Supporting Information

Discovery of JTZ-951: A HIF Prolyl Hydroxylase Inhibitor for the Treatment of Renal Anemia

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Contents

1. Abbreviations

The following abbreviations and definitions have been used:

Ac	Acetyl
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
Bu	Butyl
CDI	1,1'-Carbonyldiimidazole
DMA	*N*,*N*-Dimethylacetamide
DME	1,2-Dimethoxyethane
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethylsulfoxide
dppf	1,1'-Bis(dimethylphosphino)ferrocene
EDC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
Et	Ethyl

HOBt	1-Hydroxybenzotriazole hydrate
IPE	Diisopropyl ether
LDA	Lithium diisopropylamide
LHMDS	Lithium bis(trimethylsilyl)amide
Me	Methyl
Ms	Methanesulfonyl
NMI	1-Methylimidazole
Ph	Phenyl
Py	Pyridine
r.t.	Room temperature
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin-layer chromatography
Ts	*p*-Toluenesulfonyl
Z	Benzyloxycarbonyl
δ	Chemical shift (ppm)
J	Coupling constant
s	Singlet
br s	Broad singlet
d	Doublet
t	Triplet
q	Quartet
m	Multiplet
dd	Doublet of doublets
tt	Triplet of triplets
tq	Triplet of quartets

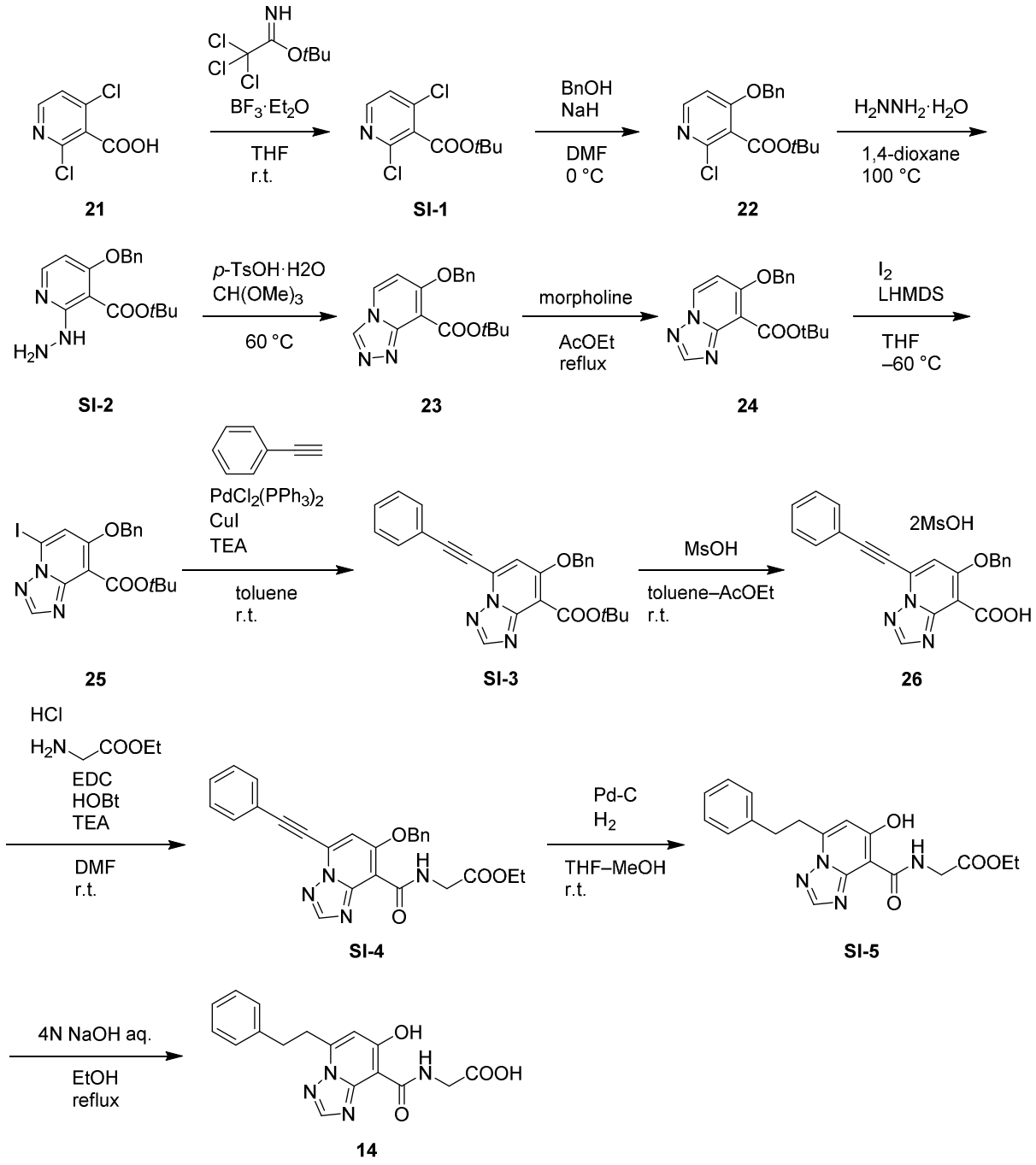
2. Chemistry

General information

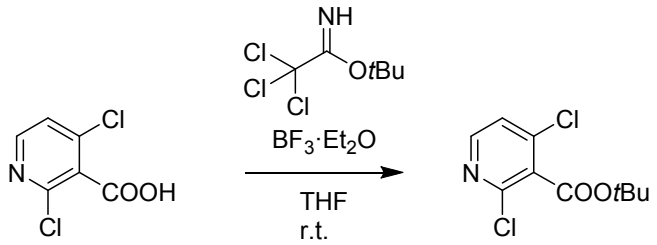
Unless otherwise specified, materials were obtained from commercial suppliers and used without further purification. TLC was performed using silica gel 60 F_{254} plates purchased from Merck. Flash chromatography was performed using pre-packed columns of silica gel (230–400 mesh, 40–63 μm) purchased from SiliCycle or using Silica gel 60N (spherical, neutral, 40–50 mesh) purchased from Kanto Chemical Co., Inc. 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 an Agilent 1290 Infinity LC and a Thermo Fisher LTQ-Orbitrap Velos. The purities of assayed compounds except for **1**, **3**, and **4** were determined by analytical HPLC under the following conditions [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: 1.0 mL/min; detection wavelength: 254 nm]. The purities of assayed compounds **1**, **3**, and **4** were also determined by analytical HPLC under the following conditions [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: 1% B to 90% B from 0 to 5 min, 90% B from 5 to 7 min, 90% B to 1% B from 7 to 7.5 min, 1% B from 7.5 to 10 min; flow rate: 1.0 mL/min; detection wavelength: 254 nm].

