Supporting Information

SAR Exploration Guided by LE and Fsp^3: Discovery of a Selective and Orally Efficacious RORγ Inhibitor

Kazuyuki Hirata,[†] Masayuki Kotoku,[†] Noriyoshi Seki,[†] Takaki Maeba,[†] Katsuya Maeda,[†] Shintaro Hirashima,[†] Takayuki Sakai,[†] Shingo Obika,[†] Akimi Hori,[†] Yasunori Hase,[†] Takayuki Yamaguchi,[§] Yoshiaki Katsuda,[§] Takahiro Hata,[§] Naoki Miyagawa,[§] Kojo Arita,[§] Yukihiro Nomura,[¶] Kota Asahina,[¶] Yusuke Aratsu,[¶] Masafumi Kamada,[#] Tsuyoshi Adachi,[#] Masato Noguchi,[#] Satoki Doi,[#] Paul Crowe,[‡] Erin Bradley,[‡,Δ] Ruo Steensma,[‡,∞] Haiyan Tao,[‡] Morgan Fenn,[‡] Robert Babine,[‡,£] Xiaolin Li,[‡,¢] Scott Thacher,[‡] Hiromasa Hashimoto[†] and Makoto Shiozaki[*,†]

[†]Chemical Research Laboratories, [§]Biological Pharmacological Research Laboratories, [¶]Drug Metabolism & Pharma-cokinetics Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc., 1-1, Murasaki-cho, Takatsuki Osaka, 569-1125, Japan, [#]Pharmaceutical Frontier Research Laboratories, Central Pharmaceutical Research Institute, Japan Tobacco Inc., 1-13-2, Fukuura, Kanazawa-ku, Yokohama Kanagawa, 236-0004, Japan, [‡]Orphagen Pharmaceuticals, 11558 Sorrento Valley Road, Suite 4, San Diego, California, 92121, United States

Present Addresses

[Δ]Corvus Pharmaceuticals Inc., 863 Mitten Road, Suite 102, Burlingame, CA 94010, United States.

[∞]Janssen Research & Development, 3210 Merryfield Row, San Diego, CA 92121, United States.

[£]Rebexsess Discovery Chemistry, 7819 Estancia St. Carlsbad, CA 92009, United States.

[¢]GIMDx Inc., 2440 Grand Ave., Ste A Vista, CA 92081, United States.

Contents

1. Abbreviations

The following abbreviations and definitions have been used:

Ac_2O	Acetic anhydride
AcOH	Acetic acid
Boc	*tert*-Butoxycarbonyl
Boc_2O	Di-*tert*-butyl dicarbonate
DIEA	*N*,*N*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
equiv	Equivalent
Et_3N	Triethylamine
EtO	Ethoxy
EtOAc	Ethyl acetate
EtOH	Ethanol
HATU	2-(7-Aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
$HOBt \cdot H_2O$	1-Hydroxybenzotriazole monohydrate
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrum
MC	Methylcellulose
MeCN	Acetonitrile
MeI	Iodomethane
MeOH	Methanol
NaOAc	Sodium acetate
NBS	*N*-Bromosuccinimide
PTX	Pertussis toxin
p-TsOMe	Methyl *p*-toluenesulfonate
TBAF	Tetrabutylammonium fluoride
TBS	*tert*-Butyldimethylsilyl
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
TMDS	1,1,3,3-Tetramethyldisilazane
WSC·HCl	1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride
DMSO-d_6	Deuterodimethylsulfoxide
MeOH-d_4	Deuteromethanol
δ	Chemical shift (ppm)
brs	Broad singlet
d	Doublet

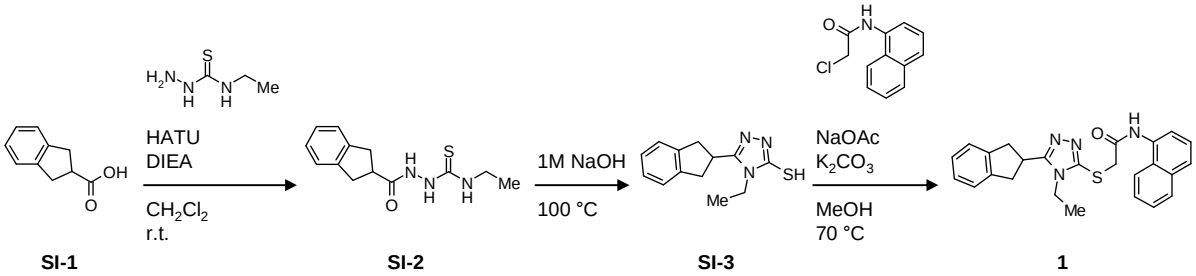
m	Multiplet
q	Quartet
quin	Quintet
s	Singlet
t	Triplet
J	Coupling constant

2. Chemistry

General information

Unless otherwise specified, materials were purchased from commercial suppliers and used without further purification. TLC was performed using silica gel 60 F_{254} plates purchased from Merck. Flash chromatography was performed using prepacked columns of silica gel (230–400 mesh, 40–63 μm) purchased from SiliCycle or using Silica gel 60N (spherical, neutral, 40–50 mesh) purchased from Kanto Chemical Co., Inc. The preparative HPLC was performed on a Japan Analytical Industry Co., Ltd. LC-908 instrument. Analytic HPLC was performed on a SHIMADZU Prominence instrument. ^{1}H NMR spectra were recorded on a JEOL JNM-AL400, Bruker AVANCEIII 400, or Varian MERCURYplus 400 spectrometer. HRMS spectra were recorded on an LC-MS system composed of Agilent 1290 Infinity LC and Thermo Fisher LTQ-Orbitrap Velos. ^{13}C NMR spectra were recorded on an Agilent DD2 500. Unless otherwise specified, ^{1}H and ^{13}C NMR were measured at 25 °C. The purity of inhibitors **1**, **2** and **3a**–**3z** was determined by HPLC [Column: SHIMADZU Shim-pack XR-ODS (3 × 50 mm, 2.2 μm); Mobile phase A: 0.1% TFA in water; Mobile phase B: 0.1% TFA in MeCN; Gradient: 10% B to 90% B from 0 to 5 min, 90% B from 5 to 7 min, 90% B to 10% B from 7 to 7.5 min, 10% B from 7.5 to 10 min; Flow rate: 8.0 mL/min; Detection wavelength: 220 nm. Optical rotation ($[\alpha]_D$) was measured at 25 °C with a Rudolph Research Analytical Autopol V spectrometer.

Synthesis of inhibitor 1



5-(2,3-Dihydro-1*H*-inden-2-yl)-4-ethyl-4*H*-1,2,4-triazole-3-thiol (SI-3)

To a solution of 2,3-dihydro-1*H*-indene-2-carboxylic acid **SI-1** (500 mg, 3.08 mmol) in CH_2Cl_2 (7.5 mL) was added HATU (1.17g, 3.08 mmol) and the reaction mixture was stirred at room temperature. After 20 min, *N*-ethylhydrazinecarbothioamide (404 mg, 3.39 mmol) and DIEA (644 μL, 3.70 mmol) were successively added and the reaction was continued at room temperature overnight. After concentrating under reduced pressure, EtOAc and THF were poured into the residue, and the mixture was washed with saturated aqueous $NaHCO_3$ solution,

water and brine, then dried over $MgSO_4$. After filtration and removal of the solvent under reduced pressure, crude **SI-2** (1.53g) was obtained as a colorless solid. The crude **SI-2** (1.53 g) was suspended in 1 M NaOH (3.7 mL, 3.7 mmol) and heated at 100 °C for 1 h. HCl (1M) was added to the reaction at 0 °C to neutralize the reaction and the organic layer was extracted with EtOAc. The mixture was washed with water, dilute HCl, and brine, then dried over $MgSO_4$. After filtration and removal of the solvent under reduced pressure, the resultant yellow solid was purified by trituration with successive extractions with diisopropyl ether (8 mL) and CH_2Cl_2 (8 mL) to afford the title compound **SI-3** (707 mg, 94% yield for 2 steps) as a colorless solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.28 (t, *J* = 7.20 Hz, 3H), 3.17 (dd, *J* = 15.60, 8.00 Hz, 2H), 3.36 (dd, *J* = 15.60, 8.80 Hz, 2H), 3.82 (quin, *J* = 8.40 Hz, 1H), 4.04 (q, *J* = 7.20 Hz, 2H), 7.17 (dd, *J* = 5.60, 3.20 Hz, 2H), 7.25 (dd, *J* = 5.20, 3.20 Hz, 2H), 13.50 (s, 1H).

2-(5-(2,3-Dihydro-1*H*-inden-2-yl)-4-ethyl-4*H*-1,2,4-triazol-3-ylthio)-*N*-(naphthalen-1-yl)acetamide (1)

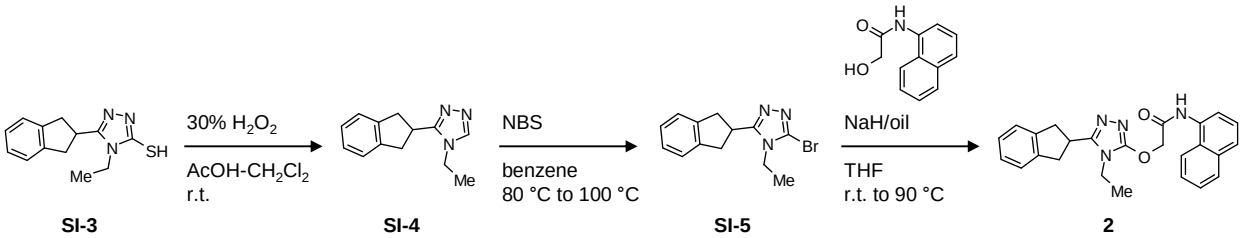
A suspension of **SI-3** (100 mg, 0.408 mmol), 2-chloro-*N*-(naphthalen-1-yl)acetamide (90 mg, 0.41 mmol), NaOAc (37 mg, 0.45 mmol) and K_2CO_3 (56 mg, 0.41 mmol) in MeOH (2 mL) was heated at 70 °C for 1 h. After removal of the solvent under reduced pressure, the residue was diluted with EtOAc and THF, washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography ($CHCl_3$:EtOAc = 67:33 (v/v)) to give the title compound **1** (145 mg, 83% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.44 (t, *J* = 7.20 Hz, 3H), 3.37 (dd, *J* = 15.20, 8.80 Hz, 2H), 3.50 (dd, *J* = 15.20, 8.80 Hz, 2H), 3.71 (quin, *J* = 8.80 Hz, 1H), 3.99 (q, *J* = 7.20 Hz, 2H), 4.15 (s, 2H), 7.21–7.28 (m, 4H), 7.44–7.48 (m, 3H), 7.65 (d, *J* = 8.40 Hz, 1H), 7.81–7.83 (m, 1H), 8.11–8.15 (m, 2H), 10.62 (s, 1H).

HRMS *m/z*: $[M+H]^+$ calcd for $C_{25}H_{24}N_4OS$, 429.1744; found, 429.1739.

Purity: 98.3%.

Synthesis of inhibitor 2



3-(2,3-Dihydro-1*H*-inden-2-yl)-4-ethyl-4*H*-1,2,4-triazole (SI-4)

A mixture of 30% H_2O_2 (2.3 mL, 22 mmol) and AcOH (8.7 mL, 0.15 mol) was added dropwise to a suspension of **SI-3** (2.48 g, 10.1 mmol) in CH_2Cl_2 (12.0 mL) at 0 °C. After stirring at room temperature overnight, the mixture was neutralized with 4M NaOH (38.0 mL, 152 mmol), and extracted with $CHCl_3$. The organic layer was washed with water and brine, dried over $MgSO_4$, and concentrated. The residue was purified by flash chromatography ($CHCl_3$:MeOH = 97: 3 (v/v)) to give the title compound **SI-4** (1.60 g, 74% yield) as a yellow oil.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.51 (t, *J* = 7.20 Hz, 3H), 3.36 (dd, *J* = 15.20, 8.40 Hz, 2H), 3.50 (dd, *J* = 15.60, 9.60 Hz, 2H), 3.72 (quin, *J* = 8.80 Hz, 1H), 4.02 (q, *J* = 7.20 Hz, 2H), 7.18–7.21 (m, 2H), 7.24–7.26 (m, 2H), 8.15 (s, 1H).

3-Bromo-5-(2,3-dihydro-1*H*-inden-2-yl)-4-ethyl-4*H*-1,2,4-triazole (SI-5)

A mixture of **SI-4** (100 mg, 0.469 mmol) and NBS (92 mg, 0.52 mmol) in benzene (2.0 mL) was stirred at 80 °C for 1 h, and the reaction was continued at 100 °C for an additional 1 h. The mixture was diluted with saturated aqueous $NaHCO_3$, and subsequently extracted with EtOAc. The organic layer was washed with water and brine, dried with Na_2SO_4, and concentrated. The residue was purified by preparative TLC (*n*-hexane:EtOAc = 25:75 (v/v)) to afford the title compound **SI-5** (65 mg, 47% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.42 (t, *J* = 7.60 Hz, 3H), 3.34 (dd, *J* = 14.80, 8.00 Hz, 2H), 3.50 (dd, *J* = 15.60, 9.60 Hz, 2H), 3.71 (quin, *J* = 8.40 Hz, 1H), 4.03 (q, *J* = 7.60 Hz, 2H), 7.18–7.21 (m, 2H), 7.24–7.26 (m, 2H).

