Discovery of TAK-272: A Novel, Potent and Orally Active Renin Inhibitor

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1. Chemistry

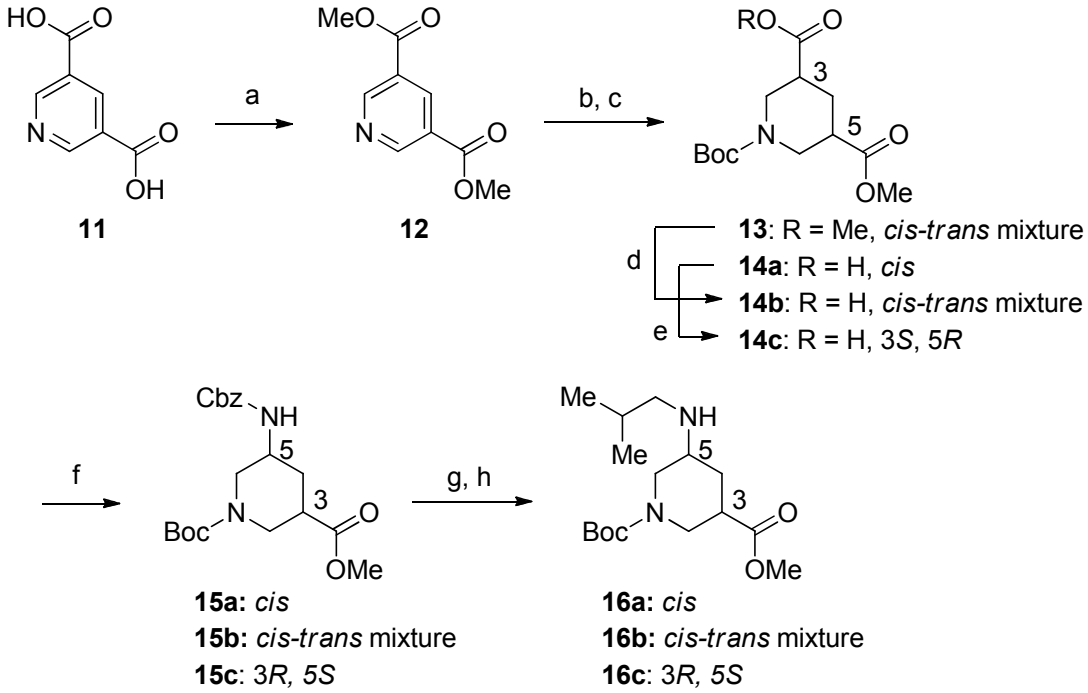
General.

^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker DPX-300 (300 MHz) spectrometer or Bruker AV-600 (600 MHz). Chemical shifts are given in parts per million (ppm) with tetramethylsilane as an internal standard. Abbreviations are used as follows: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, dd = doublet of doublets, dt = doublet of triplets, brs = broad singlet. Coupling constants (*J* values) are given in hertz (Hz). Column chromatography was performed using Merck silica gel 60 (70–230 mesh). Basic silica gel column chromatography was performed using Chromatorex NH-DM 1020 (100–200 mesh, Fuji Silysia Chemical, Ltd.). Reaction progress was determined by thin layer chromatography (TLC) analysis on Merck silica gel plates 60 F254. LC-MS analysis was performed on a Shiseido CAPCELL PACK C-18 UG120 S-3 column (1.5 mmφ x 35 mm) in a Waters Alliance 2795 or an Agilent 1100 LC system equipped with a Waters 2487 absorbance detector and a Micromass ZQ2000 mass spectrometer. Analytes were eluted using a linear gradient of water (0.05% TFA)/acetonitrile (0.04% TFA) from 90:10 to 0:100 over 4 min at a flow rate of 0.5 mL/min. UV detection was at 220 nm. Preparative HPLC was performed on a Shiseido CAPCELL PACK C-18 UG120 S-5 column (20 mmφ x 50 mm), eluting at 25 mL/min with a gradient of water (0.1% TFA)/acetonitrile (0.1% TFA). UV detection was at 220 nm. Melting points were determined on a Yanagimoto micro melting-point apparatus or a Büchi melting point apparatus B-545 and are uncorrected. The purities of all compounds tested in biological systems were assessed as being ≥90% using elemental analysis or analytical HPLC. Purity data were collected by a HPLC with Corona CAD (Charged Aerosol Detector) detector. The column was L-column 2 ODS (30 mm x 2.0 mm I.D., CERI, Japan) with a temperature of 50 ºC and a flow rate of 0.5 mL/min. Mobile phase A and B under a neutral condition were a mixture of 5 mmol/L Ammonium acetate and 5 mmol/L ammonium acetate in 98% acetonitrile, respectively. Mobile phase A and B under an acidic condition were a mixture of 0.2% formic acid in 10 mmol/L ammonium

formate and 0.2% formic acid in acetonitrile, respectively. The ratio of mobile phase B was increased linearly from 14% to 86% over 3 min, 86% over the next 1 min. Or purity measurements were carried out using a Shimadzu UFLC system employing the following conditions: column; L-column2 ODS (3.0 mmIDx30 mmL, 2 μm); mobile phase; MeCN/H_2O/TFA = 5:95:0.1 (0 min) → 90:10:0.1 (2.00 min) → 90:10:0.1 (3.3 min); flow rate; 1.2 mL/min; temperature; 40 ºC; detection; UV 220 mm. Elemental analyses were performed by Takeda Analytical Research Laboratories, Ltd. and were within 0.4% of the theoretical values unless otherwise noted. Unless otherwise specified, all solvents and reagents were obtained from commercial suppliers and used without further purification. Yields were not optimized.

Synthesis.

Scheme S1. Synthesis of piperidine intermediates [a]



[a] Reagents and conditions: (a) $SOCl_2$, MeOH, reflux, 99%; (b) H_2 (5 atm), Rh/C, 6 M aqueous HCl solution, MeOH; (c) Boc_2O, Et_3N, EtOH, rt, 94% for 2 steps; (d) 2 M aqueous NaOH solution, MeOH, rt, 76%; (e) (R)-phenylethylamine, EtOH, recrystallization, 15%; (f) DPPA, Et_3N, toluene 100 °C, then BnOH, Et_3N, 80 °C, 30–100%; (g) H_2 (5 atm), Pd/C, MeOH, rt; (h) *i*-PrCHO, AcOH, $NaBH(OAc)_3$, MeOH, rt, 89–100% for 2 steps.

Dimethyl pyridine-3,5-dicarboxylate (12).

To a solution of pyridine-3,5-dicarboxylic acid (**11**) (100 g, 0.60 mol) in MeOH (1.0 L) was added dropwise $SOCl_2$ (130 mL, 1.78 mol) at room temperature. The reaction mixture was stirred under reflux for 3 h, and then concentrated in vacuo. The residue was diluted with water and extracted with EtOAc. The aqueous layer was neutralized with 8M aqueous NaOH solution and extracted with EtOAc. The combined organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated in vacuo to give **12** as a white powder (117 g, 99%). mp 84–85 °C. MS (ESI+) *m/z*: 196.3 $(M + H)^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.00 (6H, s), 8.90 (1H, s), 9.24–9.40 (2H, m).

1-*tert*-Butyl 3,5-dimethyl piperidine-1,3,5-tricarboxylate (13), *cis*-1-(*tert*-butoxycarbonyl)-5-(methoxycarbonyl)piperidine-3-carboxylic acid (14a)[1, 2] and 1-*tert*-butyl 3,5-dimethyl piperidine-1,3,5-tricarboxylate (14b).[1, 2]

A mixture of compound **12** (15 g, 77 mmol) and Rh/C (1.5 g) in MeOH (150 mL) and 6 M aqueous HCl solution (19.2 mL) was stirred at 50 °C under H_2 atmosphere (5 atm) for 25 h. After being cooled to room temperature, the catalyst was removed by filtration, and the filtrate was concentrated in vacuo. The residue was dissolved in EtOH (100 mL), and Et_3N (16 mL, 115 mmol), and Boc_2O (18.5 g, 84.6 mmol) were added successively at 0 °C. The reaction mixture was stirred at room temperature for 15 h and concentrated in vacuo. The residue was dissolved in 0.5 M aqueous HCl solution, and the mixture was extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was subjected to silica gel column chromatography (hexane/EtOAc = 7/1 to 1/4) to give compound **13** as a white solid (15.2 g, 66%, cis/trans = 7/3, TLC: *Rf* = 0.71 or 0.79 (hexane/EtOAc = 1/2)). The other fraction (TLC: *Rf* = 0.35 (hexane/EtOAc = 1/2)) was concentrated, triturated with EtOAc, and filtered to give compound **14a** as a white solid (2.1 g, 9.5%). The filtrate was concentrated in vacuo to give compound **14b** as a colorless oil (4.2 g, 19%). **13**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.40-1.50 (9H, m), 1.63-1.78 (1H, m), 2.03–2.17 (1H, m), 2.33–2.55 (2H, m), 2.61–2.90 (2H, m), 3.70 (6H, s), 4.06–4.20 (1H,m), 4.23–4.54 (1H, m). **14a**: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.87 (9H, s), 1.64–1.81 (m, 1H), 2.41–2.63 (3H, m), 2.72 (2H, bs), 3.71 (3H, s), 4.38 (2H, d, *J* = 5.3 Hz). **14b**: MS (ESI+) *m/z*: 288 $(M + H)^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.44–1.47 (9H, m), 1.60–1.82 (1H, m), 2.10 (1H, bs), 2.38–2.61 (3H, m), 2.72 (2H, bs), 3.71 (3H, s) 4.38 (2H, bs).

1-*tert*-Butyl 3,5-dimethyl piperidine-1,3,5-tricarboxylate (14b).

To a solution of compound **13** (6.5 g, 21.6 mmol) in MeOH (30 mL) was added dropwise 2 M aqueous NaOH solution (10.8 mL, 21.6 mol) at room temperature. After being stirred at room temperature for 2 h, the reaction mixture was concentrated in vacuo. The residue was diluted with

saturated aqueous $NaHCO_3$ solution, and washed with EtOAc. The aqueous layer was acidified with 5% aqueous citric acid solution, and then extracted with EtOAc. The organic layer was dried over Na_2SO_4, and concentrated in vacuo to give compound **14b** as a white solid (4.7 g, 76%).