Synthesis of 14



***tert*-Butyl 2,4-dichloronicotinate (SI-1)**

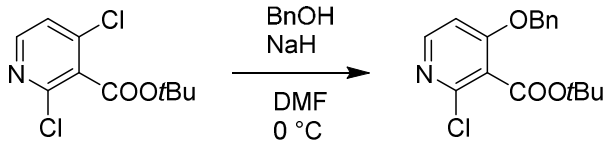


21 **SI-1**

To a solution of 2,4-dichloronicotinic acid (**21**) (234 g, 1.22 mol) in THF (1.2 L) was added $BF_3 \cdot Et_2O$ (8 mL, 0.0636 mmol) at room temperature. The mixture was cooled to 0 °C and then tert-butyl 2,2,2-trichloroacetimidate (361ml, 2.02 mol) was added dropwise under ice-cooling. To this reaction mixture were added saturated $NaHCO_3$ aq. (1.2 L) and water (1.2 L), and the aqueous layer was extracted with AcOEt (1.2 L). The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. *n*-Hexane (1.8 L) was added to the obtained residue. Insoluble material was filtered off, and the filtrate was concentrated under reduced pressure to give the title compound **SI-1** (326 g) as a crude product.

^{1}H NMR (400 MHz, CDCl3) δ: 8.31 (d, J = 5.3 Hz, 1H), 7.31 (d, J = 5.3 Hz, 1H), 1.63 (s, 9H).

***tert*-Butyl 4-(benzyloxy)-2-chloronicotinate (22)**

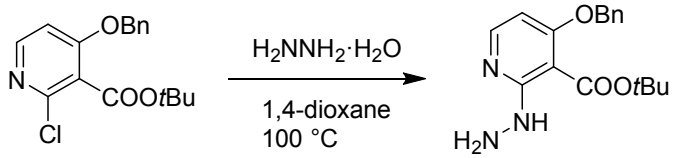


SI-1 **22**

Under a nitrogen stream, to a solution of NaH (60% oil suspension) (58 g, 1.45 mol) in DMF (1 L) was added a solution of **SI-1** (326 g) in DMF (0.3 L) at 0 °C. To this mixture was then added a solution of benzyl alcohol (136 mL, 1.32 mol) in DMF (0.2 L). After stirring under ice-cooling for 30 min, water (3 L) was added and the precipitated solid was filtered, and the filtrate was dried under reduced pressure at 50 °C. The solid was purified by column chromatography (*n*-hexane/AcOEt = 10/1–AcOEt alone). The obtained solid was further slurried in *n*-hexane to give the title compound **22** (334 g, 83% yield for 2 steps).

^{1}H NMR (400 MHz, CDCl3) δ: 8.24 (d, J = 6.0 Hz, 1H), 7.42–7.32 (m, 5H), 6.83 (d, J = 6.0 Hz, 1H), 5.17 (s, 2H), 1.55 (s, 9H).

***tert*-Butyl 4-(benzyloxy)-2-hydrazinylnicotinate (SI-2)**

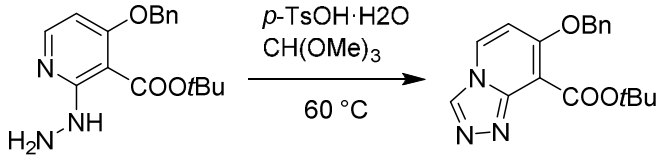


22 **SI-2**

To a solution of **22** (167 g, 0.522 mol) in dioxane (1.2 L) was added hydrazine monohydrate (127 mL, 2.62 mol). The mixture was warmed to 100 °C and then stirred for 17 h. After cooling to room temperature, AcOEt (1.7 L) was added, and the mixture was washed with saturated $NaHCO_3$ aq./water = 1/1 three times. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. By performing the operation twice, the title compound **SI-2** (266 g) was obtained as a crude product.

^{1}H NMR (400 MHz, CDCl3) δ: 8.08 (d, J = 6.0 Hz, 1H), 7.96 (br s, 1H), 7.45–7.33 (m, 5H), 6.32 (d, J = 6.0 Hz, 1H), 5.09 (s, 2H), 3.98 (br s, 2H), 1.42 (s, 9H).

***tert*-Butyl 7-(benzyloxy)-[1,2,4]triazolo[4,3-*a*]pyridine-8-carboxylate (23)**

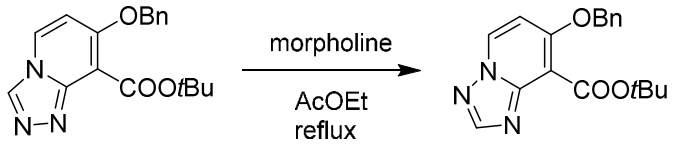


SI-2 **23**

To a mixture of **SI-2** (266 g) and trimethyl orthoformate (1 L) was added *p*-toluenesulfonic acid monohydrate (80 g, 0.421 mol). After stirring at 60 °C for 1 h, the reaction mixture was concentrated under reduced pressure. The obtained residue was slurried successively in *n*-hexane/AcOEt = 2/1 and saturated $NaHCO_3$ aq./water = 1/1 to give the title compound **23** (209 g, 76% yield for 2 steps).