2-(5-(2,3-Dihydro-1*H*-inden-2-yl)-4-ethyl-4*H*-1,2,4-triazol-3-yloxy)-*N*-(naphthalen-1-yl)acetamide (2)

To a solution of **SI-5** (36 mg, 0.12 mmol) and 2-hydroxy-*N*-(naphthalen-1-yl)acetamide (25 mg, 0.12 mmol) in THF (2.0 mL) was added NaH (60% in mineral oil, 11 mg, 0.27 mmol) at 0 °C. The mixture was stirred at room temperature for 30 min, and the reaction was further continued at 90 °C for 7 h. After removal of the solvent under reduced pressure, water was poured into the residue and the mixture was extracted with EtOAc. The organic layer was washed with water and brine, then dried over Na_2SO_4. After filtration and subsequent removal of the solvent under reduced pressure, the residue was purified by a two-step preparative TLC procedure (*n*-hexane:EtOAc = 20:80 (v/v) and $CHCl_3$:MeOH = 95:5 (v/v)) to afford the title compound **2** (5.7 mg, 11% yield).

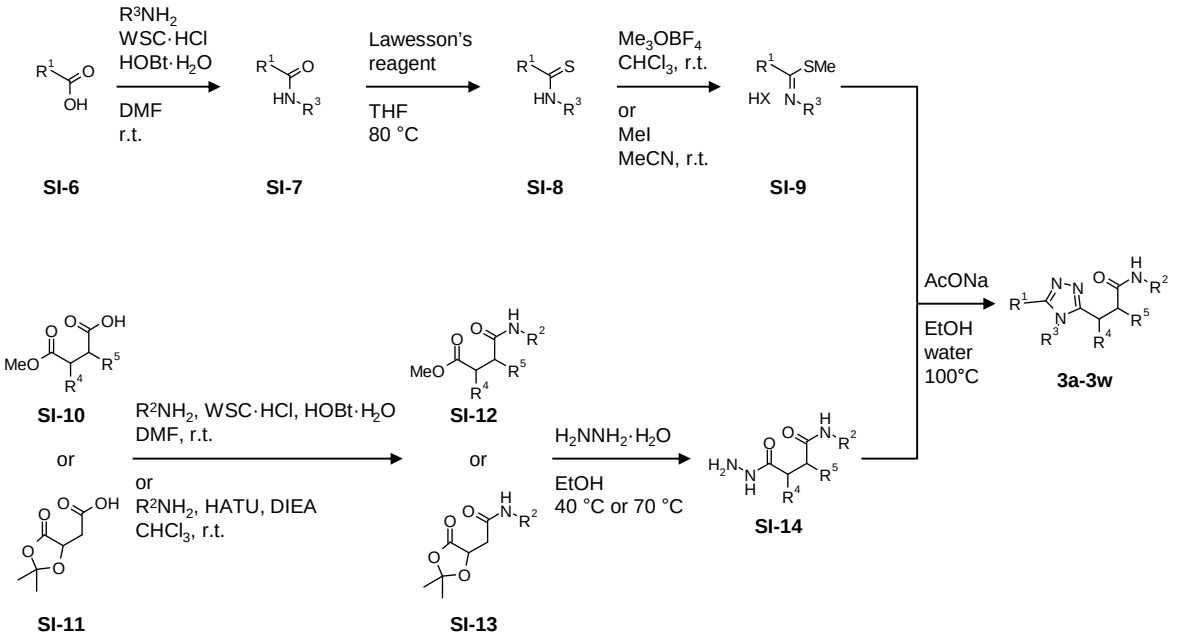
^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.32 (t, *J* = 7.20 Hz, 3H), 3.20–3.35 (m, 4H), 3.76 (quin, *J* = 8.80 Hz, 1H), 3.94 (q, *J* = 7.20 Hz, 2H), 5.18 (s, 2H), 7.16 (dd, *J* = 5.20, 3.20 Hz, 2H), 7.25 (dd, *J* = 5.20, 3.20 Hz, 2H), 7.50 (t, *J* = 8.00 Hz, 1H), 7.54 (dd, *J* = 6.80, 3.60 Hz, 2H), 7.65 (d, *J* = 7.60 Hz, 1H), 7.79 (d, *J* = 8.00 Hz, 1H), 7.93–7.95 (m, 1H), 8.06–8.08 (m, 1H), 10.19 (s, 1H).

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{25}H_{24}N_4O_2$, 413.1972; found, 413.1968.

Purity: 99.5%.

Synthesis of inhibitor 3a–3w

General procedure



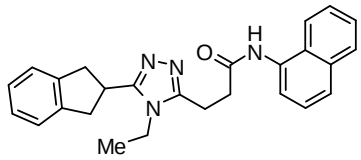
WSC·HCl (1.1 equiv) and HOBt·H_2O (1.1 equiv) were added to a mixed solution of carboxylic acid **SI-6** (1 equiv) and R^3NH_2 (1.2 equiv) in DMF (0.5 M) at room temperature. After stirring at room temperature overnight, saturated aqueous $NaHCO_3$ was poured into the reaction and the mixture was extracted with EtOAc, washed with water and brine, and then dried over $MgSO_4$. After filtration and evaporation, the residue was purified by flash chromatography to give **SI-7**. To a solution of **SI-7** (1 equiv) in THF (0.5 M), Lawesson's reagent (0.65 equiv) was added, and the mixture was heated at 80 °C for 3 h. After removal of the solvent under reduced pressure, the residue was purified by flash chromatography to give **SI-8**. **SI-8** (1 equiv) was dissolved in $CHCl_3$ (0.25 M) and treated with $Me_3O^+BF_4^-$ (1 equiv) at room temperature (alternatively, MeI (1 equiv) and MeCN (0.5 M) were used instead of $Me_3O^+BF_4^-$ and $CHCl_3$, respectively). After stirring the reaction overnight, the solvent was removed under reduced pressure, then the resultant crude thioimidate **SI-9** was ready for the next reaction.

To a mixed solution of half-ester **SI-10** or **SI-11** (1 equiv) and R^2NH_2 (1 equiv) in DMF (1 M) were added WSC·HCl (1.5 equiv) and HOBt·H_2O (1.5 equiv) **(**As an alternative condition, **SI-10** or **SI-11** (1 equiv), R^2NH_2 (1 equiv), HATU (1equiv) and DIEA (1.2 equiv) in $CHCl_3$ (0.5 M) were used). After stirring at room temperature overnight, saturated aqueous $NaHCO_3$ was poured into the reaction. The mixture was extracted with EtOAc, washed with water and brine, and then dried over $MgSO_4$. After filtration and removal of the solvent under reduced pressure, the residue was purified by flash chromatography to give **SI-12** or **SI-13**. **SI-12** or **SI-13** (1 equiv) was added to a solution of $H_2NNH_2·H_2O$ (1 equiv) in EtOH (0.75 M) and heated at 40–70 °C until the reaction was completed. After cooling to room temperature, the resulting mixture was triturated with diethyl ether to afford acylhydrazine **SI-14**.

Acylhydrazine **SI-14** (1 equiv) was added to a mixed solution of thioimidate **SI-9** (1 equiv) and NaOAc (1.5 equiv) in EtOH (0.32 M) and water (0.16 M). After stirring at 100 °C overnight, saturated aqueous $NaHCO_3$ was poured into the reaction at 0 °C. The organic layer was extracted with EtOAc, washed with water and brine, and then dried over $MgSO_4$. After filtration and removal of the solvent under reduced pressure, the residue was

purified by flash chromatography to give inhibitors **3a**–**3w**.

3-(5-(2,3-Dihydro-1*H*-inden-2-yl)-4-ethyl-4*H*-1,2,4-triazol-3-yl)-*N*-(naphthalen-1-yl)propanamide (3a)

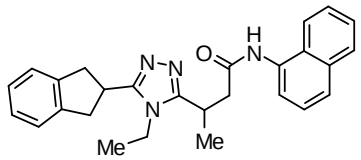


^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.34 (t, *J* = 7.28 Hz, 3H), 3.12 (t, *J* = 6.62 Hz, 2H), 3.21 (dd, *J* = 15.22, 8.38 Hz, 2H), 3.34 (td, *J* = 14.45, 8.45 Hz, 4H), 3.58 (dd, *J* = 17.97, 9.15 Hz, 1H), 3.94 (q, *J* = 7.28 Hz, 2H), 7.16 (s, 4H), 7.40 (t, *J* = 7.83 Hz, 3H), 7.64 (d, *J* = 8.16 Hz, 1H), 7.79 (t, *J* = 10.03 Hz, 2H), 7.93 (d, *J* = 9.26 Hz, 1H), 9.60 (s, 1H).

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{26}H_{26}N_4O$, 411.2179; found, 411.2177.

Purity: 98.9%.

3-(5-(2,3-Dihydro-1*H*-inden-2-yl)-4-ethyl-4*H*-1,2,4-triazol-3-yl)-*N*-(naphthalen-1-yl)butanamide (3b)

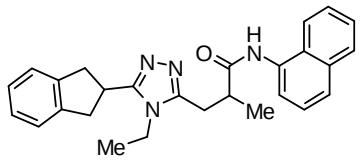


^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.34 (m, 6H), 2.94 (s, 1H), 3.33–3.90 (m, 9H), 7.17 (t, *J* = 2.65 Hz, 2H), 7.25 (s, 2H), 7.46–7.58 (m, 4H), 7.75 (d, *J* = 9.26 Hz, 1H), 7.91 (d, *J* = 7.28 Hz, 2H), 10.04 (s, 1H).

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{27}H_{28}N_4O$, 425.2336; found, 425.2334.

Purity: 97.7%.

3-(5-(2,3-Dihydro-1*H*-inden-2-yl)-4-ethyl-4*H*-1,2,4-triazol-3-yl)-2-methyl-*N*-(naphthalen-1-yl)propanamide (3c)

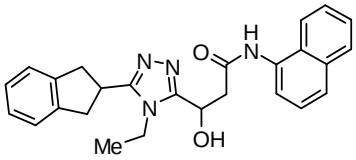


^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.28–1.37 (m, 3H), 1.50 (t, *J* = 9.81 Hz, 3H), 2.85 (dd, *J* = 16.10, 3.53 Hz, 1H), 3.09–3.69 (m, 7H), 3.83–4.02 (m, 2H), 7.21 (dt, *J* = 19.92, 4.74 Hz, 4H), 7.39–7.44 (m, 3H), 7.64 (d, *J* = 8.16 Hz, 1H), 7.83 (tt, *J* = 14.67, 5.77 Hz, 3H), 8.86 (s, 1H).

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{27}H_{28}N_4O$, 425.2336; found, 425.2334.

Purity: 95.4%.

3-(5-(2,3-Dihydro-1*H*-inden-2-yl)-4-ethyl-4*H*-1,2,4-triazol-3-yl)-3-hydroxy-*N*-(naphthalen-1-yl)propanamide (3d)

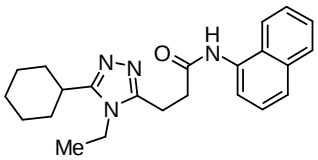


^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.37 (t, *J* = 7.17 Hz, 3H), 3.19–3.42 (m, 6H), 3.87 (t, *J* = 8.60 Hz, 1H), 4.18 (dd, *J* = 16.87, 9.37 Hz, 2H), 5.27 (s, 1H), 6.03 (brs, 1H), 7.17–7.20 (m, 2H), 7.27 (s, 2H), 7.46–7.54 (m, 3H), 7.66 (d, *J* = 7.50 Hz, 1H), 7.76 (d, *J* = 8.16 Hz, 1H), 7.92 (t, *J* = 4.63 Hz, 1H), 8.09 (d, *J* = 6.84 Hz, 1H), 10.11 (s, 1H).

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{26}H_{26}N_4O_2$, 427.2129; found, 427.2125.

Purity: 99.2%.

3-(5-Cyclohexyl-4-ethyl-4*H*-1,2,4-triazol-3-yl)-*N*-(naphthalen-1-yl)propanamide (3e)

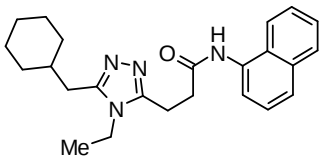


^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.32–1.34 (m, 6H), 1.74–1.77 (m, 3H), 1.87–1.90 (m, 4H), 2.57 (t, *J* = 12.02 Hz, 1H), 3.12 (t, *J* = 6.18 Hz, 2H), 3.24 (t, *J* = 6.18 Hz, 2H), 3.89 (q, *J* = 7.35 Hz, 2H), 7.47 (ddd, *J* = 24.92, 13.23, 5.51 Hz, 3H), 7.65 (d, *J* = 7.94 Hz, 1H), 7.82 (d, *J* = 8.16 Hz, 1H), 7.94 (dd, *J* = 13.56, 8.05 Hz, 2H), 9.25 (s, 1H).

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{23}H_{28}N_4O$, 377.2336; found, 377.2334.

Purity: 99.7%.

3-(5-(Cyclohexylmethyl)-4-ethyl-4*H*-1,2,4-triazol-3-yl)-*N*-(naphthalen-1-yl)propanamide (3f)

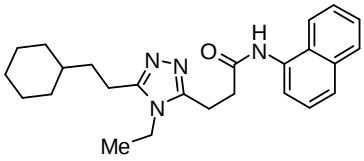


^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.90–0.99 (m, 2H), 1.06–1.21 (m, 3H), 1.26–1.34 (m, 3H), 1.60–1.80 (m, 6H), 2.56 (dd, *J* = 12.24, 7.17 Hz, 2H), 3.12 (t, *J* = 6.51 Hz, 2H), 3.27 (t, *J* = 6.51 Hz, 2H), 3.83–3.91 (m, 2H), 7.47 (tt, *J* = 14.34, 6.36 Hz, 3H), 7.65 (d, *J* = 8.16 Hz, 1H), 7.83 (dd, *J* = 18.53, 7.50 Hz, 2H), 7.96 (d, *J* = 7.94 Hz, 1H), 9.47 (s, 1H).

