory effect in NBD-Cl induced inflammatory bowel disease model mice. Pred. indicates Prednisolone. Repeated oral administration of each compounds (0.2 mg × 2 times/day) for 4 days. A) Time-dependent change of body weight for 5 days. B) Picture of Colon of day 5. C) Colon length. D) Liver weight (g) / Body weight (g). Data shown are the average (n = 3-6) ± SEM and analyzed by one-way ANOVA followed by Bonferroni test. * : $p < 0.05$ and ** : $p < 0.01$ vs. vehicle respectively.

および血中 TG 値の上昇といった副作用を示さないことが分かった.

１２．NBD-Cl 誘発腸炎モデルでの薬効評価

RXR アゴニストは PPARγ/RXRα 活性化を介した抗炎症作用が期待でき，TNBS (2,4,6-trinitrobenzenesulfonic acid) 溶液を腸注することによって誘発できるクローン病モデルマウスへの有効性が報告されている.[22] そこで，著者は TNBS 誘発クローン病モデルマウスを作成し，NEt-4IB (**15a**) を投与することで薬効評価を行うことを考えた．これまでに著者らは，幾度となく TNBS 誘発クローン病モデルマウスの作成を試み，条件検討を行ってきたが，モデルマウスの安定供給が難しかった．そのような中，名古屋大学　石黒和博　准教授らによって，NBD-Cl (4-chloro-7-nitro-2,1,3-benzoxadiazole) によっても腸炎モデルマウスが作製できることが報告された.[19] そこで著者は，NBD-Cl 誘発腸炎モデルマウスの作成および腸炎モデルに対する化合物の薬効評価を行った.

NBD-Cl 溶液をマウスに腸注することで腸炎モデルマウスを作成し，NBD-Cl 腸注日から RXR フルアゴニストである NEt-3IB (**7**)と RXR パーシャルアゴニストである NEt-4IB (**15a**)を 0.2 mg × 2 回/day で 4 日間経口投与することで化合物の薬効評価を行った．ポジティブコントロールにはステロイド薬である prednisolone を用いた.

NBD-Cl 溶液の腸注 (day 1) から 4 日後 (day 5) には解剖を行い，腸長の測定や腸の炎症所見を観察した．その結果，vehicle 群では日々の体重減少が見られ，解剖日には腸の炎症が確認でき，腸長の短縮が見られた (Figure 19)．Prednisolone 投与群は vehicle 群と体重変化，腸長等においてほとんど変化がなく，腸炎の改善が見られなかった．NEt-3IB 投与群についても体重，腸長等に改善が見られなかったものの，RXR フルアゴニストの副作用である肝肥大を認めた．一方で，NEt-4IB (**15a**)

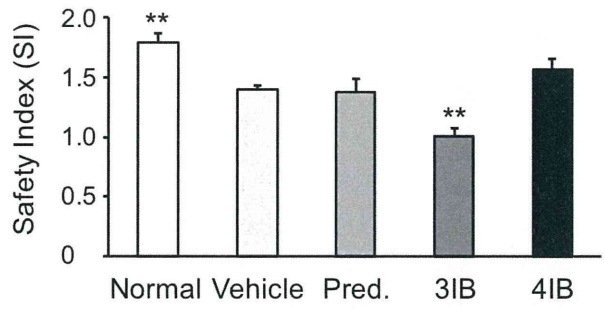


Figure 20. Safety index (SI) in each groups. Data shown are the average (n = 3-6) ± SEM and analyzed by one-way ANOVA followed by Bonferroni test. * : $p < 0.05$ and ** : $p < 0.01$ vs. vehicle respectively.