^{1}H NMR (400 MHz, DMSO-D6) δ: 9.13 (s, 1H), 8.62 (d, J = 7.7 Hz, 1H), 7.49–7.32 (m, 5H), 7.22 (d, J = 7.7 Hz, 1H), 5.36 (s, 2H), 1.49 (s, 9H).

***tert*-Butyl 7-(benzyloxy)-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxylate (24)**

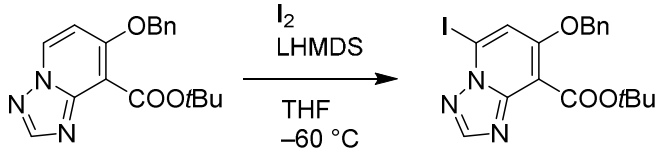


23 **24**

To a solution of **23** (200 g, 0.615 mol) in AcOEt (0.6 L) was added morpholine (160 mL, 1.84 mol). After stirring under reflux for 3 h, the reaction mixture was cooled to room temperature, and water (0.6 L) was added. The aqueous layer was extracted with AcOEt (0.4 L), the organic layers were combined and washed successively with 5% $KHSO_4$ aq. (0.6 L) and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the title compound **24** (194 g, 97% yield).

^{1}H NMR (400 MHz, CDCl3) δ: 8.50 (d, J = 7.7 Hz, 1H), 8.29 (s, 1H), 7.46–7.35 (m, 5H), 6.85 (d, J = 7.7 Hz, 1H), 5.28 (s, 2H), 1.59 (s, 9H).

***tert*-Butyl 7-(benzyloxy)-5-iodo-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxylate (25)**

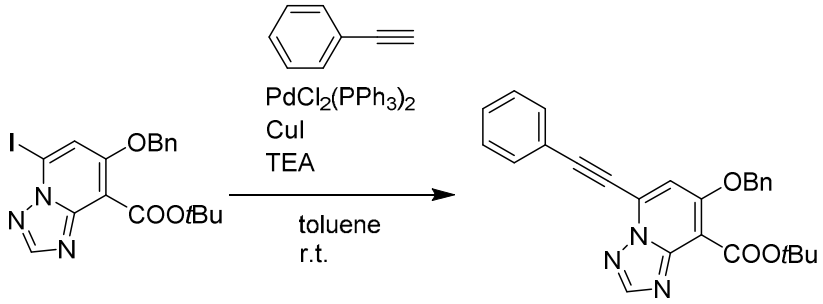


24 **25**

Under a nitrogen stream, to a solution of **24** (194 g, 0.596 mol) in THF (0.6 L) was added dropwise a solution of iodine (151 g, 0.595 mol) in THF (0.5 L) under cooling in a dry ice/*n*-hexane bath. To this mixture was added dropwise 1.6 M LHMDS in THF (788 mL, 1.26 mol) while maintaining a temperature of not less than −60 °C. After stirring under cooling in a dry ice/*n*-hexane bath for 2 h, 4N HCl in AcOEt (315 mL, 1.26 mol) was added dropwise while maintaining a temperature of not less than −60 °C. To this reaction mixture were added Na_2SO_3 (76 g, 0.603 mol), saturated NH_4Cl aq. (1 L), water (0.8 L) and *n*-hexane/AcOEt=1/1 (1 L). The organic layer was washed successively with saturated $NaHCO_3$ aq. (0.5 L) and brine (0.8 L), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the title compound **25** (188 g, 70% yield).

^{1}H NMR (400 MHz, DMSO-D6) δ: 8.43 (s, 1H), 7.87 (s, 1H), 7.49–7.33 (m, 5H), 5.39 (s, 2H), 1.46 (s, 9H).

***tert*-Butyl 7-(benzyloxy)-5-(phenylethynyl)-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxylate (SI-3)**

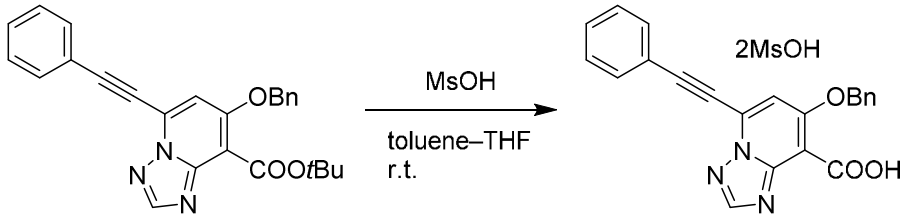


25 **SI-3**

To a solution of **25** (5.00 g, 11.1 mmol) and phenylacetylene (1.34 mL, 12.2 mmol) in toluene (35 mL) were successively added bis(triphenylphosphine)palladium dichloride (0.233 g, 0.332 mmol), copper(I) iodide (0.063g, 0.331 mmol) and triethylamine (1.85 mL, 13.3 mmol) under ice-cooling. After stirring at room temperature for 2 h, 5% NH_3 aq. (35 mL) was added to the reaction mixture. The organic layer was further washed successively with 5% NH_3 aq., saturated NH_4Cl aq. and brine, and dried over $MgSO_4$. After filtration, the filtrate was concentrated under reduced pressure, and the obtained residue was purified by column chromatography (*n*-hexane/AcOEt = 3/1–1/1). The obtained compound was slurried in *n*-hexane to give the title compound **SI-3** (3.84 g, 82% yield).

^{1}H NMR (400 MHz, DMSO-D6) δ: 8.49 (s, 1H), 7.78 (s, 1H), 7.74–7.70 (m, 2H), 7.60–7.51 (m, 3H), 7.50–7.46 (m, 2H), 7.45–7.40 (m, 2H), 7.39–7.33 (m, 1H), 5.42 (s, 2H), 1.48 (s, 9H).