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{24}H_{30}N_4O$, 391.2492; found, 391.2492.

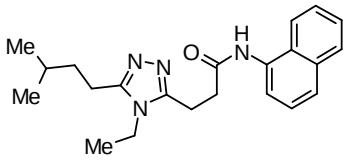
Purity: 98.5%.

3-(5-(2-Cyclohexylethyl)-4-ethyl-4*H*-1,2,4-triazol-3-yl)-*N*-(naphthalen-1-yl)propanamide (3g)



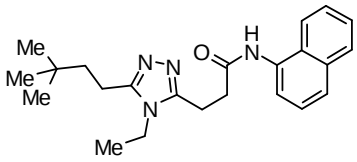
^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.73–0.82 (m, 2H), 1.09–1.22 (m, 4H), 1.27 (dt, *J* = 18.38, 5.13 Hz, 3H), 1.47–1.53 (m, 2H), 1.65 (dt, *J* = 30.07, 13.12 Hz, 5H), 2.56 (dd, *J* = 9.26, 7.28 Hz, 2H), 3.08 (t, *J* = 6.84 Hz, 2H), 3.32 (t, *J* = 6.84 Hz, 2H), 3.84 (q, *J* = 7.28 Hz, 2H), 7.42 (ddd, *J* = 17.31, 8.60, 5.18 Hz, 3H), 7.65 (d, *J* = 8.16 Hz, 1H), 7.78–7.81 (m, 2H), 7.93–7.99 (m, 1H), 9.97 (s, 1H).
HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{25}H_{32}N_4O$, 405.2649; found, 405.2646.
Purity: 99.3%.

3-(4-Ethyl-5-isopentyl-4*H*-1,2,4-triazol-3-yl)-*N*-(naphthalen-1-yl)propanamide (3h)



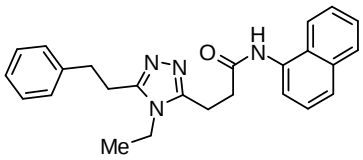
^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.95 (d, *J* = 6.40 Hz, 6H), 1.33 (t, *J* = 7.28 Hz, 3H), 1.67 (t, *J* = 5.95 Hz, 3H), 2.68 (t, *J* = 7.83 Hz, 2H), 3.13 (t, *J* = 5.95 Hz, 2H), 3.23 (t, *J* = 6.18 Hz, 2H), 3.88 (q, *J* = 7.35 Hz, 2H), 7.48 (tt, *J* = 17.64, 6.65 Hz, 3H), 7.65 (d, *J* = 7.94 Hz, 1H), 7.82 (d, *J* = 8.16 Hz, 1H), 7.94 (t, *J* = 8.49 Hz, 2H), 9.14 (s, 1H).
HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{22}H_{28}N_4O$, 365.2336; found, 365.2335.
Purity: 99.8%.

3-(5-(3,3-Dimethylbutyl)-4-ethyl-4*H*-1,2,4-triazol-3-yl)-*N*-(naphthalen-1-yl)propanamide (3i)



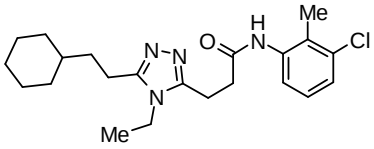
^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.94 (t, *J* = 13.78 Hz, 9H), 1.33 (t, *J* = 7.28 Hz, 3H), 1.64–1.68 (m, 2H), 2.61–2.66 (m, 2H), 3.13 (t, *J* = 6.40 Hz, 2H), 3.24 (t, *J* = 6.29 Hz, 2H), 3.88 (q, *J* = 7.28 Hz, 2H), 7.48 (ddd, *J* = 26.91, 14.22, 6.29 Hz, 3H), 7.66 (d, *J* = 8.16 Hz, 1H), 7.82 (d, *J* = 7.94 Hz, 1H), 7.93 (dd, *J* = 13.23, 7.94 Hz, 2H), 9.22 (s, 1H).
HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{23}H_{30}N_4O$, 379.2492; found, 379.2491.
Purity: 99.6%.

3-(4-Ethyl-5-phenethyl-4*H*-1,2,4-triazol-3-yl)-*N*-(naphthalen-1-yl)propanamide (3j)



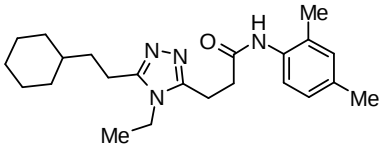
^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.20 (t, *J* = 7.28 Hz, 3H), 2.96 (t, *J* = 8.05 Hz, 2H), 3.09–3.16 (m, 4H), 3.24 (t, *J* = 6.18 Hz, 2H), 3.73 (q, *J* = 7.35 Hz, 2H), 7.15–7.25 (m, 5H), 7.49 (tt, *J* = 19.74, 6.36 Hz, 3H), 7.66 (d, *J* = 7.94 Hz, 1H), 7.83 (d, *J* = 7.94 Hz, 1H), 7.97 (dd, *J* = 17.64, 8.16 Hz, 2H), 9.13 (s, 1H).
HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{25}H_{26}N_4O$, 399.2179; found, 399.2176.
Purity: 99.8%.

_N_-(3-Chloro-2-methylphenyl)-3-(5-(2-cyclohexylethyl)-4-ethyl-4_H_-1,2,4-triazol-3-yl)propanamide (3k)



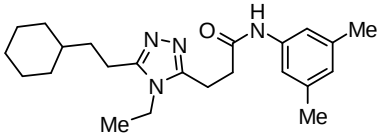
^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.92 (dt, *J* = 27.86, 7.11 Hz, 2H), 1.09–1.34 (m, 7H), 1.67 (ddt, *J* = 30.21, 13.45, 5.98 Hz, 7H), 2.25 (d, *J* = 16.76 Hz, 3H), 2.65–2.69 (m, 2H), 3.09 (dt, *J* = 34.92, 6.18 Hz, 4H), 3.88 (q, *J* = 7.28 Hz, 2H), 7.08 (t, *J* = 7.94 Hz, 1H), 7.17 (d, *J* = 7.72 Hz, 1H), 7.55 (t, *J* = 9.26 Hz, 1H), 9.15 (s, 1H).
HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{22}H_{31}ClN_4O$, 403.2259; found, 403.2256.
Purity: 97.7%.

3-(5-(2-Cyclohexylethyl)-4-ethyl-4_H_-1,2,4-triazol-3-yl)-_N_-(2,4-dimethylphenyl)propanamide (3l)



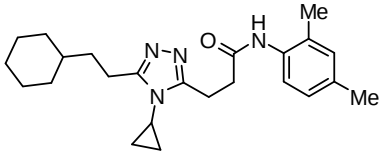
^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.90–0.99 (m, 2H), 1.10–1.38 (m, 7H), 1.62–1.78 (m, 7H), 2.17 (s, 3H), 2.28 (t, *J* = 9.15 Hz, 3H), 2.68 (dt, *J* = 7.87, 3.58 Hz, 2H), 3.07 (ddd, *J* = 15.82, 10.20, 3.58 Hz, 4H), 3.84-3.92 (m, 2H), 6.98 (d, *J* = 15.88 Hz, 2H), 7.56 (dd, *J* = 15.44, 11.03 Hz, 1H), 8.36 (s, 1H).
HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{23}H_{34}N_4O$, 383.2805; found, 383.2802.
Purity: 95.8%.

3-(5-(2-Cyclohexylethyl)-4-ethyl-4_H_-1,2,4-triazol-3-yl)-_N_-(3,5-dimethylphenyl)propanamide (3m)



^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.86–0.95 (m, 2H), 1.07–1.38 (m, 7H), 1.53–1.76 (m, 7H), 2.16–2.25 (m, 6H), 2.63–2.69 (m, 2H), 2.83 (dd, *J* = 16.43, 9.81 Hz, 2H), 2.92 (t, *J* = 6.84 Hz, 2H), 3.91 (q, *J* = 7.20 Hz, 2H), 6.66 (s, 1H), 7.21 (t, *J* = 19.19 Hz, 2H), 9.90 (d, *J* = 18.97 Hz, 1H).
HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{23}H_{34}N_4O$, 383.2805; found, 383.2803.
Purity: 99.5%.

3-(5-(2-Cyclohexylethyl)-4-cyclopropyl-4_H_-1,2,4-triazol-3-yl)-_N_-(2,4-dimethylphenyl)propanamide (3n)



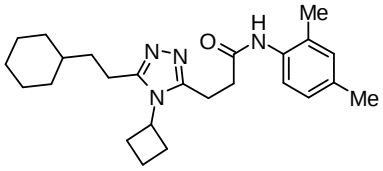
^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.94 (dt, *J* = 10.44, 5.62 Hz, 4H), 1.13–1.27 (m, 6H), 1.61–1.76 (m, 7H), 2.18 (s,

3H), 2.26 (s, 3H), 2.78 (t, *J* = 8.16 Hz, 2H), 2.94–2.98 (m, 1H), 3.06 (t, *J* = 6.18 Hz, 2H), 3.16 (t, *J* = 6.06 Hz, 2H), 6.96 (s, 2H), 7.57 (d, *J* = 8.82 Hz, 1H), 8.51 (s, 1H).

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{24}H_{34}N_4O$, 395.2805; found, 395.2803.

Purity: 99.0%.

3-(4-Cyclobutyl-5-(2-cyclohexylethyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)propanamide (3o)

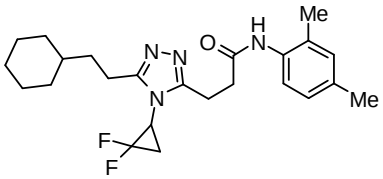


^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.92 (d, *J* = 11.69 Hz, 2H), 1.20 (dd, *J* = 22.05, 10.37 Hz, 4H), 1.57–1.75 (m, 7H), 1.86–1.96 (m, 2H), 2.19 (s, 3H), 2.26 (s, 3H), 2.55 (dd, *J* = 18.86, 8.49 Hz, 4H), 2.73 (t, *J* = 8.16 Hz, 2H), 3.11 (dd, *J* = 18.75, 6.18 Hz, 4H), 4.53 (t, *J* = 8.71 Hz, 1H), 6.95 (s, 2H), 7.61 (d, *J* = 8.60 Hz, 1H), 8.58 (s, 1H).

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{25}H_{36}N_4O$, 409.2962; found, 409.2955.

Purity: 96.8%.

3-(5-(2-Cyclohexylethyl)-4-(2,2-difluorocyclopropyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)propanamide (3p)

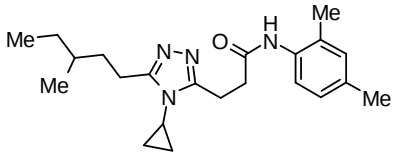


^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86–0.99 (m, 2H), 1.10–1.37 (m, 4H), 1.61–1.79 (m, 7H), 1.90–2.00 (m, 1H), 2.16 (s, 3H), 2.18–2.32 (m, 1H), 2.26 (s, 3H), 2.71–2.79 (m, 2H), 2.97–3.15 (m, 4H), 3.57–3.67 (m, 1H), 6.93–7.00 (m, 2H), 7.54 (d, *J* = 8.82 Hz, 1H), 7.84 (brs, 1H).

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{24}H_{32}F_2N_4O$, 431.2617; found, 431.2612.

Purity: 98.0%.

3-(4-Cyclopropyl-5-(3-methylpentyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)propanamide (3q)

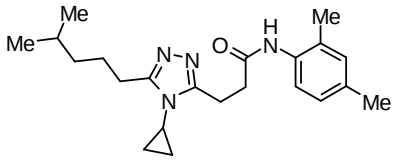


^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.89 (t, *J* = 7.28 Hz, 3H), 0.94 (d, *J* = 6.40 Hz, 3H), 0.94–0.99 (m, 2H), 1.14–1.28 (m, 3H), 1.34–1.48 (m, 2H), 1.52–1.63 (m, 1H), 1.75–1.85 (m, 1H), 2.18 (s, 3H), 2.26 (s, 3H), 2.70–2.87 (m, 2H), 2.97 (tt, *J* = 7.12, 3.96 Hz, 1H), 3.05 (t, *J* = 6.46 Hz, 2H), 3.17 (t, *J* = 6.46 Hz, 2H), 6.93–6.98 (m, 2H), 7.61 (d, *J* = 8.60 Hz, 1H), 8.28 (brs, 1H).

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{22}H_{32}N_4O$, 369.2649; found, 369.2647.

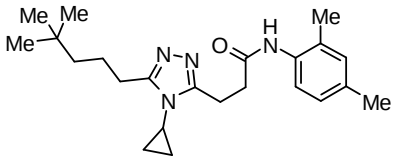
Purity: 92.9%.

3-(4-Cyclopropyl-5-(4-methylpentyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)propanamide (3r)



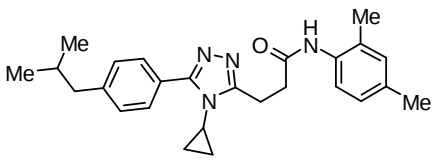
^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.89 (d, *J* = 6.62 Hz, 6H), 0.93–0.98 (m, 2H), 1.15–1.21 (m, 2H), 1.25–1.32 (m, 2H), 1.53–1.64 (m, 1H), 1.74–1.83 (m, 2H), 2.19 (s, 3H), 2.26 (s, 3H), 2.73–2.79 (m, 2H), 2.96 (tt, *J* = 7.12, 3.96 Hz, 1H), 3.02–3.09 (m, 2H), 3.14–3.19 (m, 2H), 6.93–6.98 (m, 2H), 7.62 (d, *J* = 8.82 Hz, 1H), 8.28 (brs, 1H).
HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{22}H_{32}N_4O$, 369.2649; found, 369.2647.
Purity: 96.1%.