(3*S*,5*R*)-1-(*tert*-Butoxycarbonyl)-5-(methoxycarbonyl)piperidine-3-carboxylic acid (14c). [2]

A mixture of compound **14a** (6.16 g, 21.4 mmol), (*R*)-(+)-1-phenylethylamine (2.60 g, 21.5 mmol) and EtOH (24 mL) was dissolved by heating to 70 °C, and recrystallized. The precipitated crystals were collected by filtration, dissolved in EtOH (7 mL) again and recrystallized. The precipitated crystals were collected by filtration, the obtained crystals were suspended in water, acidified by adding saturated aqueous potassium hydrogen sulfate solution, and the mixture was extracted three times with EtOAc. The extract was washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuo to give compound **14c** (95% ee; 915 mg, 15%) as a white powder . The ee value was measured by chiral HPLC using chiralpak AD-H (retention time: tR1 (enantiomer of **14c**): 15.5 min, tR2 (**14c**): 16.1 min). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.39 (9H, s), 1.52 (1H, q, J = 12.4 Hz), 2.18–2.54 (3H, m), 2.55–2.78 (2H, m), 3.63 (3H, s), 4.03–4.23 (2H, m), 12.51 (1H, bs). $[\alpha]^{20}_D$ -6.2° (c 1.006, MeOH). The absolute stereochemistry was determined by comparison of literature.[2]

***cis*-1-*tert*-Butyl 3-methyl 5-{[(benzyloxy)carbonyl]amino}piperidine-1,3-dicarboxylate (15a).**

To a solution of compound **14a** (575 mg, 2.0 mmol) and Et_3N (0.34 mL, 2.4 mmol) in toluene (10 mL) was added dropwise DPPA (0.52 mL, 2.4 mmol), and the mixture was stirred at 100 °C for 1 h, and then cooled to room temperature. Benzylalcohol (0.52 mL, 5.0 mmol) and Et_3N (0.34 mL, 2.4 mmol) were added, and the reaction mixture was stirred at 80 °C for additional 2 h. The mixture was diluted with water and extracted with toluene. The extract was washed with aqueous 5% citric acid solution, $NaHCO_3$ solution and brine, dried over Na_2SO_4 and concentrated in vacuo. The residue was subjected to silica gel column chromatography (hexane/EtOAc = 2/1 to 1/2) to give crude compound **15a** as a pale yellow oil (785 mg, 84%), which contains benzyl alcohol. MS (ESI+) *m/z*: 293.4 (M – Boc + H)$^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.45 (9H, s), 1.58–1.67 (1H, m), 2.16–2.38 (1H, m), 2.50–3.17 (3H, m), 3.53–3.78 (4H, m), 3.86–4.12 (2H, m), 5.10 (2H, brs), 7.28–7.43 (5H, m).

Compounds **15b** and **15c** were prepared following similar procedures to the synthesis of compound **15a**.

1-*tert*-Butyl 3-methyl 5-{[(benzyloxy)carbonyl]amino}piperidine-1,3-dicarboxylate (15b).

Yield: 30%, pale yellow oil. MS (ESI+) *m/z*: 293.4 (M – Boc + H)$^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.45 (9H, s), 1.59–1.90 (1H, m), 2.16–2.41 (1H, m), 2.44–3.22 (3H, m), 3.69 (4H, s), 3.83–4.18 (2H, m), 5.10 (3H, brs), 7.29-7.41 (5H, m).

1-*tert*-Butyl 3-methyl (3*R*,5*S*)-5-{[(benzyloxy)carbonyl]amino}piperidine-1,3-dicarboxylate

(15c).

Yield: quant., pale yellow oil. MS (ESI+) *m/z*: 292.9 $(M - Boc + H)^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.45 (9H, s), 1.53–1.66 (1H, m), 2.16–2.36 (1H, m), 2.48–3.17 (3H, m), 3.54–3.71 (4H, m), 3.89–4.14 (2H, m), 4.94–5.19 (2H, m), 7.32–7.40 (5H, m).

***cis*-1-*tert*-Butyl 3-methyl 5-[(2-methylpropyl)amino]piperidine-1,3-dicarboxylate (16a).**

A mixture of crude **15a** (660 mg, 1.7 mmol) and 5% palladium on carbon (70 mg) in MeOH (5 mL) was stirred at room temperature under H_2 atmosphere (1 atm) for 15 h. The palladium catalyst was removed by filtration, and the filtrate was concentrated in vacuo to give crude amine (370 mg, 1.4 mmol, 86%) as a pale yellow oil. The amine thus obtained, AcOH (85 μL, 1.5 mmol), and *i*-PrCHO (85 μL, 1.5 mmol) were dissolved in MeOH (10 mL). $NaBH(OAc)_3$ (759 mg, 3.6 mmol) was added to the mixture, and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was basified with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo to give compound **16a** as a colorless oil (439 mg, 98%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.90 (6H, d, J = 6.6 Hz), 1.15–1.37 (1H, m), 1.46 (9H, s), 1.60–1.76 (1H, m), 2.23–2.40 (2H, m), 2.41–2.60 (4H, m), 2.64–2.86 (1H, m), 3.69 (3H, s), 4.11–4.43 (2H, m).

Compounds **16b** and **16c** were prepared following similar procedures to the synthesis of compound **16a**.

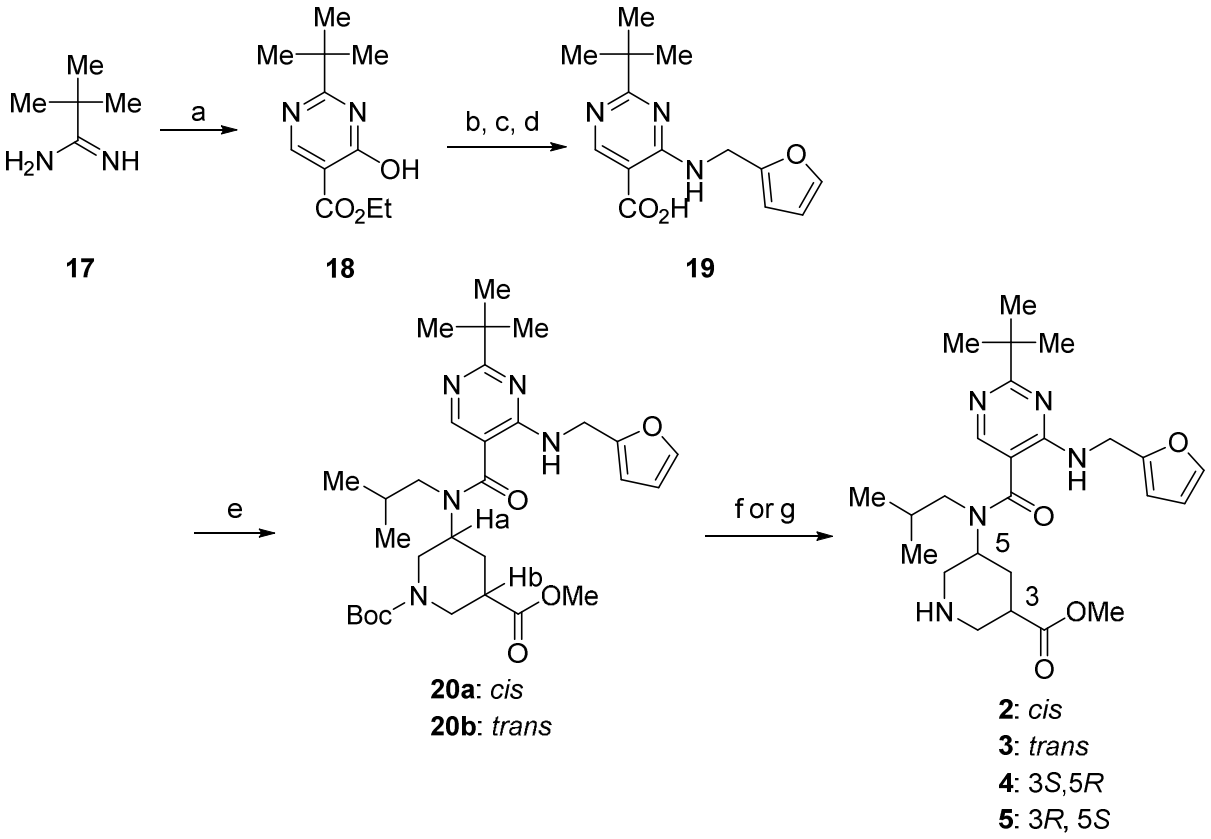
1-*tert*-Butyl 3-methyl 5-[(2-methylpropyl)amino]piperidine-1,3-dicarboxylate (16b).

Yield: quant., pale yellow oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.90 (6H, d, J = 6.8 Hz), 1.21–1.39 (1H, m), 1.39–1.54 (9H, m), 1.58–1.94 (1H, m), 2.16–2.39 (2H, m), 2.39–2.64 (4H, m), 2.65–2.87 (1H, m), 3.62–3.77 (3H, m), 4.07–4.43 (2H, m).

1-*tert*-Butyl 3-methyl (3*R*,5*S*)-5-[(2-methylpropyl)amino]piperidine-1,3-dicarboxylate (16c).

Yield: 89%, colorless oil. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.90 (6H, d, J = 6.8 Hz), 1.27–1.41 (1H, m), 1.46 (9H, s), 1.62–1.77 (1H, m), 2.21–2.40 (2H, m), 2.42–2.59 (4H, m), 2.64–2.86 (1H, m), 3.69 (3H, s), 4.14–4.47 (2H, m).

Scheme S2. Synthesis of compounds **2**–**5**.[a]



[a] Reagents and conditions: (a) diethyl (ethoxymethylidene)propanedioate, NaOEt, EtOH, 80 °C, 61%; (b) $POCl_3$, 100 °C; (c) furfurylamine, DIEA, *i*-PrOH, reflux; (d) NaOH, water, EtOH, THF, rt93% for 3 steps; (e) $SOCl_2$, DMF, toluene, 80 °C, then **16a** or **16b**, Et_3N, THF, rt, 45–53%; (f) 2 M HCl, EtOAc, rt, 49–74%; (g) preparative chiral HPLC, then 4 M HCl in EtOAc, rt, 83–85%.

Ethyl 2-*tert*-butyl-4-hydroxypyrimidine-5-carboxylate (18).