7-(Benzyloxy)-5-(phenylethynyl)-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxylic acid dimethanesulfonate (26)

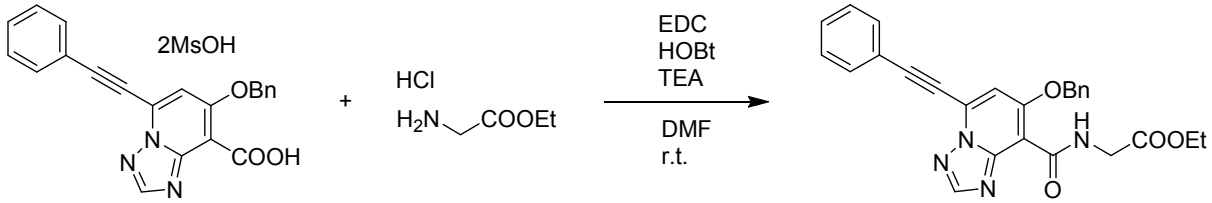


SI-3 **26**

To a mixture of **SI-3** (3.84 g, 9.02 mmol), toluene (29 mL) and AcOEt (9.5 mL) was added dropwise a solution of methanesulfonic acid (2.34 mL) in AcOEt (2.34 mL) over 10 min at room temperature. After stirring at room temperature for 3 h, AcOEt (9.5 mL) was added to the reaction mixture, and the solid was collected by filtration. The solid and DMF (30 mL) were mixed, and water (50 mL) was added dropwise over 10 min at 0 °C. The precipitated solid was collected by filtration to give the title compound **26** (3.20 g, 96% yield).

^{1}H NMR (400 MHz, DMSO-D6) δ: 8.67 (s, 1H), 7.86 (s, 1H), 7.76–7.71 (m, 2H), 7.62–7.53 (m, 3H), 7.52–7.48 (m, 2H), 7.46–7.40 (m, 2H), 7.39–7.34 (m, 1H), 5.48 (s, 2H), 2.38 (s, 6H).

Ethyl (7-(benzyloxy)-5-(phenylethynyl)-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonyl)glycinate (SI-4)

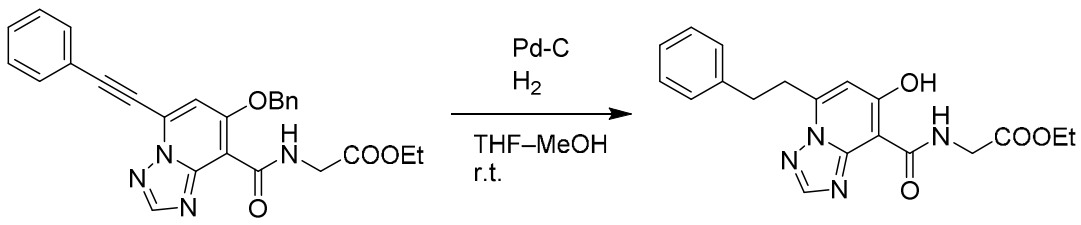


26 **SI-4**

To a solution of **26** (3.20 g, 8.66 mmol), glycine ethyl ester hydrochloride (1.33 g, 9.52 mmol) and 1-hydroxybenzotriazole hydrate (1.46 g, 9.52 mmol) in DMF (20 mL) were added successively triethylamine (2.66 mL, 19.0 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (1.83 g, 9.52 mmol) at room temperature. After stirring at room temperature for 1 h, water (20 mL) and saturated NaHCO3 aq. (20 mL) were added. The precipitated solid was collected by filtration to give the title compound **SI-4** (3.38 g, 81% yield).

^{1}H NMR (400 MHz, DMSO-d6) δ: 9.17 (t, J = 5.8 Hz, 1H), 8.51 (s, 1H), 7.74 (s, 1H), 7.73–7.70 (m, 2H), 7.58–7.51 (m, 5H), 7.43–7.38 (m, 2H), 7.36–7.31 (m, 1H), 5.43 (s, 2H), 4.12 (q, J = 7.2 Hz, 2H), 4.09 (d, J = 5.8 Hz, 2H), 1.20 (t, J = 7.2 Hz, 3H).

Ethyl (7-hydroxy-5-phenethyl-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonyl)glycinate (SI-5)

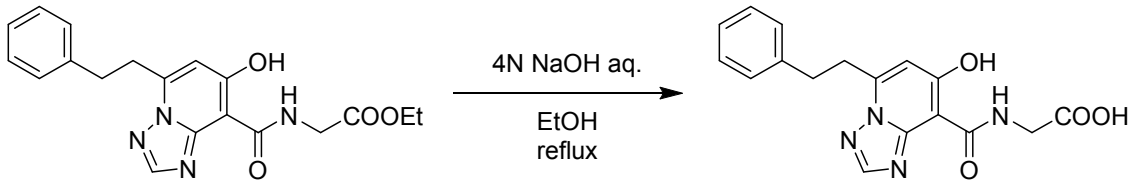


SI-4 **SI-5**

To a solution of **SI-4** (3.38 g, 7.44 mmol) in THF/MeOH = 2/1 (51 mL) was added 5% palladium carbon (0.34 g), and the mixture was stirred under a hydrogen atmosphere and normal pressure for 4 h. The reaction mixture was filtered through celite, and concentrated under reduced pressure. The obtained residue was purified by column chromatography ($CHCl_3$/MeOH = 20/0–20/1) and slurried in *n*-hexane/IPE = 1/1 to give the title compound **SI-5** (2.29 g, 83% yield).

^{1}H NMR (400 MHz, DMSO-D6) δ: 14.12 (s, 1H), 9.87 (t, J = 5.7 Hz, 1H), 8.58 (s, 1H), 7.32–7.18 (m, 5H), 6.82 (s, 1H), 4.29 (d, J = 5.7 Hz, 2H), 4.17 (q, J = 7.2 Hz, 2H), 3.41 (t, J = 7.8 Hz, 2H), 3.12 (t, J = 7.8 Hz, 2H), 1.23 (t, J = 7.2 Hz, 3H).

(7-Hydroxy-5-phenethyl-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonyl)glycine (14)



SI-5 **14**

To a solution of **SI-5** (2.28 g, 6.19 mmol) in EtOH (9.1 mL) was added 2N NaOH aq. (12.4 mL, 24.8 mmol) at room temperature. After stirring at 90 °C for 2 h, 6N HCl aq. (4.1 mL, 24.6 mmol). This was allowed to gradually cool with stirring and crystals were precipitated. The crystals were collected by filtration to give the title compound **14** (2.16 g, 103% yield).