3-(4-Cyclopropyl-5-(4,4-dimethylpentyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)propanamide (3s)



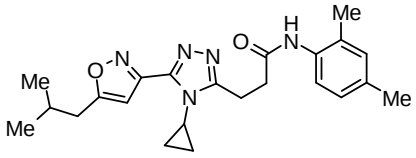
^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.90 (s, 9H), 0.95 (d, *J* = 2.65 Hz, 2H), 1.18 (d, *J* = 5.73 Hz, 2H), 1.28 (dd, *J* = 11.14, 6.06 Hz, 2H), 1.73–1.78 (m, 2H), 2.18 (s, 3H), 2.26 (s, 3H), 2.75 (t, *J* = 7.83 Hz, 2H), 2.96 (s, 1H), 3.06 (t, *J* = 6.40 Hz, 2H), 3.17 (t, *J* = 6.40 Hz, 2H), 6.95 (s, 2H), 7.59 (d, *J* = 8.60 Hz, 1H), 8.38 (s, 1H).
HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{23}H_{34}N_4O$, 383.2805; found, 383.2802.
Purity: 99.0%.

3-(4-Cyclopropyl-5-(4-isobutylphenyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)propanamide (3t)



^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.63–0.68 (m, 2H), 0.92 (d, *J* = 6.62 Hz, 6H), 1.02–1.09 (m, 2H), 1.86–1.96 (m, 1H), 2.21 (s, 3H), 2.26 (s, 3H), 2.54 (d, *J* = 7.28 Hz, 2H), 3.10–3.15 (m, 2H), 3.20–3.30 (m, 3H), 6.95–6.99 (m, 2H), 7.24 (d, *J* = 8.16 Hz, 2H), 7.61 (d, *J* = 8.38 Hz, 2H), 7.64 (d, *J* = 8.82 Hz, 1H), 8.17 (brs, 1H).
HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{26}H_{32}N_4O$, 417.2649; found, 417.2645.
Purity: 99.2%.

3-(4-Cyclopropyl-5-(5-isobutylisoxazol-3-yl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)propanamide (3u)

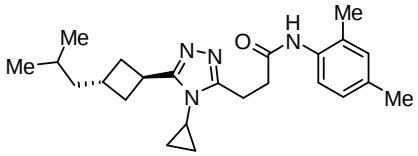


^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.87–0.94 (m, 2H), 1.00 (d, *J* = 6.62 Hz, 6H), 1.24–1.31 (m, 2H), 2.05–2.15 (m, 1H), 2.21 (s, 3H), 2.26 (s, 3H), 2.71 (d, *J* = 7.06 Hz, 2H), 3.12 (t, *J* = 6.62 Hz, 2H), 3.26–3.35 (m, 3H), 6.58 (d, *J* = 0.66 Hz, 1H), 6.95–7.00 (m, 2H), 7.60 (d, *J* = 8.82 Hz, 1H), 7.95 (brs, 1H).

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{23}H_{29}N_5O_2$, 408.2394; found, 408.2392.

Purity: 99.1%.

3-(4-Cyclopropyl-5-(*trans*-3-isobutylcyclobutyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)propanamide (3v)



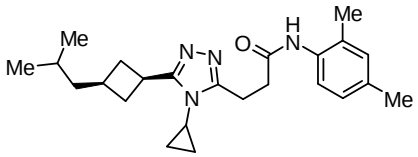
trans-3-Isobutylcyclobutanecarboxylic acid as R^1COOH was prepared according to the procedure described below.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.83–0.91 (m, 2H), 0.87 (d, *J* = 6.85 Hz, 6H), 1.08–1.15 (m, 2H), 1.43 (dd, *J* = 6.80, 7.20 Hz, 2H), 1.50–1.63 (m, 1H), 2.00–2.12 (m, 2H), 2.19 (s, 3H), 2.26 (s, 3H), 2.48–2.63 (m, 3H), 2.88 (tt, *J* = 7.20, 4.00 Hz, 1H), 3.05 (t, *J* = 6.35 Hz, 2H), 3.16 (t, *J* = 6.35 Hz, 2H), 3.55–3.66 (m, 1H), 6.92–6.99 (m, 2H), 7.60 (d, *J* = 8.87 Hz, 1H), 8.30 (brs, 1H).

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{24}H_{34}N_4O$, 395.2805; found, 395.2803.

Purity: 99.2%.

3-(4-Cyclopropyl-5-(*cis*-3-isobutylcyclobutyl)-4*H*-1, 2, 4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)propanamide (3w)



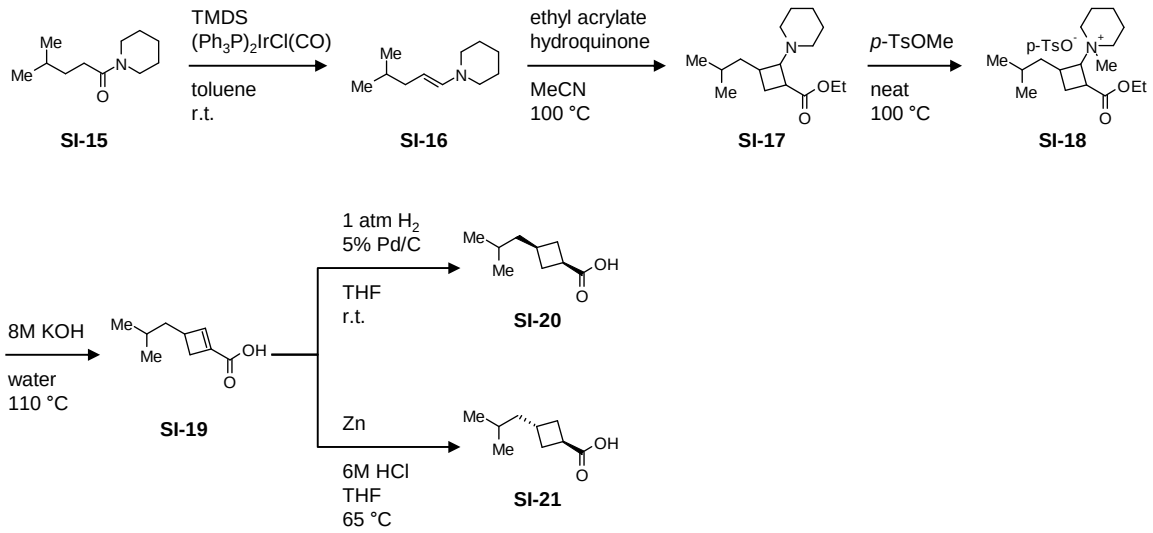
cis-3-Isobutylcyclobutanecarboxylic acid as R^1COOH was prepared according to the procedure described below.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.86 (d, *J* = 6.62 Hz, 6H), 0.87–0.93 (m, 2H), 1.09–1.17 (m, 2H), 1.34 (dd, *J* = 7.12, 6.73 Hz, 2H), 1.49–1.60 (m, 1H), 2.01–2.12 (m, 2H), 2.19 (s, 3H), 2.26 (s, 3H), 2.34–2.59 (m, 3H), 2.89 (tt, *J* = 7.12, 4.35 Hz, 1H), 3.00–3.09 (m, 2H), 3.11–3.19 (m, 2H), 3.43 (tt, *J* = 9.50, 7.92 Hz, 1H), 6.93–7.00 (m, 2H), 7.60 (d, *J* = 8.60 Hz, 1H), 8.31 (s, 1H).

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{24}H_{34}N_4O$, 395.2805; found, 395.2803.

Purity: 98.0%.

Synthesis of *cis* and *trans*-3-isobutylcyclobutanecarboxylic acid



3-Isobutylcyclobut-1-enecarboxylic acid (SI-19)

To a mixed solution of 4-methyl-1-morpholinopentan-1-one **SI-15** (169.33 g, 923.79 mmol) and $(Ph_3P)_2IrCl(CO)$ (359 mg, 0.460 mmol) in toluene (462.0 mL) was added TMDS (327.0 mL, 1850 mmol) dropwise at room temperature over 1 h. After stirring for 30 min, the solvent was removed under reduced pressure to afford crude **SI-16** (430.25 g). A mixed solution of this crude **SI-16** (430.25 g), ethyl acrylate (181.4 mL, 1663 mmol), and hydroquinone (1.08 g, 9.78mmol) in MeCN (489.0 mL) was heated at 100 °C overnight to give crude **SI-17**. After evaporation, *p*-TsOMe (177.2 mL, 1174 mmol) was added to the residue and the resultant mixture was heated at 90 °C for 3 h to yield **SI-18**. After cooling to room temperature, water (300.0 mL) and *n*-hexane (150.0 mL) were poured into the reaction and the aqueous layer was extracted. After an addition of 8M KOH (611.0 mL, 4888 mmol) to the aqueous layer, the mixture was heated at 110 °C for 4 h. The mixture was cooled to room temperature and washed with diethyl ether. The aqueous layer was acidified to pH 1 with conc. HCl and EtOAc was poured into the mixture. The organic layer was extracted, washed with water and 10% aqueous solution of NaCl, then dried over $MgSO_4$. After filtration and subsequent removal of the solvent under reduced pressure, the title compound **SI-19** (155.45 g) was obtained and used for next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.89 (d, *J* = 6.85 Hz, 6H), 1.25–1.42 (m, 2H), 1.56–1.67 (m, 1H), 2.11 (d, *J* = 12.09 Hz, 1H), 2.66–2.76 (m, 2H), 6.82 (s, 1H), 12.31 (brs, 1H).

***cis*-3-Isobutylcyclobutanecarboxylic acid (SI-20)**

A solution of **SI-19** (155.45 g) in THF (1000.0 mL) was treated with 5% palladium on activated carbon (10.70 g) and stirred under a hydrogen atmosphere (1 atm) at room temperature for 6.5 h. After removal of the palladium catalyst by Celite® filtration, the filtrate was concentrated and further purified by distillation to give the title compound **SI-20** (95.57g, 66% yield from **SI-15**).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.85 (d, J = 6.80 Hz, 6H), 1.30 (dd, J = 6.80, 6.80 Hz, 2H), 1.53 (sep, J = 6.80 Hz, 1H), 1.83–1.95 (m, 2H), 2.26–2.38 (m, 3H), 2.96–3.05 (m, 1H).

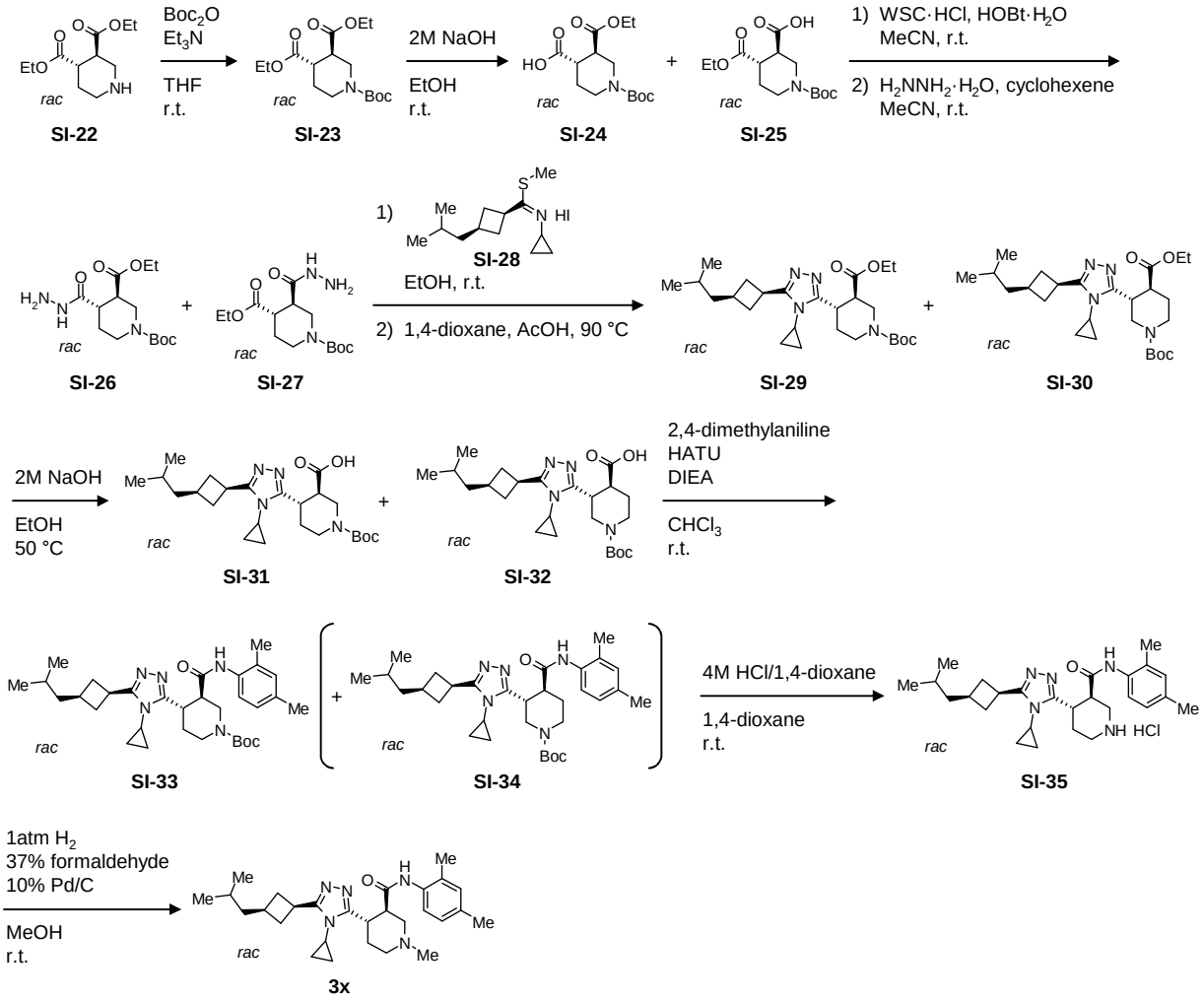
Boiling point: 94 °C/1 mm Hg

***trans*-3-Isobutylcyclobutanecarboxylic acid (SI-21)**

To a solution of **SI-19** (10.00 g) in a mixed solvent of 6 M HCl (80.0 mL) and THF (80.0 mL) was added zinc powder (16.00g) portionwise at 0 °C. After gas evolution had ceased, the mixture was heated at 65 °C and stirred for 17 h at the same temperature. Water was poured into the reaction at 0 °C and the mixture was extracted with EtOAc, washed with brine, and then dried over Na_2SO_4. After filtration and subsequent removal of the solvent under reduced pressure, the residue was purified by flash chromatography ($CHCl_3$:MeOH = 98:2 to 97:3 (v/v)) to give the title compound **SI-21** (5.37 g, 21% yield from **SI-15**).