Compound **17** (2.90 g, 21 mmol) and diethyl (ethoxymethylidene)propanedioate (4.25 mL, 21 mmol) were dissolved in EtOH (30 mL). A solution of sodium ethoxide (20% in EtOH; 14.3 g, 42 mmol) was added to the solution dropwise at 0 °C. After stirred at 80 °C for 15 h, the reaction mixture was concentrated in vacuo. The residue was acidified with 1 M aqueous HCl solution to pH 3. The precipitates were collected by filtration and washed with small amount of water to give compound **18** as a beige powder (2.90 g, 61%). MS (ESI+) *m/z*: 225.2 $(M + H)^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.37 (3H, t, J = 7.2 Hz), 1.42 (9H, s), 4.37 (2H, q, J = 6.9 Hz), 8.72 (1H, bs), 11.04 (1H, bs).

2-*tert*-Butyl-4-[(furan-2-ylmethyl)amino]pyrimidine-5-carboxylic acid (19).

Compound **18** (2.90 g, 13 mmol) was dissolved in $POCl_3$ (9.6 g). After stirred at 100 °C for 2 h, the reaction mixture was concentrated in vacuo. The residue was cooled to 0 °C, neutralized with

saturated aqueous $NaHCO_3$ solution to pH 7, and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. A solution of the product obtained, furfurylamine (1.20 mL, 12.9 mmol), and DIEA (2.26 mL, 12.9 mmol) in *i*-PrOH (10 mL) was stirred under reflux for 15 h. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was diluted with saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The extract was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 9/1 to 5/1). The product obtained was dissolved in 2 M aqueous NaOH solution (15 mL, 30 mmol), EtOH (15 mL), and THF (5 mL). After stirred at room temperature for 15 h, the mixture was acidified with 1 M aqueous HCl solution to pH 3. The precipitates were collected by filtration and washed with water to give compound **19** as a white powder (3.30 g, 93%). mp 200–202 °C. MS (ESI+) *m/z*: 276.0 $(M + H)^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.50 (9H, s), 4.81 (2H, d, *J* = 5.7 Hz), 6.27 (1H, d, *J* = 3.0 Hz), 6.29–6.34 (1H, m), 7.29–7.43 (1H, m), 8.83 (1H, s), 10.92 (1H, brs). Anal. Calcd for $C_{14}H_{17}N_3O_3 \cdot H_2O$: C, 57.33; H, 6.53; N, 14.33. Found: C, 57.22; H, 6.49; N, 14.20.

***cis*-1-*tert*-Butyl 3-methyl 5-[({2-*tert*-butyl-4-[(furan-2-ylmethyl)amino]pyrimidin-5-yl}carbonyl)(2-methylpropyl)amino]piperidine-1,3-dicarboxylate (20a).**

To a suspension of compound **19** (385 mg, 1.4 mmol) in toluene (10 mL) was added thionyl chloride (0.26 mL, 3.5 mmol) and DMF (1 drop) at room temperature. After stirred at 80 °C for 2 h, the reaction mixture was concentrated in vacuo. The residue was azeotroped with toluene and dissolved in THF (10 mL). Compound **16a** (440 mg, 1.4 mmol) and Et_3N (0.49 mL, 3.5 mmol) were added to the solution. After stirred at room temperature for 1 h, the reaction mixture was concentrated in vacuo. The residue was diluted with water and extracted with EtOAc. The extract was washed with 5% aqueous citric acid solution, saturated aqueous $NaHCO_3$ solution, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 20/1 to 4/1) to give compound **20a** as a gray powder (363 mg, 45%). MS (ESI+) *m/z*: 572.6 $(M + H)^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.85 (6H, d, *J* = 6.4 Hz), 1.36 (9H, s), 1.43 (9H, s), 2.05 (1H, s), 2.16 (2H, bs), 2.43–2.83 (2H, m), 3.05 (1H, bs), 3.17 (2H, d, *J* = 7.2 Hz), 3.70 (4H, s), 4.12 (1H, d, *J* = 7.2 Hz), 4.30 (1H, bs), 4.68 (2H, m), 6.23 (1H, d, *J* = 3.4 Hz), 6.27–6.31 (1H, m), 6.67 (1H, bs), 7.33 (1H, s), 8.14 (1H, s). ^{1}H-^{1}H NOESY (300 MHz, DMSO-d_6, 333 K): Ha/Hb.

1-*tert*-Butyl 3-methyl *trans*-5-[({2-*tert*-butyl-4-[(furan-2-ylmethyl)amino]pyrimidin-5-yl}-carbonyl)(2-methylpropyl)amino]piperidine-1,3-dicarboxylate (20b).

To a suspension of compound **19** (639 mg, 2.0 mmol) in toluene (10 mL) was added thionyl chloride (0.42 mL, 5.8 mmol) and DMF (1 drop) at room temperature. After stirred at 80 °C for 2 h, the reaction mixture was concentrated in vacuo. The residue was azeotroped with toluene and dissolved in THF (10 mL). Compound **16b** (730 mg, 2.3 mmol) and Et_3N (0.81 mL, 5.8 mmol) were added to the solution. After stirred at room temperature for 1 h, the reaction mixture was concentrated in vacuo. The residue was diluted with water and extracted with EtOAc. The extract was washed with 5% aqueous citric acid solution, saturated aqueous $NaHCO_3$ solution, and brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexane/EtOAc = 20/1 to 4/1) to give compound **20a** as a beige solid (TLC: *Rf* = 0.35 (hexane/EtOAc = 3/1); 700 mg, 53%) and compound **20b** as a beige solid (TLC: *Rf* = 0.22 (hexane/EtOAc = 3/1); 300 mg, 23%). **20b**: MS (ESI+) *m/z*: 572.6 $(M + H)^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.85 (6H, d, *J* = 6.1 Hz), 1.36 (9H, s), 1.40 (9H, bs), 2.05 (1H, dd, *J* = 13.5, 6.7 Hz), 2.23 (2H, bs), 2.77 (1H, bs), 2.96 (1H, bs), 3.07 (1H, bs), 3.27 (2H, dd, *J* = 13.6, 7.2 Hz), 3.65 (3H, bs), 4.09 (1H, bs), 4.46 (1H, d, *J* = 13.6 Hz), 4.69 (2H, dd, *J* = 8.7, 5.7 Hz), 6.22–6.25 (1H, m), 6.28-6.31 (1H, m), 6.55 (1H, bs), 7.33 (1H, s), 8.16 (1H, bs).

***cis*-Methyl**
5-[({2-*tert*-butyl-4-[(furan-2-ylmethyl)amino]pyrimidin-5-yl}carbonyl)(2-methylpropyl)amino]piperidine-3-carboxylate dihydrochloride (2).

A mixture of compound **20a** (100 mg, 0.17 mmol) and 2 M HCl in EtOAc was stirred at room temperature for 15 h. The insoluble material was removed by filtration, and the filtrate was concentrated in vacuo. The residue was diluted with EtOAc-hexane. The precipitate was collected by filtration to give compound **2** (70 mg, 74%) as a beige powder. MS (ESI+) *m/z*: 472.5 $(M + H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.53–1.01 (6H, m), 1.18–1.47 (9H, m), 1.70–2.37 (3H, m), 2.85–3.39 (7H, m), 3.67 (3H, s), 4.13–4.49 (1H, m), 4.53–4.83 (2H, m), 6.28–6.35 (1H, m), 6.40 (1H, dd, *J* = 3.2, 1.9 Hz), 7.54–7.62 (1H, m), 8.31 (1H, brs), 9.48 (2H, brs). ^{13}C-NMR (151 MHz, DMSO-d_6) δ 13.9, 19.4, 20.5, 21.0, 22.0, 26.9, 27.8, 27.8, 27.9, 29.5, 30.9, 37.3, 37.7, 38.4, 42.4, 43.5, 44.7, 48.7, 50.9, 51.5, 52.0, 54.9, 60.7, 107.6, 110.5, 112.0, 142.3, 150.6, 158.1, 164.1, 170.1, 171.0, 171.9, 171.7, 172.2. 120.4, 126.8, 129.5, (observed complexity is due to rotameric effects at the experimental temperature). Anal. Calcd for $C_{25}H_{37}N_5O_4 \cdot 2HCl \cdot 1.2H_2O \cdot 0.3EtOAc$: C, 53.10; H, 7.45; N, 11.82. Found: C, 52.94; H, 7.72; N, 11.60. HPLC purity: 99.7%.

Compound **3** was prepared following similar procedures to the synthesis of **2.**

***trans*-Methyl**
5-[({2-*tert*-butyl-4-[(furan-2-ylmethyl)amino]pyrimidin-5-yl}carbonyl)(2-methylpropyl)amino]piperidine-3-carboxylate dihydrochloride (3)

Yield: 49%, beige solid. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.53–1.01 (6H, m), 1.18–1.54 (9H, m), 1.75–2.42 (3H, m), 2.81–3.46 (9H, m), 3.96 (1H, brs), 4.54–4.88 (2H, m), 6.33 (1H, brs), 6.39–6.44 (1H, m), 7.60 (1H, s), 8.20 (1H, brs), 8.47–9.66 (2H, m), 9.78-10.05 (1H, m). ^{13}C-NMR (151 MHz, DMSO-d_6) δ 19.5, 20.5, 21.0, 26.9, 27.7 (3C), 36.7, 37.8, 38.4, 42.0, 44.7, 48.7, 50.5, 52.2, 107.9, 110.5, 111.9, 142.4, 150.5, 158.2, 164.1, 170.0, 171.2, 171.9. Anal. Calcd for $C_{25}H_{37}N_5O4{\cdot}2HCl{\cdot}1.5H_2O$: C, 52.54; H, 7.41; N, 12.25. Found: C, 52.63; H, 7.60; N, 11.85.

Methyl (3*S*,5*R*)-5-[({2-*tert*-butyl-4-[(furan-2-ylmethyl)amino]pyrimidin-5-yl}carbonyl)(2-methylpropyl) amino]piperidine-3-carboxylate dihydrochloride (4).