^{1}H NMR (400 MHz, DMSO-D6) δ: 14.22 (s, 1H), 12.98 (br s, 1H), 9.84 (t, J = 5.6 Hz, 1H), 8.58 (s, 1H), 7.33–7.18 (m, 5H), 6.80 (s, 1H), 4.22 (d, J = 5.6 Hz, 2H), 3.40 (t, J = 7.7 Hz, 2H), 3.12 (t, J = 7.7 Hz, 2H).

^{13}C NMR (126 MHz, CDCl3) δ: 170.28, 167.70, 165.32, 152.95, 148.53, 146.49, 140.05, 128.33, 128.20, 126.17, 106.72, 95.56, 41.00, 31.95, 31.72.

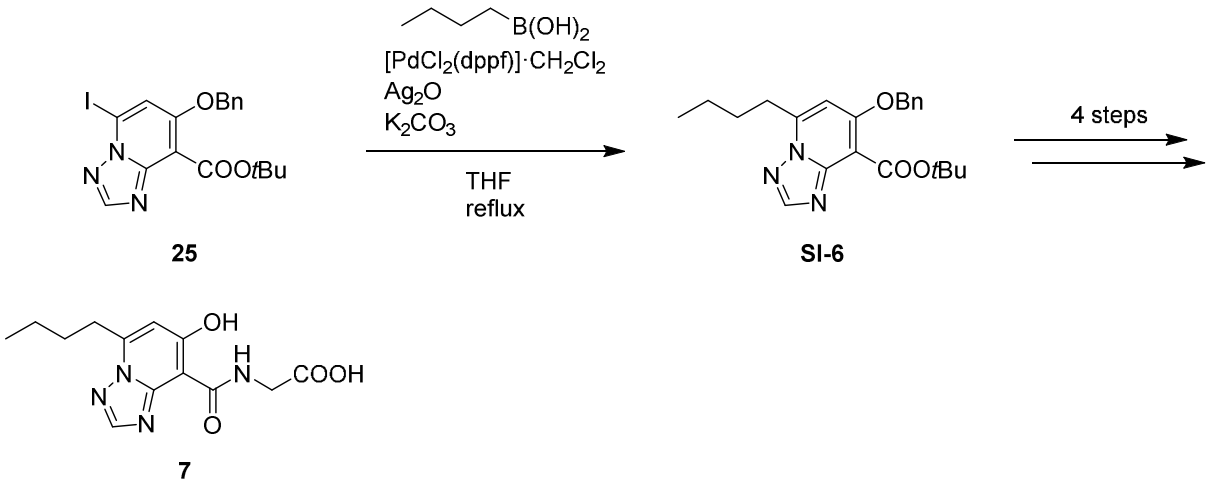
HRMS m/z: $[M+H]^+$ calcd for C17H17N4O4, 341.1244; found, 341.1243.

Anal. (C17H16N4O4) calcd C 59.99%, H 4.74%, N 16.46%; found C 60.02%, H, 4.78%, N, 16.42%.

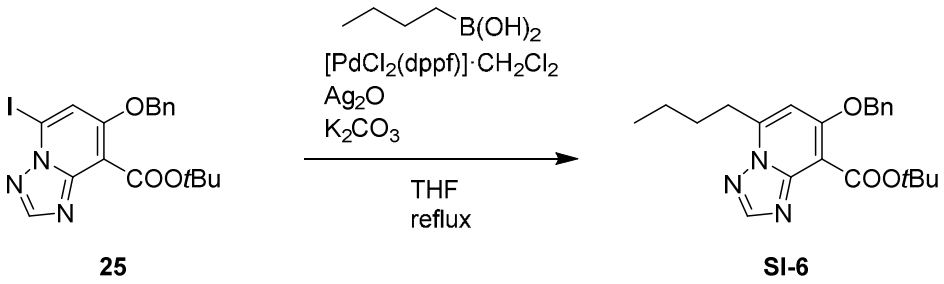
Melting point: 186 °C

Purity: 100.0%.

Synthesis of inhibitor 7



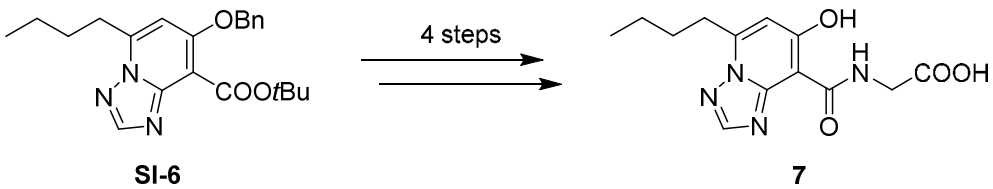
***tert*-Butyl 7-(benzyloxy)-5-butyl-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxylate (SI-6)**



Compound **25** (200 mg, 0.443 mmol), butylboronic acid (50 mg, 0.490 mmol), [1,1'-bis(diphenylphosphino)ferrocene]palladium(II) dichloride dichloromethane complex (1:1) (11.0 mg, 0.0135 mmol), silver(I) oxide (123 mg, 0.530 mmol), potassium carbonate (153 mg, 1.11 mmol) and THF (1.6 mL) were mixed, and the mixture was stirred at 80 °C for 40 h. The reaction mixture was filtered through celite, and concentrated under reduced pressure. The obtained residue was purified by column chromatography (*n*-hexane/AcOEt = 8/2–6/4) to give the title compound **SI-6** (130 mg, 77% yield).

^{1}H NMR (400 MHz, DMSO-D6) δ: 8.38 (s, 1H), 7.51–7.45 (m, 2H), 7.44–7.38 (m, 2H), 7.38–7.32 (m, 1H), 7.23 (s, 1H), 5.38 (s, 2H), 3.11 (t, J = 7.5 Hz, 2H), 1.75 (tt, J = 7.5, 7.3 Hz, 2H), 1.48 (s, 9H), 1.34 (tq, J = 7.5, 7.3 Hz, 2H), 0.91 (t, J = 7.5 Hz, 3H).