^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.82 (d, J = 6.62 Hz, 6H), 1.31 (dd, J = 7.28, 7.28 Hz, 2H), 1.43–1.54 (m, 1H), 1.75–1.85 (m, 2H), 2.18–2.27 (m, 2H), 2.31–2.39 (m, 1H), 2.91–3.02 (m, 1H), 12.01 (brs, 1H).

Synthesis of inhibitor 3x



(3*R,4*S**)-*tert*-butyl 4-(4-cyclopropyl-5-(*cis*-3-isobutylcyclobutyl)-4*H*-1,2,4-triazol-3-yl)-3-(2,4-dimethylphenylcarbamoyl)piperidine-1-carboxylate (SI-33)**

To a solution of (3*R**,4*S**)-diethylpiperidine-3,4-dicarboxylate **SI-22** (4.51 g, 19.7 mmol) in THF (50.0 mL) were added Et_3N (3.0 mL, 21.7 mmol) and Boc_2O (4.5 mL, 19.7 mmol) at 0 °C. After stirring at room temperature for 3 days, water was poured into the reaction. The organic layer was extracted with EtOAc, washed with brine, and

then dried over $MgSO_4$. After filtration and subsequent removal of the solvent under reduced pressure, crude **SI-23** was obtained. To the crude **SI-23** in EtOH (50.0 mL), 2 M NaOH (12.8 mL, 25.6 mmol) was added at 0 °C and stirred at room temperature overnight. The reaction was further continued at 60 °C for 1 h, and subsequently neutralized with 6 M HCl (4.3 mL, 25.6 mmol) at 0 °C. After removal of the solvent under reduced pressure, the mixture was extracted with EtOAc, washed with brine, and then dried over $MgSO_4$. After filtration and concentration, the residue was purified by flash chromatography ($CHCl_3$:MeOH = 100:0 to 90:10 (v/v)) to afford a crude mixture of **SI-24** and **SI-25** (3.33g). The crude mixture (1.74 g) was dissolved in MeCN (17.4 mL), then treated with WSC·HCl (1.06 g, 6.93 mmol) and HOBt·H_2O (1.33g, 6.93 mmol) at 0 °C. After stirring at room temperature for 2 h, the resultant mixture was transferred to a mixed solution of hydrazine monohydrate (545 μL, 11.5 mmol) and cyclohexene (100 μL, 0.987 mmol) in MeCN (10.0 mL) at 0 °C. After stirring at room temperature for 4.5 h, water was added to the reaction. The organic layer was extracted with EtOAc, washed with saturated aqueous $NaHCO_3$ and brine, then dried over $MgSO_4$. After filtration and concentration, a crude mixture of **SI-26** and **SI-27** (1.37g) was obtained as a yellow oil. To the crude mixture in EtOH (3.0 mL) was added **SI-28** (200 mg, 0.568 mmol) which was prepared from *cis*-3-isobutylcyclobutanecarboxylic acid following the same procedure as described for the preparation of **SI-9**. The resultant mixture was stirred at room temperature overnight and saturated aqueous $NaHCO_3$ was afterward poured into the reaction. The mixture was extracted with EtOAc, washed with saturated aqueous $NaHCO_3$, and then dried over $MgSO_4$. After filtration and concentration, the residue was dissolved in 1,4-dioxane (4.0 mL) and treated with AcOH (0.4 mL). The mixture was heated at 90 °C for 5 h and cooled to 0 °C. The mixture was diluted with saturated aqueous $NaHCO_3$ at 0 °C. The organic layer was extracted with EtOAc, washed with saturated aqueous $NaHCO_3$, and then dried over Na_2SO_4. After filtration and concentration, the residue was purified by flash chromatography ($CHCl_3$:MeOH = 100:0 to 94:6 (v/v)) to afford a mixture of **SI-29** and **SI-30** (53mg). The crude mixture (50 mg) was dissolved in EtOH (0.5 mL), then treated with 2 M NaOH (80 μL, 0.16 mmol). The mixture was heated at 50 °C for 3 h, and water was subsequently poured into the reaction at 0 °C. The aqueous layer was washed with diethyl ether, and afterward acidified with 2 M HCl at 0 °C. The organic layer was in turn extracted with EtOAc and dried over Na_2SO_4. After filtration and concentration, a mixture of **SI-31** and **SI-32** (29 mg) was obtained as a yellow oil. The mixture (28 mg) was dissolved in $CHCl_3$ (1 mL), and DIEA (10 μL), HATU (28 mg) and 2, 4-dimethylaniline (9 μL) were added thereto. After stirring at room temperature for 3 h, the solvent was removed under reduced pressure and the residue was purified by preparative TLC ($CHCl_3$:MeOH = 94:6 (v/v)) to afford two isomers. The faster moving isomer was the title compound **SI-33** (8 mg, 0.9% yield from **SI-22**).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.83–0.87 (m, 6H), 0.88–1.02 (m, 1H), 1.08–1.19 (m, 2H), 1.29–1.35 (m, 2H), 1.47 (s, 9H), 1.50–1.56 (m, 1H), 1.56–1.65 (m, 6H), 1.86–2.02 (m, 3H), 2.03 (s, 3H), 2.04–2.16 (m, 1H), 2.23 (s, 3H), 2.29–2.55 (m, 2H), 2.83–2.96 (m, 1H), 3.15–3.29 (m, 1H), 3.32–3.47 (m, 2H), 4.06–4.49 (m, 2H), 6.88–6.94 (m, 2H), 7.30–7.36 (m, 1H).

Purity: >90% (measured by ^{1}H NMR).

(3*R,4*S**)-4-(4-Cyclopropyl-5-(*cis*-3-isobutylcyclobutyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)-1-methylpiperidine-3-carboxamide (3x)**

To a solution of **SI-33** (8 mg, 15 μmol) in 1,4-dioxane (0.1 mL) was added 4 M HCl in 1,4-dioxane (0.1 mL).

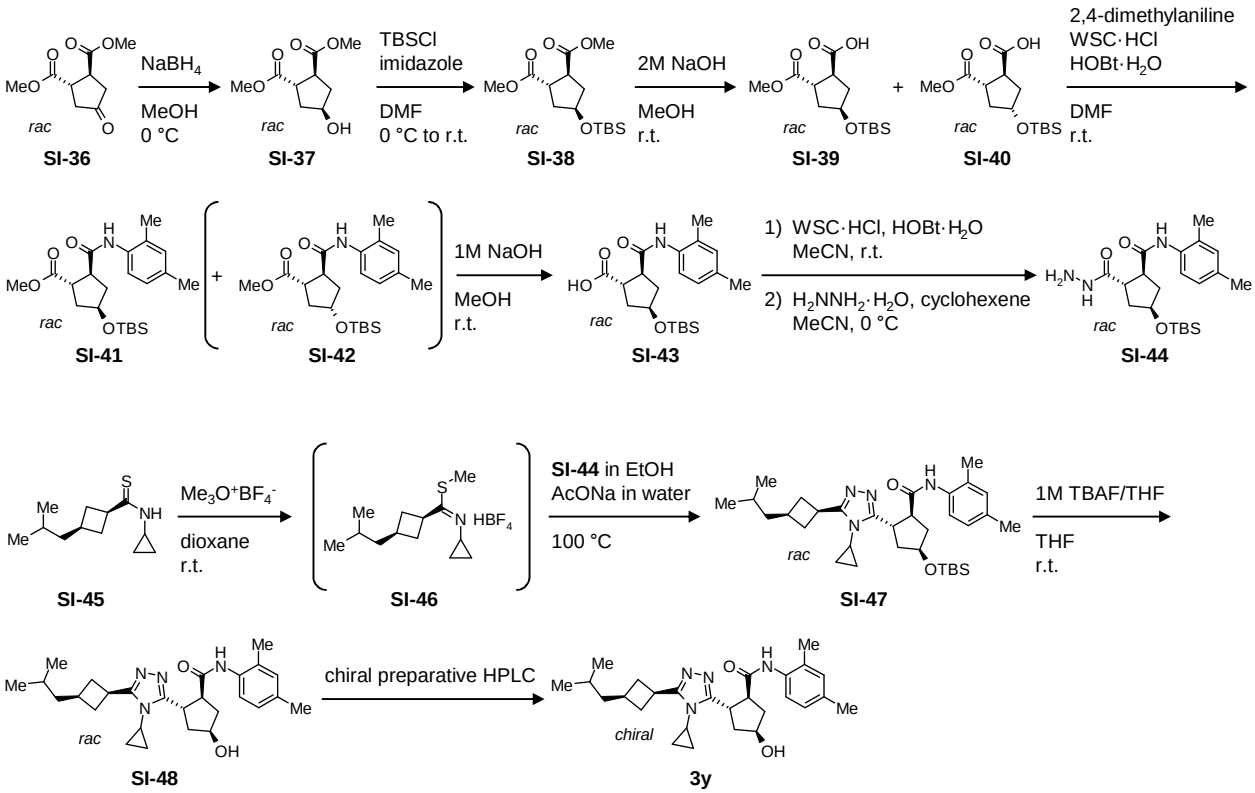
After stirring at room temperature for 3 days, the solvent was removed under reduced pressure. A solution of thus obtained residue in MeOH (1 mL) was treated with 37% aqueous formaldehyde (10 μL) and 10% palladium on activated carbon (10 mg) at room temperature. The mixture was stirred under hydrogen atmosphere (1 atm) at room temperature for 7 h, then the catalyst was removed by Celite® filtration. The filtrate was concentrated and the residue was purified by preparative TLC ($CHCl_3$:MeOH = 94:6 (v/v)) to give the title compound **3x** (3 mg, 45% yield for 2 steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.74–0.81 (m, 1H), 0.82–0.88 (m, 7H), 1.07–1.16 (m, 2H), 1.27–1.35 (m, 2H), 1.47–1.58 (m, 2H), 1.85–1.94 (m, 2H), 1.98–2.02 (m, 3H), 2.02–2.12 (m, 1H), 2.21–2.24 (m, 4H), 2.31–2.35 (m, 4H), 2.35–2.52 (m, 2H), 2.80–2.88 (m, 1H), 2.93–3.06 (m, 4H), 3.32–3.43 (m, 1H), 3.58–3.70 (m, 1H), 6.86–6.94 (m, 2H), 7.31–7.36 (m, 1H), 7.43–7.50 (m, 1H).

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{28}H_{41}N_5O$, 464.3384; found, 464.3380.

Purity: 89.1%.

Synthesis of inhibitor 3y



(1*R,2*R**)-Dimethyl 4-hydroxycyclopentane-1,2-dicarboxylate (SI-37)**

To a solution of (1*R**,2*R**)-dimethyl 4-oxocyclopentane-1,2-dicarboxylate **SI-36** (5.00 g, 25.0 mmol) in MeOH (200.0 mL) was added $NaBH_4$ (1.14 g, 30.0 mmol) portionwise at 0 °C. After stirring at the same temperature for 1 h, brine was poured into the reaction and the solvent was removed under reduced pressure. The mixture was extracted with EtOAc, washed with brine, then dried over $MgSO_4$, After filtration and concentration, the obtained residue was purified by flash chromatography ($CHCl_3$:EtOAc = 100:0 to 66:34 (v/v)) to give the title compound **SI-37** (2.87 g, 57% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.89–2.08 (m, 2H), 2.08–2.20 (m, 2H), 2.20–2.30 (m, 1H), 3.21–3.30 (m, 1H),

3.37–3.47 (m, 1H), 3.71 (s, 3H), 3.73 (s, 3H), 4.35–4.45 (m, 1H).

(1*R,2*R**)-Dimethyl 4-(*tert*-butyldimethylsilyloxy)cyclopentane-1,2-dicarboxylate (SI-38)**

To a solution of **SI-37** (2.81 g, 13.9 mmol) in DMF (20.0 mL) were added imidazole (1.23 g, 18.1 mmol) and *tert*-butyldimethylsilyl chloride (2.52 g, 16.7 mmol) at 0 °C. After stirring at room temperature overnight, water was poured into the reaction, and the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. After filtration and concentration, the residue was purified by flash chromatography (*n*-hexane:EtOAc = 100:0 to 90:10 (v/v)) to give the title compound **SI-38** (4.40 g, quantitative).