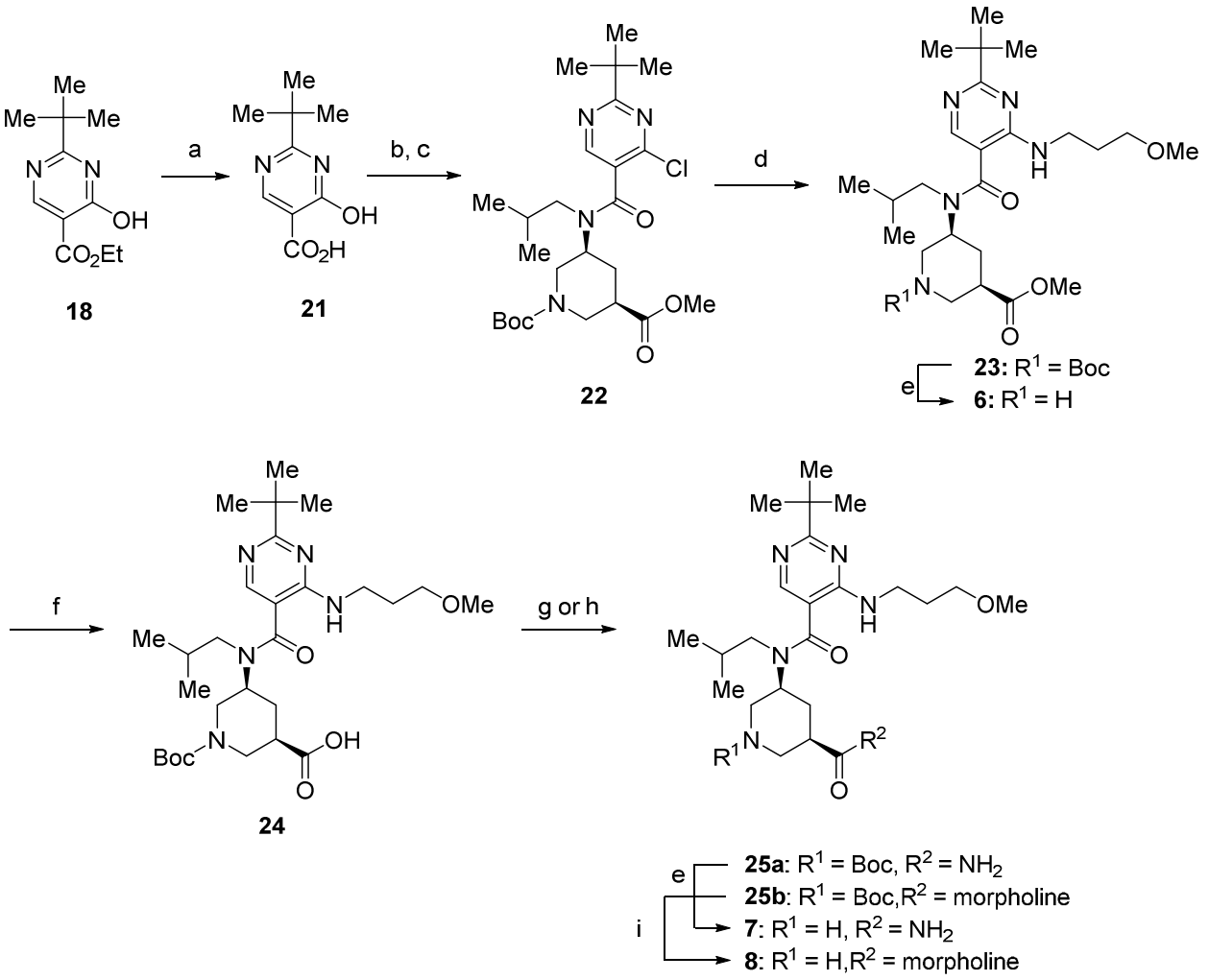
Compound **20a** (3.0 g) was resolved using normal phase preparative HPLC [CHIRALPAK AD column, 50 mmID 500 mmL, mobile phase hexane/EtOH (97/3); flow rate 85 mL/min; UV 220 nm; temperature 30 ˚C]. [Analysis: CHIRALPAK AD-H column, 4.6 mmID 250 mmL, mobile phase hexane/ *i*-PrOH (80/20); flow rate 0.5 mL/min; UV 220 nm; temperature 30 ˚C]. A solution of obtained tR1 of compound **20a** (>99.9% ee, 50 mg, 0.087 mmol) in 1 M HCl in EtOAc (3mL) was stirred at room temperature for 2h. The reaction mixture was concentrated in vacuo to give compound **4** as a white solid (40.6 mg, 85%). MS (ESI+) *m/z*: 472.5 $(M + H)^+$**.** ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.50–1.03 (6H, m), 1.37 (9H, s), 1.66–2.41 (3H, m), 2.72–3.38 (9H, m), 3.67 (3H, s), 4.33 (1H, bs), 4.70 (2H, bs), 6.33 (1H, bs), 6.37–6.43 (1H, m), 7.58 (1H, s), 8.32 (1H, s), 9.55 (2H, bs). Anal. Calcd for $C_{25}H_{39}N_5O_4Cl_2{\cdot}1.5H_2O$: C, 52.54; H, 7.41; N, 12.25. Found: C, 52.73; H, 7.40; N, 11.85. $[\alpha]^{25}_D$ -9.8° (c 0.2870, MeOH)

Compound **5** was prepared following similar procedures to the synthesis of compound **4** by using tR2 (99.6% ee) instead of tR1 of compound **20a**.

Methyl (3*R*,5*S*)-5-[({2-*tert*-butyl-4-[(furan-2-ylmethyl)amino]pyrimidin-5-yl}carbonyl)(2-methylpropyl) amino]piperidine-3-carboxylate dihydrochloride (5)

Yield: 83%. MS (ESI+) *m/z*: 472.0 $(M + H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.54-1.01 (6H, m), 1.37 (9H, s), 1.73–2.40 (3H, m), 2.79–3.31 (9H, m), 3.67 (3H, s), 4.32 (1H, bs), 4.71 (2H, bs), 6.32 (1H, bs), 6.40 (1H, bs), 7.58 (1H, s), 8.30 (1H, bs), 9.52 (2H, bs). Anal. Calcd for $C_{23}H_{37}N_5O_4{\cdot}2HCl{\cdot}1.4H_2O{\cdot}0.1EtOAc$: C, 52.73; H, 7.42; N, 12.11. Found: C, 52.73; H, 7.40; N, 11.85. $[\alpha]^{25}_D$ +6.1° (c 0.4740, MeOH). HPLC purity: 98.8%.

Scheme S3. Synthesis of compounds **6**–**8**.[a]



[a] Reagents and conditions: (a) 2 M aqueous NaOH solution, EtOH, rt, 85%; (b) $SOCl_2$, DMF, THF, reflux; (c) **16c**, DIEA, THF, rt, 94%; (d) 3-methoxypropylamine, DIEA, DMF, 80 °C, 96%; (e) 2 M HCl, EtOAc, rt, 97%; (f) 2 M aqueous NaOH solution, MeOH, THF, rt, quant.; (g) HOBt·NH_3 complex, WSC, Et_3N, CH_2Cl_2, rt, 85%; (h) morpholine, BOP reagent, DIEA, DMF, rt, 79%; (i) 4 M HCl in EtOAc, rt, then fumaric acid, EtOAc, MeOH, 59%.

2-*tert*-Butyl-4-hydroxypyrimidine-5-carboxylic acid (21).

To a solution of compound **18** (43.9 g, 196 mmol) in EtOH (200 mL) was added 2 M aqueous NaOH solution (330 mL, 660 mmol). After being stirred at room temperature for 40 h, the reaction mixture was concentrated in vacuo. The resulting aqueous solution was acidified with 6 M aqueous HCl solution to pH 8, then concentrated in vacuo, and azeotroped with *i*-PrOH. The residue was triturated with acetone, and the insoluble materials were filtered. The powder thus obtained was suspended in 1 M aqueous HCl solution, adjusting pH 3, and the mixture was concentrated in vacuo.

After being azeotroped with *i*-PrOH, the residue was triturated with acetone. Insoluble materials were removed by filtration, and the filtrate was concentrated in vacuo to give compound **21** as a white solid (32.8 g, 85%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 1.45 (9H, s), 8.99 (1H, s), 10.59 (1H, bs), 12.47 (1H, bs).

1-*tert*-Butyl 3-methyl (3*R*,5*S*)-5-{[(2-*tert*-butyl-4-chloropyrimidin-5-yl)carbonyl](isobutyl)amino}piperidine-1,3-dicarboxylate (22).

Compound **21** (3.25 g, 16.6 mmol) was dissolved in THF (60 mL), thionyl chloride (4.3 mL, 59.0 mmol) and DMF (5 drops) were added and the mixture was stirred with heating to reflux for 2.5 h. The reaction mixture was cooled to room temperature, and concentrated in vacuo, and the residue was subjected to azeotropic distillation with toluene. The obtained residue was suspended in THF (50 mL), a solution of compound **16c** (4.13 g, 13.1 mmol) and DIEA (9.15 mL, 52.5 mmol) in THF (50 mL) was added and the mixture was stirred at room temperature for 8 h. The reaction mixture was concentrated in vacuo, and diluted with water, and the mixture was extracted with EtOAc. The extract was washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuo and the residue was purified by silica gel column chromatography. The fraction eluted with EtOAc-hexane (1:19–2:3) was concentrated in vacuo to give compound **22** (6.29 g, 94%). MS (ESI+) *m/z*: 511.1 $(M + H)^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.84 (4H, d, J = 6.4 Hz), 1.01 (2H, dd, J = 6.2, 3.6 Hz), 1.34–1.50 (18H, m), 1.83–1.99 (1H, m), 2.15–2.31 (1H, m), 2.60 (2H, bs), 2.80 (2H, bs), 3.11 (1H, bs), 3.47 (2H, d, J = 6.1 Hz), 3.59–3.75 (3H, m), 4.30 (2H, bs), 8.51 (1H, s).

1-*tert*-Butyl 3-methyl (3*R*,5*S*)-5-[({2-*tert*-butyl-4-[(3-methoxypropyl)amino]pyrimidin-5-yl}carbonyl)(2-methylpropyl)amino]piperidine-1,3-dicarboxylate (23).