(5-butyl-7-hydroxy-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonyl)glycine (7)



Compound **7** was prepared by a method similar to the synthesis of **14**.

^{1}H NMR (400 MHz, DMSO-D6) δ: 14.24 (s, 1H), 12.94 (s, 1H), 9.83 (t, J = 5.3 Hz, 1H), 8.54 (s, 1H), 6.84 (s, 1H), 4.21 (d, J = 5.3 Hz, 2H), 3.10 (t, J = 7.6 Hz, 2H), 1.76 (tt, J = 7.6, 7.5 Hz, 2H), 1.39 (tq, J = 7.3, 7.5 Hz, 2H), 0.93 (t, J = 7.3 Hz, 3H).

^{13}C NMR (500 MHz, DMSO-D6) δ: 170.30, 167.76, 165.45, 152.85, 148.57, 147.54, 106.28, 95.45, 41.02, 29.95, 28.09, 21.65, 13.50.

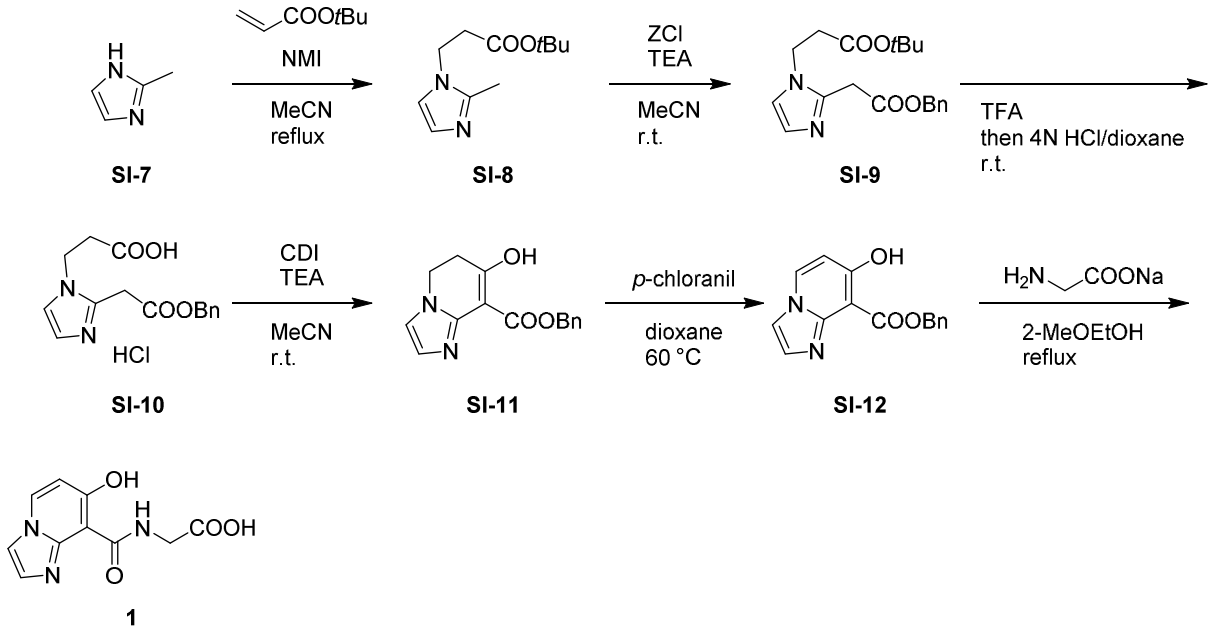
HRMS m/z: $[M+H]^+$ calcd for C13H17N4O4, 293.1244; found, 293.1239.

Anal. (C13H16N4O4) calcd C 53.42%, H 5.52%, N 19.17%; found C 53.35%, H, 5.53%, N, 19.21%.

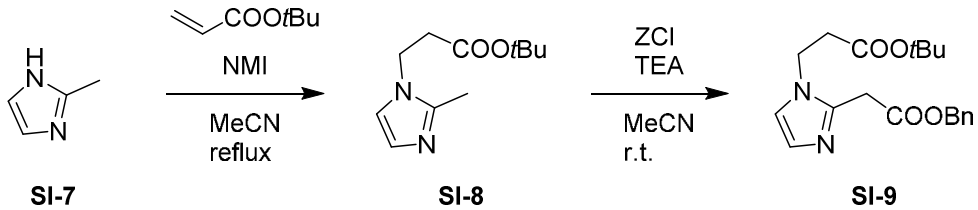
Melting point: 162 °C

Purity: 100.0%.

Synthesis of inhibitor 1



***tert*-Butyl 3-(2-(2-(benzyloxy)-2-oxoethyl)-1H-imidazol-1-yl)propanoate (SI-9)**

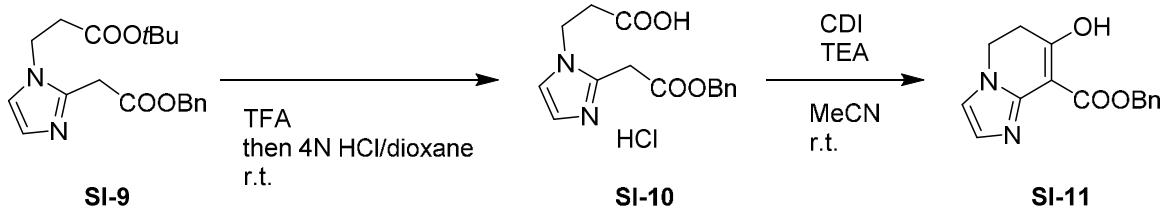


To a solution of **SI-7** (2.00g, 24.4 mmol) in MeCN (20 mL) were added successively *tert*-butyl acrylate (7.1 mL, 48.7 mmol) and 1-methylimidazole (0.19 mL, 2.40 mmol). After stirring at 100 °C for 6 h, the reaction mixture was concentrated under reduced pressure. To the obtained residue were added brine and AcOEt, the aqueous layer was extracted with AcOEt, the organic layers were combined and dried over Na_2SO_4. After filtration and removal of the solvent under reduced pressure, crude **SI-8** (5.32 g) was obtained as a yellow oil. To a solution of crude

SI-8 (3.32 g (92% purity), 14.5 mmol) in MeCN (33 mL) was added TEA (5.95 mL, 42.7 mmol). The mixture was cooled to 0 °C and then benzyl chloroformate (5.55 mL, 39.4 mmol) was added dropwise under ice-cooling. The mixture was gradually warmed to room temperature and stirred overnight. To this reaction mixture were added water and brine, and the resulting mixture was extracted with AcOEt. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The obtained residue was purified by column chromatography (*n*-hexane/AcOEt = 3/1–1/9) to give the title compound **SI-9** (1.97 g, 39% yield for 2 steps).