^{1}H NMR (400 MHz, $CDCl_3$) δ: −0.01 (s, 3H), 0.00 (s, 3H), 0.81 (s, 9H), 1.79–1.90 (m, 1H), 1.90–2.03 (m, 2H), 2.10–2.22 (m, 1H), 3.07–3.18 (m, 1H), 3.44–3.53 (m, 1H), 3.65 (s, 3H), 3.67 (s, 3H), 4.22–4.31 (m, 1H).

(1*R,2*R**,4*S**)-Methyl 4-(*tert*-butyldimethylsilyloxy)-2-(2,4-dimethylphenylcarbamoyl)cyclopentanecarboxylate (SI-41)**

To a solution of **SI-38** (4.32 g, 13.7 mmol) in MeOH (32.0 mL) was added 2 M NaOH (7.5 mL, 15 mmol) at 0 °C. After stirring at room temperature overnight, the reaction was acidified with 6 M HCl (2.5 mL, 15 mmol) at 0 °C and the solvent was removed under reduced pressure. The organic layer was extracted with EtOAc, washed with brine, then dried over $MgSO_4$. After filtration and concentration, the residue was purified by flash chromatography (*n*-hexane:EtOAc = 91:9 to 66:34 (v/v)) to give a mixture of **SI-39** and **SI-40** (2.27 g). The obtained mixture of **SI-39** and **SI-40** (2.20 g) was dissolved in DMF (16.6 mL) and successively treated with 2,4-dimethylaniline (899 μL, 7.27 mmol), HOBt·H_2O (1.34 g, 8.72 mmol) and WSC·HCl (1.67 g, 8.72 mmol) at 0 °C. After stirring at room temperature overnight, water was poured into the reaction and the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over $MgSO_4$. After filtration and concentration, the residue was purified by flash chromatography (*n*-hexane:EtOAc = 98:2 to 80:20 (v/v)) to give (1*R**,2*R**,4*R**)-methyl 4-(*tert*-butyldimethylsilyloxy)-2-(2,4-dimethylphenylcarbamoyl)cyclopentanecarboxylate **SI-42** (1.86 g, 34% yield for 2 steps) followed by the title compound **SI-41** (224 mg, 4% yield for 2 steps).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.05–0.08 (m, 6H), 0.86 (s, 9H), 1.95–2.13 (m, 2H), 2.18–2.25 (m, 5H), 2.29 (s, 3H), 3.06–3.17 (m, 1H), 3.34–3.44 (m, 1H), 3.74 (s, 3H), 4.32–4.42 (m, 1H), 6.94–7.02 (m, 2H), 7.57–7.61 (m, 1H), 7.86 (brs, 1H). The relative configuration of the substituents on the cyclopentane ring for **SI-41** and **SI-42** was determined by NOESY.

(1*R,2*R**,4*S**)-4-(*tert*-Butyldimethylsilyloxy)-2-(2,4-dimethylphenylcarbamoyl)cyclopentanecarboxylic acid (SI-43)**

To a solution of **SI-41** (197 mg, 0.486 mmol) in MeOH (3.0 mL) was added 1 M NaOH (580 μL, 0.580 mmol) at room temperature and stirred overnight. The reaction was neutralized with 2 M HCl (290 μL, 0.580 mmol) and afterward diluted with water. The organic layer was extracted with EtOAc, washed with brine, then dried over $MgSO_4$. After filtration and concentration, the title compound **SI-43** (182 mg, 95% yield) was obtained as a yellow solid.

^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.03–0.06 (m, 6H), 0.85 (s, 9H), 1.60–1.72 (m, 1H), 1.77–1.87 (m, 1H), 1.91–2.03 (m, 1H), 2.11 (s, 3H), 2.24 (s, 3H), 2.28–2.37 (m, 1H), 2.99–3.11 (m, 1H), 3.20–3.28 (m, 1H), 4.23–4.38 (m,

1H), 6.91–6.97 (m, 1H), 7.00 (s, 1H), 7.13–7.23 (m, 1H), 9.15 (s, 1H), 12.26 (brs, 1H).

(1*R,2*R**,4*S**)-4-(*tert*-Butyldimethylsilyloxy)-*N*-(2,4-dimethylphenyl)-2-(hydrazinecarbonyl)cyclopentanecarboxamide (SI-44)**

A suspension of **SI-43** (179 mg, 0.457 mmol) in MeCN (6.0 mL) were treated with HOBt·H_2O (84 mg, 0.55 mmol) and WSC·HCl (105 mg, 0.550 mmol) at 0 °C. After stirring at room temperature for 2 h, the solvent was removed under reduced pressure and MeCN (2.0 mL) was poured into the residue. The mixture was thus transferred into a mixed solution of hydrazine monohydrate (43 μL, 0.92 mmol) and cyclohexene (10 μL, 99 μmol) in MeCN (2.0 mL) at 0 °C. The reaction was continued at the same temperature for 1.5 h and water was poured into the mixture. The organic layer was extracted with EtOAc, washed with brine, then dried over Na_2SO_4. After filtration and concentration, the title compound **SI-44** (180 mg, 97% yield) was obtained and used in next step without further purification.

^{1}H NMR (400 MHz, DMSO-d_6) δ: 0.02-0.06 (m, 6H), 0.85 (s, 9H), 1.65-1.88 (m, 3H), 2.11 (s, 3H), 2.23 (s, 3H), 2.28-2.38 (m, 1H), 3.06-3.14 (m, 2H), 4.31-4.42 (m, 1H), 6.89-6.96 (m, 1H), 6.99 (brs, 1H), 7.17-7.24 (m, 1H), 9.05-9.11 (m, 2H).

(1*R,2*R**,4*R**)-4-(*tert*-Butyldimethylsilyloxy)-2-(4-cyclopropyl-5-(*cis*-3-isobutylcyclobutyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)cyclopentanecarboxamide (SI-47)**

SI-45 (102 mg, 0.484 mmol) which was prepared from **SI-20** following the same procedure described earlier, was dissolved in 1,4-dioxane (1.5 mL) and treated with $Me_3O^+BF_4^-$ (80 mg, 0.48 mmol) under a nitrogen atmosphere at room temperature. After stirring at the same temperature for 1.5 h, the mixture was cooled to 0 °C, then a mixed solution of **SI-44** (179 mg, 0.441 mmol) and NaOAc (99 mg, 1.2 mmol) in EtOH:water = 2:1 (3.0 mL) was transferred. The mixture was heated at 100 °C for 4 h and saturated aqueous $NaHCO_3$ was poured into the mixture at 0 °C. The organic layer was extracted with $CHCl_3$, washed with brine, then dried over Na_2SO_4. After filtration and concentration, the residue was purified by flash chromatography (*n*-hexane:EtOAc = 90:10 to 50:50 (v/v)) to give the title compound **SI-47** (44 mg, 18% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.05–0.12 (m, 6H), 0.83–0.88 (m, 9H), 0.90 (s, 9H), 1.06–1.17 (m, 1H), 1.31–1.40 (m, 2H), 1.46–1.55 (m, 1H), 1.88–2.03 (m, 1H), 2.03–2.15 (m, 5H), 2.19–2.27 (m, 4H), 2.32–2.45 (m, 3H), 2.45–2.57 (m, 2H), 2.83–2.93 (m, 1H), 3.35–3.47 (m, 1H), 3.59–3.71 (m, 1H), 3.71–3.83 (m, 1H), 4.41–4.52 (m, 1H), 6.88–6.99 (m, 2H), 7.64–7.75 (m, 1H), 8.30–8.38 (m, 1H).

(1*R,2*R**,4*R**)-2-(4-Cyclopropyl-5-(*cis*-3-isobutylcyclobutyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)-4-hydroxycyclopentanecarboxamide (SI-48)**

To a solution of **SI-47** (43 mg, 76 μmol) in THF (1.0 mL) was added 1 M TBAF in THF (0.3 mL, 0.3 mmol) at 0 °C. After stirring at room temperature for 1 h, the mixture was heated at 60 °C and stirred for 10 min. Water was poured into the reaction at 0 °C and the mixture was extracted with EtOAc. The organic layer was washed with brine and dried over Na_2SO_4. After filtration and concentration, the residue was purified by preparative TLC ($CHCl_3$:MeOH = 90:10 (v/v)) to afford the title compound **SI-48** (34 mg, 99% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.83–0.99 (m, 8H), 1.11–1.20 (m, 2H), 1.31–1.39 (m, 2H), 1.48–1.61 (m, 1H),

1.78–1.90 (m, 1H), 2.01–2.17 (m, 5H), 2.26 (s, 3H), 2.28–2.34 (m, 2H), 2.35–2.46 (m, 1H), 2.46–2.56 (m, 3H), 2.85–2.92 (m, 1H), 3.38–3.50 (m, 2H), 3.69–3.81 (m, 1H), 3.97–4.05 (m, 1H), 4.46–4.53 (m, 1H), 6.92–7.00 (m, 2H), 7.65–7.72 (m, 1H), 8.32 (brs, 1H).

(1*S*,2*S*,4*S*)-2-(4-Cyclopropyl-5-(*cis*-3-isobutylcyclobutyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)-4-hydroxycyclopentanecarboxamide (3y)

SI-48 (16 mg, 36 μmol) was subjected to chiral preparative HPLC (Column: DAICEL CHIRALPAK AD (20 × 250 mm, 10 μm); Mobile phase: EtOH; Flow rate: 8.0 mL/min; Detection wavelength: 220nm) to afford two enantiomers:

(1*S*,2*S*,4*S*)-2-(4-cyclopropyl-5-(*cis*-3-isobutylcyclobutyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)-4-hydroxycyclopentanecarboxamide **3y** (8 mg) and (1*R*,2*R*,4*R*)-2-(4-cyclopropyl-5-(*cis*-3-isobutylcyclobutyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)-4-hydroxycyclopentanecarboxamide ***ent*-3y** (8 mg).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.83–0.99 (m, 8H), 1.11–1.20 (m, 2H), 1.31–1.39 (m, 2H), 1.48–1.61 (m, 1H), 1.78–1.90 (m, 1H), 2.01–2.17 (m, 5H), 2.26 (s, 3H), 2.28–2.34 (m, 2H), 2.35–2.46 (m, 1H), 2.46–2.56 (m, 3H), 2.85–2.92 (m, 1H), 3.38–3.50 (m, 2H), 3.69–3.81 (m, 1H), 3.97–4.05 (m, 1H), 4.46–4.53 (m, 1H), 6.92–7.00 (m, 2H), 7.65–7.72 (m, 1H), 8.32 (brs, 1H).

^{13}C NMR (126 MHz, CDCl3) δ: 174.51, 160.40, 157.86, 134.72, 133.20, 131.15, 129.19, 127.05, 122.62, 73.20, 47.86, 46.14, 42.78, 40.23, 37.67, 34.34, 34.30, 30.95, 27.69, 26.51, 24.76, 22.74, 20.82, 17.87, 7.81, 7.10.

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{27}H_{38}N_4O_2$, 451.3068; found, 451.3063.

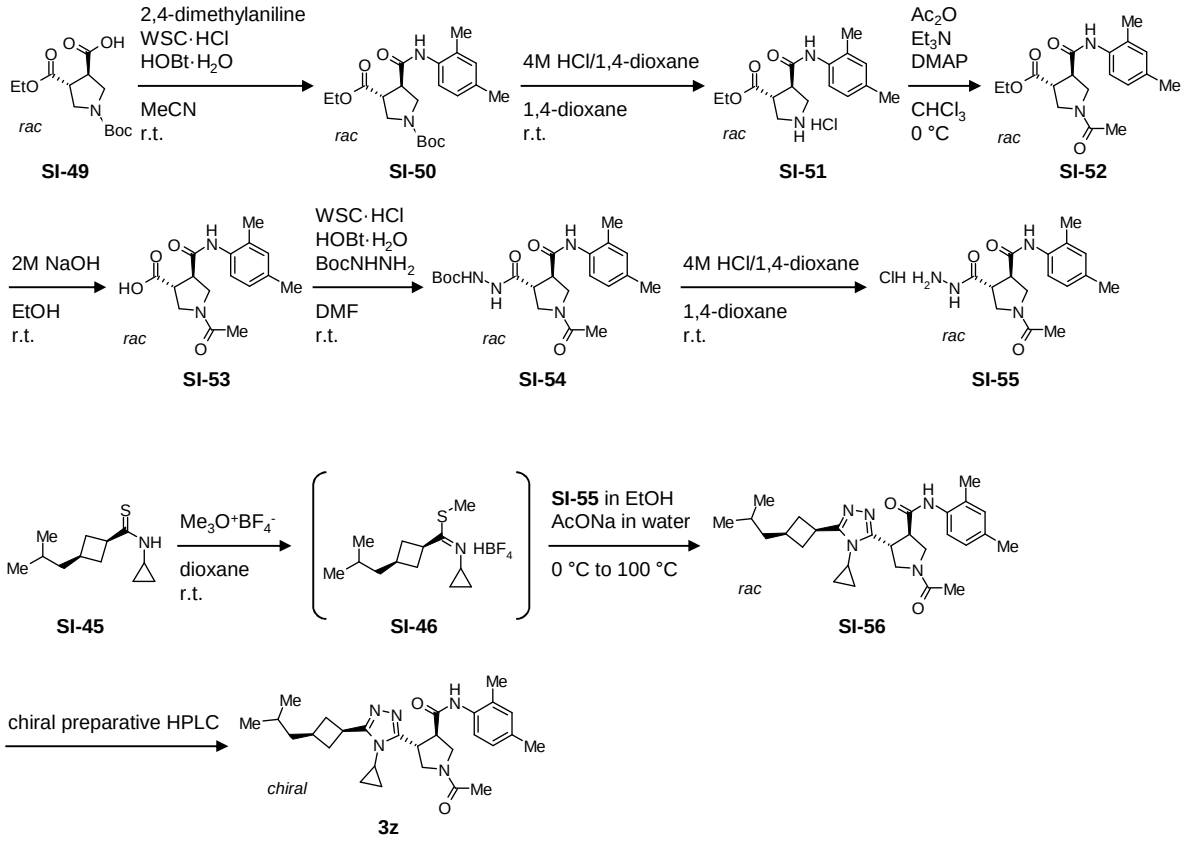
Optical purity: >99% ee [HPLC condition; Column: DAICEL CHIRALPAK AD (4.6 × 250 mm, 10 µm); Mobile phase: EtOH; Flow rate: 0.5 mL/min; Detection wavelength: 220nm; Retention time: **3y** 10.8 min, ***ent*-3y** 36.3 min].