To a mixture of compound **22** (2.98 g, 5.83 mmol) and DIEA (3 mL, 17.5 mmol) in DMF (60 mL), 3-methoxypropylamine (1.19 mL, 11.7 mmol) was added dropwise. The mixture was stirred at 80 °C for 1.5 h. The mixture was concentrated in vacuo and the residue was diluted with sat aqueous $NaHCO_3$ solution and extracted with EtOAc. The extract was dried over $MgSO_4$, concentrated in vacuo and purified by silica gel column chromatography. The fraction eluted with EtOAc-hexane (0:100–35:65) was concentrated in vacuo to give compound **23** as a pale brown powder (3.15 g, 96%). MS (ESI+) *m/z*: 564.1 $(M + H)^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.86 (6H, d, J = 6.6 Hz), 1.34 (9H, s), 1.44 (9H, s), 1.88 (2H, quin, J = 6.2 Hz), 1.92–2.08 (1H, m), 2.10–2.29 (2H, m), 2.47–2.83 (2H, m), 2.97–3.26 (3H, m), 3.34 (3H, s), 3.48 (2H, t, J = 6.0 Hz), 3.52–3.64 (2H, m), 3.63–3.74 (1H, m) 3.70 (3H, s), 3.96–4.20 (1H, m), 4.22–4.42 (1H, m), 6.53 (1H, bs), 8.08 (1H, s).

Compound **6** was prepared following similar procedures to the synthesis of compound **2**.

Methyl (3R,5S)-5-[({2-tert-butyl-4-[(3-methoxypropyl)amino]pyrimidin-5-yl}carbonyl)(2-methylpropyl) amino]piperidine-3-carboxylate dihydrochloride (6).

Yield: 97%, white powder. MS (ESI+) *m/z*: 464.2 $(M + H)^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.52-1.04 (6H, m), 1.39 (9H, s), 1.81 (2H, quin, *J* = 6.5 Hz), 1.88–2.42 (3H, m), 2.77–3.61 (11H, m), 3.23 (3H, s), 3.66 (3H, s), 4.17–4.61 (1H, m), 8.27 (1H, s), 8.72 (1H, brs), 9.19–10.76 (2H, m), 14.01 (1H, brs). Anal. Calcd for $C_{24}H_{41}N_5O_4 \cdot 2HCl \cdot 1.5H_2O$: C, 51.15; H, 8.23; N, 12.43. Found: C, 50.90; H, 8.00; N, 12.17. $[\alpha]^{25}_D$ +11.9° (c 0.4050, MeOH).

(3*R*,5*S*)-1-(*tert*-Butoxycarbonyl)-5-[({2-*tert*-butyl-4-[(3-methoxypropyl)amino]pyrimidin-5-yl}carbonyl)(isobutyl)amino]piperidine-3-carboxylic acid (24)

Compound **23** (1.08 g, 1.91 mmol) was dissolved in MeOH (25 mL) and THF (12 mL), 2 M aqueous NaOH solution (4.79 mL) was added, and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo, the residue was diluted with saturated aqueous NH_4Cl solution and the mixture was extracted with EtOAc. The extract was dried over anhydrous $MgSO_4$, and concentrate in vacuo to give compound **24** (1.05 g, quant.). MS (ESI+) *m/z*: 550.1 $(M + H)^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.88 (6H, bs), 1.33 (9H, s), 1.38 (9H, s), 1.78–1.94 (2H, m), 1.92-2.18 (2H, m), 2.19–3.24 (8H, m), 3.34 (3H, s), 3.47, (2H, t, *J* = 5.1 Hz), 3.52–3.84 (2H, m), 3.86–4.15 (1H, m), 4.16–4.44 (1H, m) 7.95–8.46 (1H, m).

***tert*-Butyl (3*S*,5*R*)-3-[({2-*tert*-butyl-4-[(3-methoxypropyl)amino]pyrimidin-5-yl}carbonyl)(2-methylpropyl) amino]-5-carbamoylpiperidine-1-carboxylate (25a).**

To a solution of compound **24** (301 mg, 0.55 mmol), HOBt·NH_3 complex (167 mg, 1.10 mmol), and Et_3N (0.23 mL, 1.64 mmol) in dichloroethane (10 mL) was added WSC (210 mg, 1.10 mmol) at room temperature. After being stirred at room temperature for 2 h, the reaction mixture was poured into saturated aqueous $NaHCO_3$ solution and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. The residue was purified by silica-gel column chromatography (eluted with hexane/EtOAc = 1/1 to EtOAc) to give compound **25a** as a white solid (62 mg, 85%). MS (ESI+) *m/z*: 549.2 $(M + H)^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.88 (6H, d, *J* = 6.8 Hz), 1.34 (9H, s), 1.41 (9H, s), 1.88 (2H, quin, *J* = 6.2 Hz), 1.96–2.30 (3H, m), 2.35–2.51 (1H, m), 2.65–2.86 (1H, m), 3.00 (1H, dd, *J* = 12.5, 11.7 Hz), 3.12 (1H, dd, *J* = 13.6, 7.2 Hz), 3.26 (1H, dd, *J* = 13.3, 7.6 Hz), 3.35 (3H, s), 3.48 (2H, t, *J* = 6.1 Hz), 3.51–3.69 (2H, m), 3.71–3.88 (1H, m), 3.94–4.11 (1H, m), 4.13–4.27 (1H, m), 5.35 (1H, bs), 5.86 (1H, bs), 6.63 (1H, bs), 8.10 (1H, s).

***tert*-Butyl**

(3*S*,5*R*)-3-[({2-*tert*-butyl-4-[(3-methoxypropyl)amino]pyrimidin-5-yl}carbonyl)(isobutyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate (25b)

Compound **24** (110 mg, 0.20 mmol), morpholine (52 mL) and DIEA (140 mL) were dissolved in DMF (8 mL), BOP reagent (265 mg, 0.60 mmol) was added, and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was diluted with saturated aqueous $NaHCO_3$ solution, and the mixture was extracted with EtOAc. The extract was dried over anhydrous $MgSO_4$, and the solvent was evaporated in vacuo. The residue was purified by silica gel column chromatography, and the fraction eluted with EtOAc-hexane (1:9) to EtOAc was concentrated to give compound **25b** (105 mg, 79%) as a white powder. MS (ESI+) *m/z*: 619.3 $(M + H)^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.89 (6H, d, *J* = 6.4 Hz), 1.34 (9H, s), 1.40 (9H, s), 1.88 (2H, m), 1.93–2.09 (2H, m), 2.23–2.44 (1H, m), 2.58–2.84 (2H, m), 2.92–3.06 (1H, m), 3.09–3.33 (2H, m), 3.35 (3H, s), 3.48 (2H, t, *J* = 6.0 Hz), 3.50–3.79 (10H, m), 3.79–3.94 (1H, m), 3.95–4.16 (2H, m), 6.66 (1H, bs), 8.09 (1H, s).

Compound **7** was prepared following similar procedures to the synthesis of compound **2**.

2-*tert*-Butyl-*N*-[(3*S*,5*R*)-5-carbamoylpiperidin-3-yl]-4-[(3-methoxypropyl)amino]-*N*-(2-methylpropyl)pyrimidine-5-carboxamide dihydrochloride (7).

Yield: 79%, white solid. MS (ESI+) *m/z*: 449.2 $(M + H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.71–1.00 (6H, m), 1.39 (9H, s), 1.74–1.87 (2H, m), 1.87–2.28 (3H, m), 2.67–2.94 (2H, m), 2.94–3.73 (12H, m), 4.05–4.44 (1H, m), 7.12 (1H, bs), 7.63 (1H, bs), 8.22 (1H, bs), 8.44–9.73 (2H, m). Anal. Calcd for $C_{23}H_{40}N_6O_3 \cdot 2HCl \cdot 2H_2O$: C, 49.55; H, 8.32; N, 15.07. Found: C, 49.63; H, 8.10; N, 15.08. $[\alpha]^{25}_D$ -1.0° (c 0.3000, MeOH)

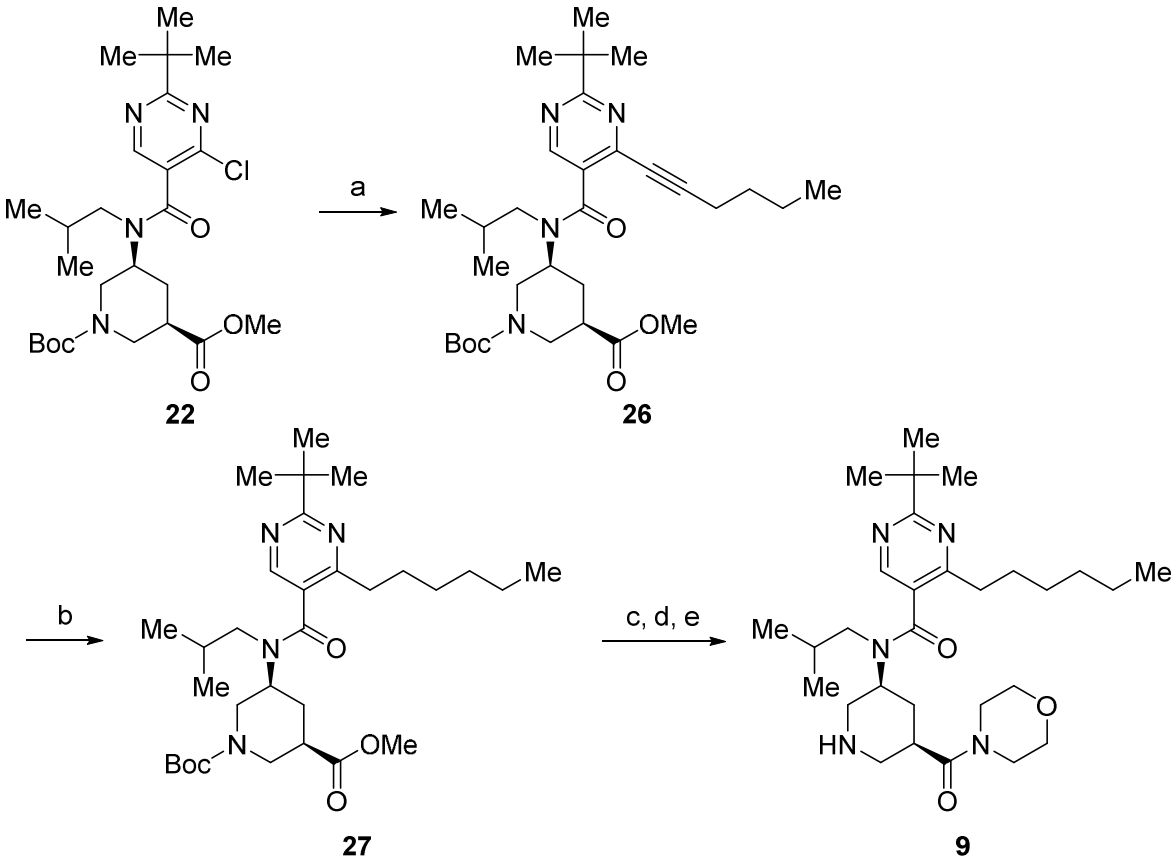
Methyl

(3*R*,5*S*)-5-{[(2-*tert*-butyl-4-{[3-(methylsulfonyl)propyl]amino}pyrimidin-5-yl)carbonyl](2-methylpropyl)amino}piperidine-3-carboxylate fumarate (8).