^{1}H NMR (400 MHz, CDCl3) δ: 7.39–7.30 (m, 5H), 6.99 (d, J = 1.4 Hz, 1H), 6.93 (d, J = 1.4 Hz, 1H), 5.17 (s, 2H), 4.13 (t, J = 6.7 Hz, 2H), 3.92 (s, 2H), 2.64 (t, J = 6.7 Hz, 2H), 1.42 (s, 9H).

Benzyl 7-hydroxy-5,6-dihydroimidazo[1,2-*a*]pyridine-8-carboxylate (SI-11)

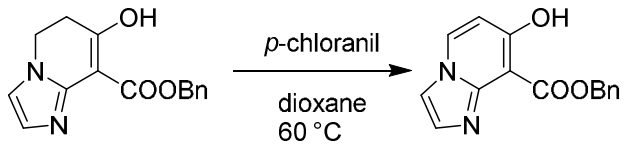


SI-9 **SI-10** **SI-11**

SI-9 (1.97 g, 5.72 mmol) and TFA (10 mL) were mixed under ice-cooling. After stirring at room temperature for 1 h, the reaction mixture was concentrated under reduced pressure. To the obtained residue was added 4N HCl in dioxane, and the solvent was removed under reduced pressure, then crude **SI-10** was ready for the next reaction. To a solution of crude **SI-10** in MeCN (20 mL) were added successively TEA (40 mL) and 1,1'-carbonyldiimidazole (1.39 g, 8.59 mmol) under ice-cooling. The mixture was gradually warmed to room temperature and stirred overnight. After the reaction mixture was concentrated under reduced pressure, and the obtained residue was purified by column chromatography (*n*-hexane/AcOEt = 1/1–AcOEt alone then $CHCl_3$/MeOH = 95/5–85/15). To the obtained compound were added water and $CHCl_3$, the aqueous layer was extracted with $CHCl_3$. The organic layers were combined and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The obtained residue was purified by preparative TLC ($CHCl_3$/MeOH = 9/1) to give the title compound **SI-11** (580 mg, 37% yield for 2 steps).

^{1}H NMR (400 MHz, CDCl3) δ: 11.07 (br s, 1H), 7.54–7.50 (m, 2H), 7.37–7.32 (m, 2H), 7.28–7.23 (m, 1H), 6.79 (d, J = 2.2 Hz, 1H), 6.64 (d, J = 2.2 Hz, 1H), 5.36 (s, 2H), 4.05 (t, J = 7.3 Hz, 2H), 2.76 (t, J = 7.3 Hz, 2H).

Benzyl 7-hydroxyimidazo[1,2-*a*]pyridine-8-carboxylate (SI-12)



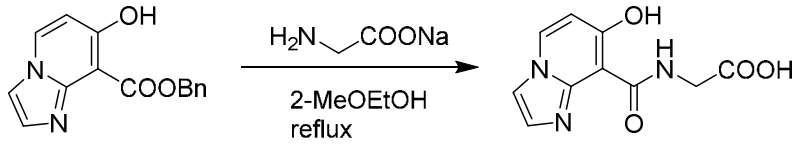
SI-11 **SI-12**

To a solution of **SI-11** (250 mg, 0.926 mmol) in dioxane (5 mL) was added *p*-chloranil (342 mg, 1.39 mmol). After stirring under an argon atmosphere at 60 °C for 2 h, the mixture was cooled to room temperature. The reaction mixture was filtered through celite, and concentrated under reduced pressure. The obtained residue was

purified twice by preparative TLC ($CHCl_3$/MeOH/acetone = 8/1/1) to give the title compound **SI-12** (126 mg, 51% yield).

^{1}H NMR (400 MHz, MeOH-D4) δ: 8.05 (d, J = 7.7 Hz, 1H), 7.52–7.47 (m, 2H), 7.50 (s, 1H), 7.35–7.30 (m, 2H), 7.32 (s, 1H), 7.29–7.23 (m, 1H), 6.50 (d, J = 7.7 Hz, 1H), 5.42 (s, 2H).

(7-Hydroxyimidazo[1,2-*a*]pyridine-8-carbonyl)glycine (1)