Purity: 98.0%.

$[\alpha]_D^{25}$ 55.6 (*c* 0.09, MeOH)

Absolute stereochemistry of **3y** was putatively assigned based on the docking experiment described in the section 6.

Synthesis of inhibitor 3z



(3*R,4*R**)-1-*tert*-Butyl 3-ethyl 4-(2,4-dimethylphenylcarbamoyl)pyrrolidine-1,3-dicarboxylate (SI-50)**

To a mixed solution of (3*R**,4*R**)-1-(*tert*-butoxycarbonyl)-4-(ethoxycarbonyl)pyrrolidine-3-carboxylic acid **SI-49** (500 mg, 1.74mmol) and 2,4-dimethylaniline (215 μL, 1.74 mmol) in DMF (2.5 mL) were added HOBt·H_2O (320 mg, 2.09 mmol) and WSC·HCl (401 mg, 2.09 mmol) at room temperature. After stirring overnight, the mixture was diluted with 10% aqueous Na_2CO_3, and extracted with EtOAc. The organic layer was washed with 10% aqueous Na_2CO_3, water, 5% aqueous $KHSO_4$, water and 10% aqueous NaCl, then dried over $MgSO_4$. After filtration and concentration, the title compound **SI-50** (656 mg, 97% yield) was obtained and used in next step without further purification.

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.28 (t, *J* = 7.20 Hz, 3H), 1.47 (s, 9H), 2.22 (s, 3H), 2.28 (s, 3H), 3.34–3.45 (m, 3H), 3.73–3.97 (m, 3H), 4.20–4.22 (m, 2H), 6.99–7.01 (m, 2H), 7.66–7.68 (m, 1H), 7.86 (brs, 1H).

(3*R,4*R**)-Ethyl 4-(2,4-dimethylphenylcarbamoyl)pyrrolidine-3-carboxylate hydrochloride (SI-51)**

A solution of **SI-50** (650 mg, 1.67 mmol) in 1,4-dioxane (3.5 mL) was treated with 4 M HCl in 1,4-dioxane (3.5 mL) at room temperature. After stirring for 1.5 h, the solvent was removed under reduced pressure to give the title compound **SI-51** (546 mg, quantitative).

^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.21 (t, *J* = 7.20 Hz, 3H), 2.15 (s, 3H), 2.25 (s, 3H), 3.39–3.61 (m, 6H), 4.15 (q, *J* = 7.60 Hz, 2H), 6.98 (d, *J* = 8.00 Hz, 1H), 7.04 (s, 1H), 7.21 (d, *J* = 8.00 Hz, 1H), 9.28 (brs, 1H), 9.69 (s, 1H).

(3*R,4*R**)-Ethyl 1-acetyl-4-(2,4-dimethylphenylcarbamoyl)pyrrolidine-3-carboxylate (SI-52)**

To a solution of **SI-51** (544 mg, 1.66 mmol) in $CHCl_3$ (5.5 mL) were added Et_3N (698 μL, 5.01mmol), DMAP (10

mg, 83 μmol) and Ac_2O (205 μL, 2.17 mmol) at 0 °C. After stirring at the same temperature for 1 h, the mixture was diluted with 5% aqueous $KHSO_4$ and extracted with $CHCl_3$. The organic layer was washed with 5% aqueous $KHSO_4$, water, 10% aqueous Na_2CO_3, water and 10% aqueous NaCl, then dried over $MgSO_4$. After filtration, the filtrate was concentrated to give the title compound **SI-52** (527 mg, 95% yield).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.29 and 1.30 (each t, *J* = 7.60 and 7.20 Hz, total 3H), 2.08 and 2.09 (each s, total 3H), 2.22 and 2.27 (each s, total 3H), 2.29 (s, 3H), 3.33–4.27 (m, 8H), 7.00–7.02 (m, 2H), 7.50 and 8.04 (each brs, total 1H), 7.60 and 7.69 (each d, each *J* = 8.80 Hz, total 1H).

(3*R,4*R**)-1-Acetyl-4-(2,4-dimethylphenylcarbamoyl)pyrrolidine-3-carboxylic acid (SI-53)**

To a solution of **SI-52** (522 mg, 1.57 mmol) in EtOH (2.5 mL) was added 2 M NaOH (2.0 mL, 4.0 mmol) dropwise at 0 °C. The mixture was stirred at room temperature for 4 h and thus diluted with water (5.0 mL). After the mixture was acidified with 2 M HCl (2.0 mL, 4.0 mmol) at 0 °C, the resulting precipitate was collected by filtration to give the title compound **SI-53** (376 mg, 79% yield).

^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.95 (s, 3H), 2.13 (d, *J* = 5.20 Hz, 3H), 2.24 (s, 3H), 3.38–3.87 (m, 6H), 6.95 (d, *J* = 8.00 Hz, 1H), 7.01 (s, 1H), 7.20 (dd, *J* = 7.20, 7.20 Hz, 1H), 9.52 (s, 1H), 12.73 (s, 1H).

***tert*-Butyl**
2-((3*R,4*R**)-1-acetyl-4-(2,4-dimethylphenylcarbamoyl)pyrrolidine-3-carbonyl)hydrazinecarboxylate (SI-54)**

To a mixed solution of **SI-53** (373 mg, 1.23 mmol) and *tert*-butyl carbazate (194 mg, 1.47 mmol) in DMF (8.0 mL) were added HOBt·H_2O (225 mg, 1.47 mmol) and WSC·HCl (282 mg, 1.47 mmol) at 0 °C. After stirring at room temperature overnight, the mixture was diluted with water and extracted with EtOAc. The organic layer was washed with 10% aqueous Na_2CO_3, 5% aqueous $KHSO_4$, water and 10% aqueous NaCl, then dried over $MgSO_4$. After filtration, the filtrate was concentrated to give the title compound **SI-54** (515 mg, quantitative).

^{1}H NMR (400 MHz, $CDCl_3$) δ: 1.45 and 1.46 (each s, total 9H), 2.05 (s, 3H), 2.18 and 2.20 (each s, total 3H), 2.29 and 2.30 (each s, total 3H), 3.19 (dd, *J* = 19.20, 10.40 Hz, 0.5H), 3.33 (dd, *J* = 18.80, 10.00 Hz, 0.5H), 3.50–3.64 (m, 2H), 3.77 (dd, *J* = 10.40, 8.40 Hz, 0.5H), 3.81 (dd, *J* = 8.20, 8.20 Hz, 0.5H), 3.93 (dd, *J* = 10.00, 10.00 Hz, 0.5H), 3.98 (dd, *J* = 10.00, 10.00 Hz, 0.5H), 4.15 (dd, *J* = 11.60, 9.20 Hz, 0.5H), 4.24 (dd, *J* = 11.20, 8.40 Hz, 0.5H), 6.47 (d, *J* = 14.40 Hz, 1H), 6.98–7.02 (m, 2H), 7.35 and 7.55 (each d, *J* = 8.00 Hz, total 1H), 7.82 and 8.25 (each s, total 1H), 8.42 and 8.51 (each s, total 1H).

(3*R,4*R**)-1-acetyl-*N*-(2,4-dimethylphenyl)-4-(hydrazinecarbonyl)pyrrolidine-3-carboxamide hydrochloride (SI-55)**

To a solution of **SI-54** (512 mg, 1.22 mmol) in 1,4-dioxane (2.5 mL) was added 4 M HCl in 1,4-dioxane (2.5 mL) at 0 °C. After stirring at room temperature for 15 min, 2 M HCl in MeOH (2.5 mL) was added to the mixture and stirred at room temperature for 1 h. The solvent was removed under reduced pressure to afford the title compound **SI-55** (432 mg, quantitative).

^{1}H NMR (400 MHz, DMSO-d_6) δ: 1.96 (d, *J* = 4.40 Hz, 3H), 2.13 (d, *J* = 4.80 Hz, 3H), 2.23 (s, 3H), 3.70–4.03 (m, 6H), 6.95 (d, *J* = 8.00 Hz, 1H), 7.01 (s, 1H), 7.17 (dd, *J* = 7.60 Hz, 1H), 9.61 (d, *J* = 7.60 Hz, 1H), 11.40 (d, *J* =

11.20 Hz, 1H).

(3*R,4*R**)-1-acetyl-4-(4-cyclopropyl-5-(*cis*-3-isobutylcyclobutyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)pyrrolidine-3-carboxamide (SI-56)**

To a solution of **SI-45** (59 mg, 0.28 mmol) in 1,4-dioxane (450 μL) was added $Me_3O^+BF_4^-$ (46 mg, 0.28 mmol) under a nitrogen atmosphere and stirred at room temperature for 1 h. After cooling to 0 °C, a mixed solution of **SI-55** (90 mg, 0.25 mmol) and NaOAc (52 mg, 0.63 mmol) in EtOH:water = 2:1 (1.5 mL) was transferred to the reaction and stirred for 1 h at 0 °C. The reaction was further continued at 100 °C for 4 h and afterward diluted with 10% aqueous Na_2CO_3 at 0 °C. The organic layer was extracted with $CHCl_3$, washed with water and 10% aqueous NaCl, then dried over $MgSO_4$. After filtration and concentration, the residue was purified by preparative TLC ($CHCl_3$:MeOH = 90:10 (v/v)) to afford the title compound **SI-56** (98 mg, 82% yield).

^{1}H NMR (400 MHz, MeOH-d_4) δ: 0.89 (d, *J* = 6.62 Hz, 6H), 0.92–1.39 (m, 6H), 1.45–1.63 (m, 1H), 1.91–2.07 (m, 2H), 2.03 (s, 3H), 2.09 and 2.12 (each s, total 3H), 2.26 (s, 3H), 2.41–2.61 (m, 3H), 3.02–3.11 (m, 1H), 3.41–3.91 (m, 4H), 4.09–4.31 (m, 3H), 6.96 (d, *J* = 7.94 Hz, 1H), 7.01 (s, 1H), 7.10 and 7.13 (each d, *J* = 7.94 and 7.94 Hz, total 1H).

(3*R*,4*R*)-1-acetyl-4-(4-cyclopropyl-5-(*cis*-3-isobutylcyclobutyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)pyrrolidine-3-carboxamide (3z)

SI-56 (25 mg, 52 μmol) was subjected to chiral preparative HPLC (Column: DAICEL CHIRALCEL OD (20 × 250 mm); Mobile phase: EtOH; Flow rate: 7.0 mL/min; Detection wavelength: 220 nm) to afford two enantiomers as a beige foam: (3*R*,4*R*)-1-acetyl-4-(4-cyclopropyl-5-(*cis*-3-isobutylcyclobutyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)pyrrolidine-3-carboxamide **3z** (13 mg) and (3*S*,4*S*)-1-acetyl-4-(4-cyclopropyl-5-(*cis*-3-isobutylcyclobutyl)-4*H*-1,2,4-triazol-3-yl)-*N*-(2,4-dimethylphenyl)pyrrolidine-3-carboxamide ***ent*-3z** (12 mg).

^{1}H NMR (400 MHz, MeOH-d_4) δ: 0.89 (d, *J* = 6.62 Hz, 6H), 0.92–1.39 (m, 6H), 1.45–1.63 (m, 1H), 1.91–2.07 (m, 2H), 2.03 (s, 3H), 2.09 and 2.12 (each s, total 3H), 2.26 (s, 3H), 2.41–2.61 (m, 3H), 3.02–3.11 (m, 1H), 3.41–3.91 (m, 4H), 4.09–4.31 (m, 3H), 6.96 (d, *J* = 7.94 Hz, 1H), 7.01 (s, 1H), 7.10 and 7.13 (each d, *J* = 7.94 and 7.94 Hz, total 1H).

^{13}C NMR (126 MHz, MeOH-d_4) δ: 171.96, 171.53, 171.13, 161.66, 161.60, 156.33, 156.15, 137.64, 137.59, 134.03, 133.87, 133.77, 133.75, 132.18, 127.95, 127.93, 126.80, 126.63, 52.81, 51.35, 51.25, 50.71, 49.93, 49.69, 47.55, 39.41, 38.45, 35.42, 35.29, 35.25, 32.10, 28.52, 27.69, 25.69, 23.13, 21.98, 21.88, 20.94, 17.95, 17.93, 7.89, 7.85, 7.49, 7.42.

HRMS *m*/*z*: $[M+H]^+$ calcd for $C_{28}H_{39}N_5O_2$, 478.3177; found, 478.3173.

Optical purity: >99% ee [HPLC condition; Column: DAICEL CHIRALCEL OD (4.6 × 250 mm, 10 μm); Mobile phase: EtOH; Flow rate: 0.5 mL/min; Detection wavelength: 220 nm; Retention time: **3z** 9.0 min, ***ent*-3z** 10.8 min].

Purity: 95.4%.