A mixture of compound **25a** (8.25 g, 13.3 mmol) and 4 M HCl in EtOAc was stirred for 6 h at room temperature. The reaction mixture was concentrated in vacuo. The residue was diluted with saturated aqueous K_2CO_3 solution and extracted with EtOAc. The extract was washed with brine, dried over $MgSO_4$ and concentrated in vacuo to give the corresponding free amine (6.30 g, 12.1 mmol, 91%) as a white solid. The obtained amine (5.22 g, 10.1 mmol) and fumaric acid (1.17 g, 10.1 mmol) were dissolved in EtOAc/ MeOH (4/1; v/v, 100 mL), and the mixture was stirred at 70 °C for 20 min. Insoluble materials were removed by filtration, and the filtrate was concentrated in vacuo. The residue was dissolved in warmed (70 °C) EtOAc/MeCN (1/1; v/v, 380 mL) and cooled to room temperature over 15 h, stirring vigorously. After being stirred at 0 °C for additional 2 h, resulting precipitates were

collected by filtration and dried in vacuo at 100 °C for 12 h to give compound **8** as a white powder (4.16 g, 65%). mp 134–135 °C. MS (ESI+) *m/z*: 519.2 $(M + H)^+$. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.80 (6H, d, J = 4.7 Hz), 1.29 (9H, s), 1.71–1.82 (2H, m), 1.82–2.08 (3H, m), 2.71–2.98 (3H, m), 2.98–3.18 (4H, m), 3.21 (3H, s), 3.35 (2H, t, J = 6.4 Hz), 3.39–4.03 (14H, m), 6.55 (2H, s), 6.75–6.92 (1H, m), 7.99 (1H, s). ^{13}C-NMR (151 MHz, DMSO-d_6) δ 19.8 (2C), 27.1, 28.8, 29.2 (3C), 30.4, 36.9, 37.5, 38.8, 41.5, 41.4, 45.3, 46.0, 49.3, 53.2, 57.8, 66.0, 66.1, 70.1, 110.9, 134.5 (2C), 152.3, 158.0, 167.2 (2C), 168.2, 169.7, 175.6. Anal. Calcd for $C_{27}H_{46}N_6O_4 \cdot 1.0$fumarate$\cdot 0.5H_2O$: C, 57.84; H, 7.98; N, 13.05. Found: C, 58.08; H, 7.92; N, 13.01. $[\alpha]^{25}_D$ -3.2° (c 0.4900, MeOH).

Scheme S4. Synthesis of compound **9**.[a]



22 26 27 9

[a] Reagents and conditions: (a) CuI, DIEA, 1-hexyne, DMF, 70 °C, 67%; (b) H_2, Pd/C, MeOH, rt, quant.; (c) 1 M aqueous NaOH solution, THF, MeOH, rt; (d) morpholine, WSC, HOBt, Et_3N, $ClCH_2CH_2Cl$, rt; (e) 1 M HCl in EtOAc, rt, 27% for 3 steps.

1-*tert*-Butyl 3-methyl (3*R*,5*S*)-5-{[(2-*tert*-butyl-4-(hex-1-yn-1-yl)pyrimidin-5-yl)carbonyl](2-methylpropyl)amino}piper

idine-1,3-dicarboxylate (26).

Compound **22** (412 mg, 0.59 mmol), copper iodide (112 mg, 0.59 mmol) and DIEA (0.51 μL, 2.9 mmol) were dissolved in DMF (8 mL), and the mixture was stirred at room temperature for 15 min. 1-Hexyne (0.08 mL) was added and the mixture was stirred at room temperature for 2 h, and further at 70 °C for 8 h. The mixture was cooled to room temperature, adsorbed to silica gel (10 g), and a fraction eluted with EtOAc was concentrated in vacuo. The residue was subjected to silica gel column chromatography, and a fraction eluted with hexane-EtOAc (95:5–30:70) was concentrated in vacuo to give compound **26** (219 mg, 67%) as a pale brown solid. MS (ESI+) *m/z*: 557.2 $(M + H)^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.80–0.87 (10H, m), 1.01–1.20 (2H, m), 1.34–1.50 (12H, m), 1.83–1.99 (1H, m), 2.15–2.48 (4H, m), 2.60 (2H, bs), 2.76–2.92 (7H, m), 3.49–3.70 (3H, m), 4.30–4.47 (4H, m), 5.67–5.98 (2H, m), 8.61 (1H, s).

1-*tert*-Butyl 3-methyl (3*R*,5*S*)-5-{[(2-*tert*-butyl-4-hexylpyrimidin-5-yl)carbonyl](2-methylpropyl)amino}piperidine-1,3-dicarboxylate (27).

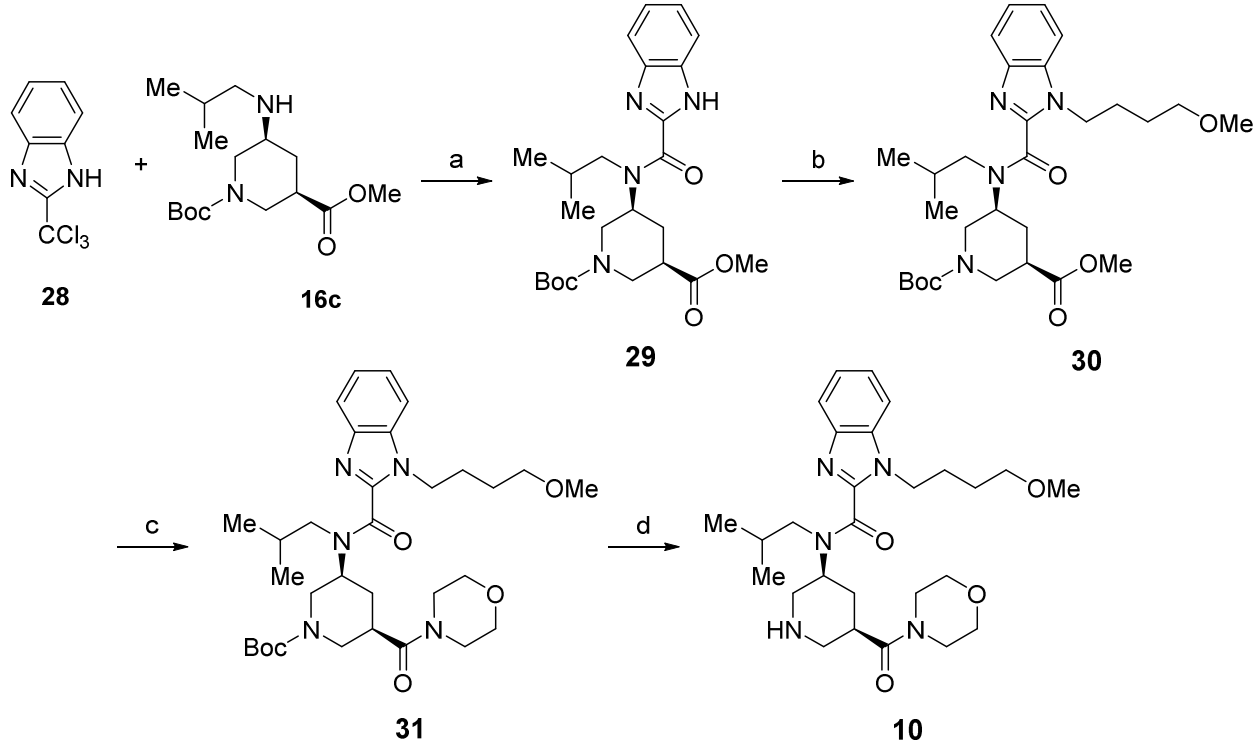
A mixture of compound **26** (218 mg, 0.39 mmol) and 10% palladium on carbon (20 mg) in MeOH (6 mL) was stirred at room temperature under H_2 atmosphere for 16 h. The palladium catalyst was removed by filtration through a pad of celite. The filtrate was concentrated in vacuo to give compound **27** (219 mg, quant.) as a pale yellow solid. MS (ESI+) *m/z*: 561.0 $(M + H)^+$. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.80–1.20 (12H, m), 1.34–1.50 (16H, m), 1.83–1.99 (1H, m), 2.02–2.35 (4H, m), 2.42–2.65 (2H, m), 2.76–2.95 (7H, m), 3.49–3.70 (3H, m), 3.99–4.35 (4H, m), 4.20–4.36 (2H, m), 8.59 (1H, s).

2-*tert*-Butyl-4-hexyl-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-ylcarbonyl)piperidin-3-yl]pyrimidine-5-carboxamide dihydrochloride (9).

Compound **27** (219 mg, 0.39 mmol) was dissolved in MeOH (3 mL) and THF (2 mL), 1 M aqueous NaOH solution (2 mL) was added and the mixture was stirred at room temperature for 2 h. The reaction mixture was concentrated in vacuo, and the aqueous layer of the mixture was adjusted to pH 5-6 with saturated aqueous NH_4Cl solution, and the mixture was extracted with EtOAc. The extract was washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuo. The obtained residue, morpholine (41 μL, 0.47 mmol), HOBt (30 mg, 0.12 mmol) and Et_3N (140 μL, 1.00 mmol) were dissolved in 1,2-dichloroethane (4 mL), and WSC (115 mg, 0.599 mmol) was added, and the mixture was stirred at room temperature for 3 days. The reaction mixture was poured into water, and the mixture was extracted with EtOAc. The extract was washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuo. The residue was subjected to silica gel column chromatography, and a fraction eluted with EtOAc-hexane (5:95–80:20) was concentrated in

vacuo. The residue was then subjected to preparative HPLC, and a fraction was concentrated in vacuo to give *tert*-butyl (3*S*,5*R*)-3-{[(2-*tert*-butyl-4-hexylpyrimidin-5-yl)carbonyl](2-methylpropyl)amino}-5-(morpholin-4-yl carbonyl)piperidine-1-carboxylate (88 mg, 0.14 mmol) as a white solid. The morpholine amide thus obtained (88 mg) was dissolved in 1 M HCl in EtOAc (3 mL), and the mixture was stirred at room temperature for 1.5 h. The reaction mixture was concentrated to give compound **9** (63 mg, 27% for 3 steps) as a white solid. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.61–0.78 (2H, m), 0.78–0.88 (2H, m), 0.88–1.04 (2H, m), 1.12–1.30 (4H, m), 1.30–1.49 (7H, m), 1.57–1.85 (2H, m), 1.85–2.18 (2H, m), 2.29–2.55 (10H, m), 2.55–2.74 (4H, m), 2.74–3.02 (2H, m), 3.05–3.31 (6H, m), 3.52–3.83 (6H, m), 8.44–8.71 (1H, m), 9.76 (1H, bs). ESI-HRMS Calcd for $C_{29}H_{49}N_5O_3$ m/z 516.3908 (M+H), Found 516.3876 (M+H). $[\alpha]^{25}_D$ -4.5° (c 0.0880, MeOH).