$[\alpha]_D^{25}$ 68.9 (*c* 1.02, MeOH)

Absolute stereochemistry of **3z** was putatively assigned based on the docking experiment described in the section 6.

3. Biological assay experimental procedure

Cellular Assay (LUC)

Human RORγ and mouse RORγ Luc assays were carried out in accordance with the method of Madoux *et al.* (Madoux, F. *et al*. Potent, selective and cell penetrant inhibitors of SF-1 by functional uHTS. *Mol. Pharmacol.* **2008**, *73*, 1776–1784.) with some modifications and described previously (Kotoku, M. *et al*. Triazole-isoxazole compound and medical use thereof. PCT Int. Appl. WO 2014065413, 2014.). Briefly, a cDNA encoding human or mouse RORγ ligand binding domain (LBD) was obtained based on the reported sequences (Genbank, accession number, human RORγ, NM_005060.3; mouse RORγ accession, NM_011281.2, LBD: human RORγ, from Ser253 to Lys518; mouse RORγ, from Ile251 to Lys516). The cDNA of human RORγ or mouse RORγ was inserted into pFA-CMV vector (Strategene), which in turn expresses GAL4-DNA binding domain-RORγ LBD fusion proteins. The construct, GAL4-RORγ plasmid, was transiently co-transfected into CHO-K1 cells with pG5-Luc plasmid. The test article was applied to the co-transfected CHO-K1 cells and incubated at 37°C, 6% CO_2, for two days. After the incubation, cell viability was checked using resazurin. Then, the transcriptional activity of RORγ was measured by means of the SteadyLite Plus Reporter Gene Assay System. LUC assays for other nuclear receptors were conducted similarly after construction of Gal4 hybrids of each nuclear receptor.

Biochemical Assay (FRET)

hRORγ-LBD (aa 253–518) was expressed as a GST fusion protein in Sf9 cells The lysate of hRORγ-LBD expressing Sf9 cells was incubated with a biotinylated co-activator peptide derived from the NR2 motif of PGC1α (EEPSLLKKLLLAPA) in the presence or absence of test compounds. The binding of co-activator peptide to hRORγ LBD was detected by the FRET signal derived from anti-GST-APC and Streptavidin-RPE.

CD3-induced mouse PD model

Peptide derived from myelin oligodendrocyte glycoprotein (MOG35–55) were dissolved in D-PBS (−) at concentration of 3 mg/mL, and then emulsified with equal volume of complete Freund’s adjuvant. Female C57BL/6 mice were immunized withthe MOG35–55 emulsion by subcutaneous injection at two sites of the back (75 μg of MOG35–55 per site) and 0.2 μg/200 μL of pertussis toxin (PTX) in D-PBS (−) was injected interperitoneally on day 1. On day3, same amount of PTX was injected. On day5, 1 μg / 200 μL of the anti-CD3 antibody dissolved in saline was injected intravenouslyto induce IL-17 production. The blood samples were collected 2 hours after the CD3 injection, then the IL-17A level in plasma was measured by ELISA. The test article was administered orally 3 h or 8 h before the CD3 antibody injection into 5 mice per each group.

4. Pharmacokinetics

Metabolic stability in liver S9

Test compounds were incubated with mouse, rat or human liver S9 (Xenotech, US) for up to 60 min and the remaining ratios were determined.

CYP inhibition assay

A cocktail of typical substrates of each CYP isozyme was incubated with Human liver microsomes (Xenotech, US) in the presence or absence of the test compounds and the inhibitory effects of the test compounds were evaluated according to the published method (Dierks, E. A. *et al*. A method for the simultaneous evaluation of the activities of seven major human drug-metabolizing cytochrome P450 using in vitro cocktail of probe substrates and fast gradient liquid chromatography tandem mass spectrometry. *Drug Metab. Dispos*., **2001**, *29*, 23–29. and Polasek, T. M. *et al*. Time-dependent inhibition of human drug metabolizing cytochromes P450 by tricyclic antidepressants. *Br. J. Clin. Pharmacol*. **2007**, *65*, 87–97.) with some modifications.

Mouse PK (IV, PO)

Female C57BL/6 mice (Charles River Laboratories (Japan)) were intravenously or orally administered a single dose of test compounds at 1 mg/kg (dimethyl sulfoxide solution) or 30 and 100 mg/kg (aqueous suspension in 0.5% methylcellulose), respectively. After the administration, the plasma samples were collected over a period of 24 h and mean concentration data at each time point were used for pharmacokinetic analysis.

Table S1. Pharmacokinetic profiles of inhibitor **3z** (HCl salt).

Route	Dose (mg/kg)	Vehicle	C_{5min} (h)	$T_{1/2\beta}$ (h)	$AUC_{0\text{-inf.}}$ (μM·h)	CL_{tot} (L/h/kg)	MRT (h)	Vdss (L/kg)
i.v.	1	DMSO	1.0	0.3	0.8	2.5	0.4	1.0

Route	Dose (mg/kg)	Vehicle	T_{max} (h)	C_{max} (μM)	$AUC_{0\text{-inf.}}$ (μM·h)	$T_{1/2}$ (h)	MRT (h)	BA (%)
p.o.	30	MC	1.0	10	18	3.3	1.6	78
p.o.	100	MC	0.5	38	100	2.2	2.5	130

Protein Binding

Mouse plasma protein binding was determined using an equilibrium dialysis method (Lin, Z. J. *et al.* Simultaneous determination of glipizide and rosiglitazone unbound drug concentrations in plasma by equilibrium dialysis and liquid chromatography–tandem mass spectrometry. *J. Chromatogr. B.* **2004**, *801*,265–272.) with some modifications and the percentage of test compound bound to plasma protein (% Bound) was calculated according to the following equation: % Bound = $[(C_{tot}-C_{buf})/C_{tot}] \times 100$ where C_{tot} is the concentration of the incubated standard plasma sample at the time of the measurement and C_{buf} is the compound concentration in the buffer sample at the time of the measurement.

Plasma Concentrations of Compound 3z in CD3-induced mouse PD model

Plasma samples were extracted with 90% acetonitrile and spiked with internal standard. The organic and aqueous phases were separated by centrifugation. The upper organic phase was transferred to another tube and mixed well with 0.1% HCOOH aqueous. Aliquots of the solution were injected into the LC-MS/MS system for analysis.

Free Plasma Concentrations of Compound 3z in CD3-induced mouse PD model

Free plasma concentrations of **3z** were calculated according to the following equation: Free plasma concentration = [Total plasma concentration × (100−% Bound) / 100]

Table S2. Plasma Concentrations of Compound **3z** in CD3-induced mouse PD model

Mouse (C57BL/6)					
Dose	Plasma concentration (μM)		Protein Binding	Free Plasma concentration (μM)	
(mg/kg)	3 hr	8 hr	(%)	3 hr	8 hr
30	5.696	0.771	97.2	0.159	0.022
100	14.994	8.627		0.420	0.242

5. X-ray crystal analysis

Method

The His-tagged human RORγ-LBD (residues 261-518aa) cloned into pET28 plasmid was produced in *Escherichia coli* BL21 (DE3) at 22 °C. The fusion protein obtained by affinity chromatography on Ni-NTA Superflow agarose column (Qiagen) was treated with AcTEV protease (Invitrogen), and further purified by gel-filtration using a Superdex75 column (GE healthcare science). The 0.2 mg/mL purified protein was incubated with three times molar excess of compound **3g** at 4 °C and then concentrated to 16 mg/mL. Crystals grew in sitting drops at 20 °C with a reservoir solution (0.1 M Tris-HCl pH 7.5, 0.5 M Na/K tartrate, 2.5 M MPD).

Diffraction data of the RORγ-LBD were collected at BL17A beamline of the Photon Factory (KEK, Tsukuba, Japan). The data were integrated with XDS (Kabsch, W. XDS. *Acta. Crystallogr. D Biol. Crystallogr*. **2010**, *66*, 125–132.) and scaled using SCALA. The RORγ structure was solved by molecular replacement with PHASER in CCP4 suite using the RORγ-LBD structure (PDB ID: 3BOW) as a starting model. The structural model was built in Coot (Emsley, P. *et al.* Coot: model-building tools for molecular graphics. *Acta.Crystallogr. D Biol. Crystallogr*. **2004**, *60*, 2126–2132.) and refined using REFMAC5 in CCP4 suite (Murshudov, G. N. *et al*. REFMAC5 for the refinement of macromolecular crystal structures. *Acta. Crystallogr. D Biol. Crystallogr*. **2011**, *67*, 355–367.) . Figure was created in PyMOL4 (The PyMOL Molecular Graphics System, Version1.4 (Schrödinger, LLC); http://www.pymol.org). Crystallization data and refinement statics are summarized in Supplemental Table S3. The structure has been deposited in the RCSB Protein Data Bank database (PDB ID: 5AYG).

Table S3. Crystal data-collection and refinement statistics for RORγ crystal-packing.

	Compound **3g**
Space group	$P6_1$
Unit-cell parameters	
a, b, c (Å)	99.24 99.24 127.84
α, β, γ (°)	90.00 90.00 120.00
Resolution range(Å) [a]	85.94–2.60 (2.74–2.60)
Total reflections	234253
Unique reflections	22021
Completeness (%)	100 (100)
Redundancy	10.6 (7.5)
I/δ (%l)	29.7 (6.6)
R_{merge} % (%) [b]	5.7 (30.9)
Refinement statistics	
Resolution range (Å)	85.94–2.60
No. of reflections	20852
R_{cryst} (R_{free}) [c]	18.35 (22.67)
No. of atms	
Protein	3521
Ligand	60
Water	85
B-factors	
Protein	57.2
Ligand or Ion	43.9
Water	53.4
R.m.s. deviations	
Bond length (Å)	0.012
Bond angles (°)	2.052

[a]Values in parenthesis are for the highest resolution shell.

[b]Rmerge=$\sum|I_h(I)_h|\sum I_h$, where $(I)_h$ is average intensity over symmetry equivalents.

[c]R-factor=$\sum|F_{obs}F_{calc}|\sum|F_{obs}$. The free R-factor is calculated from 10% of the reflections that are omitted from the refinement.

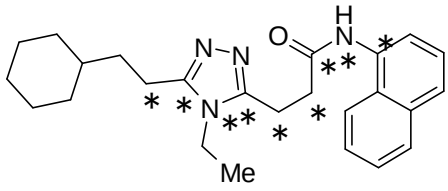
Values in parenthesis are for the highest resolution shell.

6. Docking study

Modeling procedure

In order to generate conformers of **3y** and **3z**, the mixed torsional/low mode sampling method was employed using MacroModel from Schrödinger Suite 2013. The maximum number of total steps for conformational search was 10,000 and the option to specify the number of search steps per rotatable bond was not used. The energy window for saving structures was set to 42.0 kJ/mol (10.04 kcal/mol). Each enumerated conformation was energy minimized using the OPLS2005 force field in combination with the Generalized Born/Surface Area (GB/SA) continuum water solvation model and the Polak-Ribière conjugate gradient method with a gradient convergence threshold of 0.05 and a maximum of 50,000 iterations. To remove redundant conformations, the maximum atom deviation cutoff was set to 0.5 Å. The generated conformers were overlaid with the binding structure **3g** using 9 points (marked with * in Figure S1). Stable conformers of **3y** and **3z** were reasonably fitted with **3g** while any conformers of their enantiomers (*anti*-**3y** and *anti*-**3z**) were not (Figure S2).

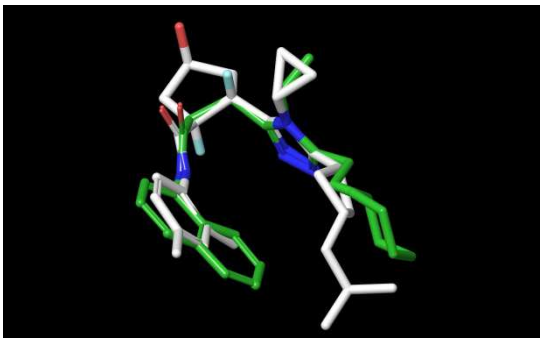
Figure S1. Selected atoms of **3g** for the superimposition onto **3y** and **3z**



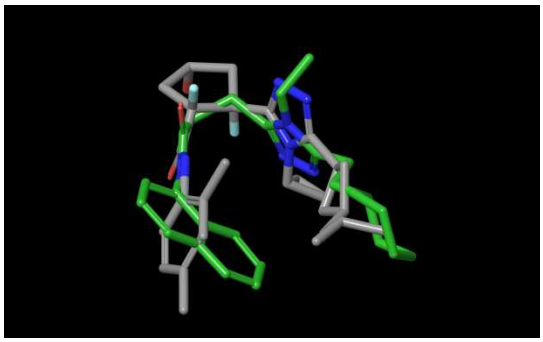
3g

Figure S2. (A) Superimposition of **3y** onto **3g**. (B) Superimposition of *anti*-**3y** onto **3g**. (C) Superimposition of **3z** onto **3g**. (D) Superimposition of *anti*-**3z** onto **3g**.

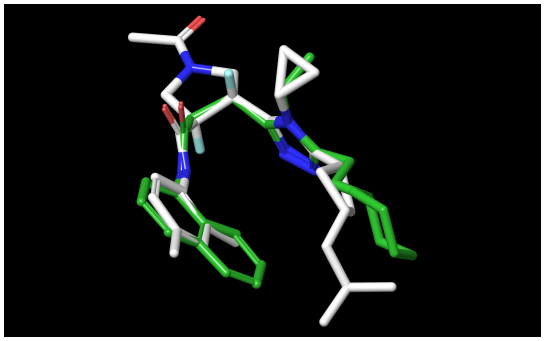
(A)



(B)



(C)



(D)