Scheme S5. Synthesis of compound **10**.[a]



[a] Reagents and conditions: (a) aq. $NaHCO_3$, THF, rt to 50 °C, 84%; (b) 4-MeO$(CH_2)_4$OMs, Cs_2CO_3, DMA, 70 °C, 80%.; (c) 1) 4 M aqueous NaOH solution, MeOH, 50 °C; 2) morpholine, WSC, HOBt, DMF, rt, 89%; (d) 4 M HCl in EtOAc, MeOH, rt, 50%.

1-*tert*-Butyl **3-methyl**

(3*R*,5*S*)-5-[(1*H*-benzimidazol-2-ylcarbonyl)(2-methylpropyl)amino]piperidine-1,3-dicarboxylate (29).

2-(Trichloromethyl)-1*H*-benzimidazole[3] (**28**; 19 g, 79.5 mmol) and compound **16c** (25 g, 79.5 mmol) were dissolved in THF (1200 mL), $NaHCO_3$ (67 g, 84.0 mmol) and water (600 mL) were added, and the mixture was stirred at room temperature for 1 h and at 50°C for 1 h. After evaporation of the solvent, the residue was extracted 3 times with EtOAc (700 mL). The extract was washed successively with 10% aqueous citric acid solution (500 mL) and brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo. The residue was dissolved in EtOAc (1000 mL), subjected to basic silica gel column chromatography, and a fraction eluted with EtOAc was concentrated in vacuo to give compound **29** (30.6 g, 84%) as a white solid. MS (ESI+) *m/z*: 459.0 (M+1). ^{1}H-NMR ($CDCl_3$) δ 0.78–1.09 (6 H, m), 1.17–1.55 (9 H, m), 1.77–2.95 (5 H, m), 3.11–3.79 (6 H, m), 3.99–4.73 (4 H, m), 7.24–7.41 (2 H, m), 7.45–7.59 (1 H, m), 7.72–7.88 (1 H, m), 10.66–10.98 (1 H, m).

1-*tert*-Butyl 3-methyl (3*R*,5*S*)-5-[{[1-(4-methoxybutyl)-1*H*-benzimidazol-2-yl]carbonyl}(2-methylpropyl)amino]piperidine-1,3-dicarboxylate (30).

Compound **29** (30 g, 65.4 mmol) and 4-methoxybutyl methanesulfonate (12.5 g, 68.7 mmol) were dissolved in DMA (600 mL), cesium carbonate (32 g, 98.1 mmol) was added, and the mixture was stirred at 70°C for 12 h. The reaction mixture was poured into ice water (1000 mL), and the mixture was extracted twice with EtOAc (1000 mL). The extract was washed with brine and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo. The residue was subjected to silica gel column chromatography, and a fraction eluted with EtOAc-hexane (1:4 – 1:1) was concentrated in vacuo to give compound **30** (28.7 g, 80%) as a white solid. MS (ESI+) *m/z*: 545.2 (M+1). ^{1}H-NMR ($CDCl_3$) δ 0.66–1.07 (6H, m), 1.29–1.52 (9H, m), 1.63–1.74 (2H, m), 1.75–2.04 (3H, m), 2.11–2.37 (1H, m), 2.41–2.67 (2H, m), 2.81 (1H, d, *J* = 13.2 Hz), 3.27–3.36 (4H, m), 3.37–3.46 (3H, m), 3.46–3.81 (5H, m), 4.16–4.56 (4H, m), 7.28–7.40 (2H, m), 7.41–7.48 (1H, m), 7.72–7.85 (1H, m).

***tert*-Butyl (3*S*,5*R*)-3-[{[1-(4-methoxybutyl)-1*H*-benzimidazol-2-yl]carbonyl}(2-methylpropyl)amino]-5-(morpholin-4-ylcarbonyl)piperidine-1-carboxylate (31)**.

Compound **30** (15 g, 27.5 mmol) was dissolved in methanol (150 mL), 4 M aqueous NaOH solution (250 mL) was added, and the mixture was stirred at 50°C for 1 h. The solvent was evaporated in vacuo, and the residue was ice-cooled, neutralized with 2 M aqueous hydrochloric acid solution, and extracted twice EtOAc (500 mL). The extract was washed with brine and dried over anhydrous

Na_2SO_4. The solvent was evaporated in vacuo. The residue was dried in vacuo to give (3*R*,5*S*)-1-(*tert*-butoxycarbonyl)-5-[{[1-(4-methoxybutyl)-1*H*-benzimidazol-2-yl]carbonyl}(2-methyl propyl)amino]piperidine-3-carboxylic acid (15.0 g, quant.) as a white solid. (3*R*,5*S*)-1-(tert-Butoxycarbonyl)-5-[{[1-(4-methoxybutyl)-1*H*-benzimidazol-2-yl]carbonyl}(2-methyl propyl)amino]piperidine-3-carboxylic acid (10 g, 16.7 mmol) and morpholine (1.6 g, 18.3 mmol) were dissolved in DMF (100 mL), WSC·HCl (4.8 g, 25.0 mmol), and HOBt (3.1 g, 20.2 mmol) were added, and the mixture was stirred at 50°C for 12 h. The reaction mixture was poured into 10% aqueous sodium bicarbonate, and the mixture was extracted with EtOAc. The extracts were combined, washed with brine, and dried over anhydrous Na_2SO_4. The solvent was evaporated in vacuo. The residue was subjected to silica gel column chromatography, and a fraction eluted with EtOAc-hexane (1:1 –1:0) was concentrated in vacuo to give compound **31** (8.9 g, 89%) as a white solid. ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.63–0.80 (2H, m), 0.89–1.07 (4H, m), 1.41–1.59 (9H, m), 1.59–1.80 (2H, m), 1.87–2.23 (4H, m), 2.30–2.98 (3H, m), 3.21–3.46 (6H, m), 3.49–3.91 (10H, m), 3.95–4.47 (5H, m), 7.18–7.51 (3H, m), 7.56–7.84 (1H, m). MS (ESI+) *m/z*: 600.2 (M+1).

1-(4-Methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-yl)carbonylpiperidin-3-yl]-1*H*-benzimidazole-2-carboxamide hydrochloride (10).

Compound **31** (5.85 g, 9.75 mmol) was dissolved in MeOH (20 mL), 4 M HCl in EtOAc (20 mL) was added, and the mixture was stirred at room temperature for 15 h. The reaction mixture was concentrated, and the residue was diluted with aqueous Na_2CO_3 solution, and the mixture was extracted with EtOAc. The extract was washed with brine and dried over anhydrous $MgSO_4$. The solvent was evaporated in vacuo. The residue was subjected to basic silica gel column chromatography, and a fraction eluted with EtOAc-MeOH (90:10) was concentrated in vacuo to give 1-(4-methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-yl)carbonylpiperidin-3-yl]-1*H*-be nzimidazole-2-carboxamide (4.40 g, 90%) as a white solid. ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.66–1.04 (6H, m), 1.55–1.73 (2H, m), 1.86–2.40 (5H, m), 2.54–3.00 (4H, m), 3.03–3.44 (8H, m), 3.46–3.76 (9H, m), 4.13–4.46 (3H, m), 7.27–7.40 (2H, m), 7.40–7.47 (1H, m), 7.62–7.82 (1H, m). MS (ESI+) *m/z*: 499.9 (M+1)+.

1-(4-Methoxybutyl)-*N*-(2-methylpropyl)-*N*-[(3*S*,5*R*)-5-(morpholin-4-yl)carbonylpiperidin-3-yl]-1*H*-b enzimidazole-2-carboxamide (24.4 g, 48.8 mmol) was dissolved in EtOAc (225 mL), and the mixture was heated to 50 °C. 4 M HCl in EtOAc (12.8 mL, 51.2 mmol) was added dropwise to the mixture. Heptane (75 mL) was added dropwise, and the mixture was cooled to 30 °C. The seed crystal was added and the mixture was stirred for 30 min. The mixture was heated to 50 °C, and heptane (150 mL) was added dropwise. After stirring at 50 °C for 1 h, the mixture was cooled to 0 °C and stirred for 1 h.

The precipitated crystals were collected by filtration, washed with EtOAc-heptane and dried to give crude **10** (15.3 g, 63%). The obtained crude **10** (3.0 g, 5.6 mmol) was recrystallized from *i*-PrOH-EtOAc-heptane to give compound **10** as a white crystalline (2.6 g, 87%). ^{1}H-NMR (300 MHz, DMSO-d_6) δ 0.67–0.99 (6H, m), 1.41–1.61 (2H, m), 1.68–2.42 (5H, m), 2.81–3.81 (20H, m), 4.18–4.40 (3H, m), 7.24–7.43 (2H, m), 7.62–7.79 (2H, m), 8.26–9.88 (2H, m). ^{13}C-NMR (151 MHz, DMSO-d_6, the minor rotamer's signals are omitted) δ 20.6, 20.7, 26.8, 27.1, 27.9, 31.6, 35.5, 42.1, 44.1, 44.4, 45.1, 45.9, 48.9, 52.9, 58.4, 66.5, 66.5, 71.8, 111.6, 120.4, 123.2, 124.2, 135.0, 141.8, 146.2, 163.0, 169.3. Anal. Calcd for $C_{27}H_{41}N_5O_4$·HCl: C, 60.49; H, 7.90; N, 13.06. Found: C, 60.39; H, 7.89; N, 13.03. mp 158 °C. $[\alpha]^{25}_{D}$ +27.8º (c 1.0055, MeOH).

2. Calculation of in silico properties.

Values for clogD (pH 7.4, 25 ºC) were calculated using Advanced Chemistry Development (ACD/Labs) Software (ACD/Labs Software version 12, Advanced Chemistry Development, Inc., Toronto, Ontario, Canada; http://www.acdlabs.com/products/percepta/predictors/logd/). Values for TPSA were calculated using Daylight software (Daylight Software, version 4.82, Daylight Chemical Information Systems, Inc., Aliso Viejo, CA; http://www.daylight.com.)

3. Procedures for biological tests

Purification of recombinant human renin

Human preprorenin was expressed using the FreeStyle 293 Expression System (Invitrogen). The recombinant prorenin was exported by FreeStyle 293 cells into the tissue culture media. The cell culture supernatants were processed by filtration, concentration and dialysis in 20 mM Tris-HCl buffer (pH 8.0). Prorenin was purified using Resource Q column (GE Healthcare) and HiLoad 16/60 Superdex 200pg (GE Healthcare). Prorenin was activated to renin by trypsin digestion. Renin was purified using TSKgel DEAE-5PW (Tosoh).

Rh-renin inhibition assay (enzyme-linked immunosorbent assay (ELISA)).

The inhibitory potency of the compounds against human renin was determined by the following protocol. In 384−well plates (ABgene), 1 μL of test compound in 100% DMSO was incubated with 14 μL of enzyme (at a final concentration of 40 pM human renin) in buffer (20 mM Phosphate buffer, 1 mM EDTA, pH 7.4, with 0.004% Tween 20) at 37 ºC. After 10 min, 5 μL of recombinant human angiotensinogen was added to a final concentration of 1.5 μM and incubated at 37 ºC for 30 min. The enzymatic reaction was terminated by adding 20 μL of stop solution (1 μM CGP-29287 in diluent buffer (20 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1% BSA, 0.05% Tween20)). The diluent buffer was used for diluting each reagent in the ELISA. Angiotensin I generated during the incubation was

measured by ELISA. Aliquots (10 μL) of the incubates or angiotensin I peptide standards were transferred to 384−well immuno plates which were previously coated with anti-angiotensin I antibody (Peninsula Laboratories) and incubated with 15 μL of 1.6 nM biotin-conjugated angiotensin I (AnaSpec) at room temperature for 1 h. After washing the plates 5 times with wash buffer (0.05% Tween20 in PBS), 25 μL of 100 ng/mL streptavidin-HRP (Pierce) was incubated at room temperature for 30 min. After washing, 25 μL of substrate of HRP (Pierce) was added and chemiluminescence was detected using a microplate reader. The raw data for the specifically bound counts were normalized between 0% and 100% activity and nonlinear fitted to a sigmoidal equation to calculate the IC_{50} and its 95% confidence interval (CI) using PRISM 3.0 (GraphPad Software Inc.).

Human plasma renin activity (hPRA) assay.

The inhibitory effect of each compound on the human plasma renin activity was tested using the radioimmunoassay kit (Fujirebio Inc., Tokyo, Japan. http://www.info.pmda.go.jp/tgo/pack/20500AMZ00702000_A_02_01/20500AMZ00702000_A_02_01?view=body). IC_{50} values were calculated from concentration-response curves with SAS software (SAS Institute Japan Ltd., Tokyo, Japan). The raw data for the specifically bound counts were normalized between 0% and 100% activity and nonlinear fitted to a sigmoidal equation to calculate the IC_{50} and its 95% confidence interval (CI) using PRISM 3.0 (GraphPad Software Inc.).

CYP inhibition and time-dependent inactivation of CYP activity

Compound **10** had no obvious issues about CYP inhibition (3A, 2C9, 2D6) and time-dependent inactivation of CYP3A activity.

Animal care

All animal protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Shonan Research Center, Takeda Pharmaceutical Company Limited.

Pharmacokinetic analysis in rat cassette dosing.

Test compounds were administered intravenously (0.1 mg/kg) or orally (1 mg/kg) by cassette dosing to non-fasted mice rats. After administration, blood samples were collected and centrifuged to obtain the plasma fraction. The plasma samples were deproteinized followed by centrifugation. The compound concentrations in the supernatant were measured by LC/MS/MS.

Plasma concentrations after administration of TAK-272 to rats.

For oral administration, TAK-272 was suspended in a 0.5% aqueous solution of methylcellulose. The suspension of TAK-272 was administered orally at a dose of 1 mg/10 mL/kg as TAK-272 free base to Crl:CD(SD) rats (male, 8 weeks old, n=3). For intravenous administration, TAK-272 was

dissolved in *N*,*N*-Dimethylacetamide/saline (1:3, v/v). The solution of TAK-272 was administered intravenously at a dose of 0.2 mg/mL/kg as TAK-272 free base to rats. The blood was collected from the tail vein of the rats into heparinized pipettes at 5, 10, 15, 30 min, 1, 2, 3, 4, 6, 8, 24, 32 and 48 h after oral and intravenous administrations. After collection, the blood was kept under ice-chilled conditions and then centrifuged at 4 °C to obtain plasma. The concentrations of TAK-272 free base in the plasma were determined by high-performance liquid chromatography/tandem mass spectrometry (LC-MS/MS) method. To plasma samples, the internal standard (TAK-272-d_8) solution and methanol was added and mixed with a vortex mixer, and then centrifuged at 4 °C. To the supernatants, 10 mmol/L ammonium acetate/acetic acid solution (100:0.3, v/v) was added and mixed with a vortex mixer. The mixtures were filtered through a Centricut ultra-mini (W-MO, 0.45 μm, Kurabo, Osaka, Japan) by centrifugation at 4 °C. The processed solutions were injected into the LC-MS/MS system. The column was a CAPCELL PAK C_{18} AQ (2.0 mm I.D. × 10 mm, particle size 3 μm, Shiseido, Tokyo, Japan). The mobile phase (A) (MP(A)) was a mixture of 10 mmol/L ammonium acetate and acetic acid solution (100:0.3, v/v) and the mobile phase (B) (MP(B)) was methanol. The column temperature and the flow-rate were 40 °C and 0.2 mL/min, respectively. The time program for the gradient elution was as follows: the concentration of MP(B) was held at 55% for 4.3 minutes, and then increased to 95% for 4.5 min. Detection was performed by API4000 (AB SCIEX, MA, USA) with a lower limit of quantification of 1 ng/mL.

Measurement of blood pressure.

Five week-old male human angiotensinogen and renin double transgenic (hAOGEN-hREN dTg) rats were used. Systolic blood pressure (SBP) was measured by the tail-cuff method (BP-98A, Softron, Tokyo, Japan) before administration, and at 5 and 24 h after oral administration of TAK-272, aliskiren or vehicle. TAK-272 and aliskiren were dissolved in 0.5% methylcellulose solution.

4. X-ray co-crystallography of inhibitors with human renin.

Crystallization of mature human renin (1-340) was carried out by the sitting drop vapor diffusion method (Nanovolume CrystallizationTM methods[4]). Conditions for the reservoir solution were 19.95%−40.95% PEG600, 100 mM citric acid buffer pH 4.5–6.0, or 24%−45% PEG600, citric acid buffer pH 4.5–6.0, 50 mM NaH_2PO_4 aqueous solution. A mixture of the reservoir solution and a solution of human renin (ca. 6 mg/mL in 25 mM Tris pH 7.9 and 150 mM NaCl aqueous solution) was left at 20 ºC until crystals of apoprotein were generated. Cocrystals of inhibitors and human renin were prepared by a soaking method. The apoprotein crystals were soaked into a soaking buffer, which was prepared by adding inhibitors to the reservoir solution to 1 – 10 mM, for 30 min to 1 day. The crystals thus obtained were soaked into the soaking buffer, to which was added ethylene glycol

to 0–12%, and the mixture was frozen. X-ray diffraction analysis was carried out by using beamline 5.0.3 at the Advanced Light Source (ALS). The data obtained was processed by HKL2000[5] to generate initial complex structures, addressing molecular replacement method with MOLREP of CCP4 (Ver. 4.0). Structure refinements of the models, which were generated by Xfit[6] based on the initial complex structures, were carried out by using REFMAC.[7]

Table 1. Data reduction and refinement statistics for the X-ray structures of the compounds complexed with renin.

Data Collection		
Compound	**1**	**8**
PDB code	5KOQ	5KOS
Beamline	ALS 5.0.3	ALS 5.0.3
Wavelength (Å)	1	1
Space group	$P2_13$	$P2_13$
Unit cell dimensions (Å)	a=b=c=138.6 α=β=γ=90°	a=b=c=137.1 α=β=γ=90°
Resolution (Å)	2.7	2.4
Unique reflections	24655	33577
Redundancy	5.9	6.1
Completeness (%)	100 (100)	99.8 (100)
I/σ(I)	20.2 (2.4)	15.9 (2.3)
R_{sym}[a]	0.071 (0.721)	0.099 (0.769)
Refinement		
Molecules in assymteric unit	2	2
Reflections used	23173	31736
RMS Bonds (Å)	0.009	0.007
RMS Angles (°)	1.25	1.28
Average B value ($Å^2$)	66.2	47.9
R-value[b]	0.206	0.187
R_{free}[b]	0.252	0.23

[a]Rsym = ΣhΣj |<I(h)> - I(h)j | / ΣhΣj <I(h)>, where <I(h)> is the mean intensity of symmetry-related reflections. [b]R-value = Σ | |Fobs| - |Fcalc| | / Σ |Fobs|. Rfree for 5% of reflections excluded from refinement. Values in parentheses are for the highest resolution shell.

ABBREVIATIONS WSC, (1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide; HOBt, 1-hydroxybenzotriazole hydrate; EtOH, ethanol; DIEA, N,N-diisopropylethylamine; *i*-PrOH, isopropanol; AcOH, acetic acid; MeOH, methanol; EtOAc, ethyl acetate; Et_3N, triethylamine; ELISA, enzyme-linked immunosorbent assay; hPRA, human plasma renin activity; TFA, trifluoroacetic acid; DPPA, diphenylphosphoryl azide; BOP, 1*H*-benzotriazol-1-yloxytris(dimethylamino)phosphonium hexafluorophosphate

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