SI-12 **1**

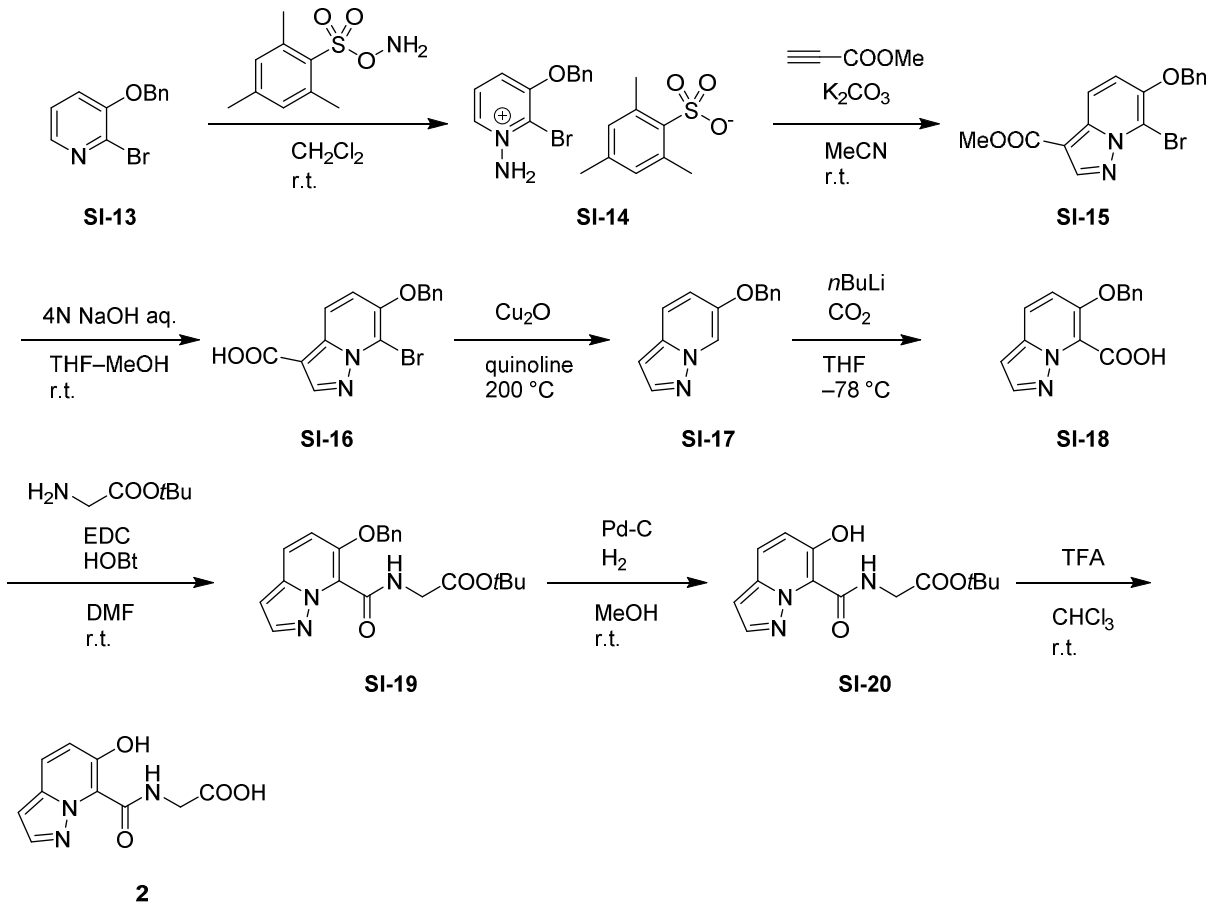
To a solution of **SI-12** (30 mg, 0.112 mmol) in 2-MeOEtOH (1 mL) was added glycine sodium salt (54 mg, 0.560 mmol). After stirring at 130 °C for 20 min, the reaction mixture was acidified with 2N HCl aq. (0.308 mL, 0.616 mmol) and concentrated under reduced pressure. The obtained residue was slurried in water to give the title compound **1** (6.4 mg, 24% yield).

^{1}H NMR (400 MHz, DMSO-d6) δ: 11.97 (s, 1H), 10.76 (t, J = 5.6 Hz, 1H), 8.10 (d, J = 7.7 Hz, 1H), 7.58 (d, J = 4.4 Hz, 1H), 7.28 (d, J = 4.4 Hz, 1H), 6.40 (d, J = 7.7 Hz, 1H), 4.03 (d, J = 5.6 Hz, 2H).

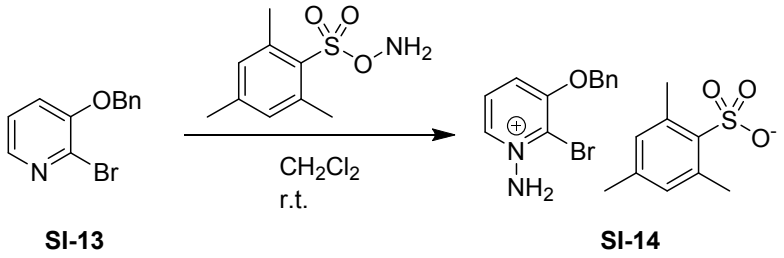
HRMS m/z: $[M-H]^-$ calcd for C10H8N3O4, 234.0520; found, 234.0519.

Purity: 95.7%.

Synthesis of inhibitor 2



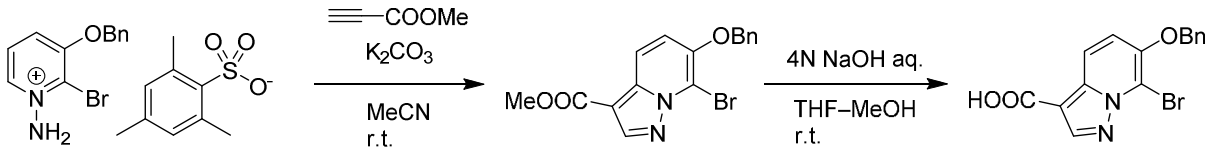
1-Amino-3-(benzyloxy)-2-bromopyridin-1-ium 2,4,6-trimethylbenzenesulfonate (SI-14)



To a solution of **SI-13** (5.00 g, 18.9 mmol) in CH_2Cl_2 (5 mL) was added dropwise a freshly prepared solution of *O*-(mesitylsulfonyl)hydroxylamineglycine (22.8 mmol) in CH_2Cl_2 under ice-cooling.[1–4] After stirring at room temperature for 1 h, diethyl ether (35 mL) was added. The solid was collected by filtration to give the title compound **SI-14** (5.36 g, 59% yield).

^{1}H NMR (400 MHz, DMSO-D6) δ: 8.63 (dd, J = 6.2, 1.2 Hz, 1H), 8.48 (s, 2H), 8.05 (dd, J = 8.7, 1.2 Hz, 1H), 7.93 (dd, J = 8.7, 6.2 Hz, 1H), 7.52–7.37 (m, 5H), 6.73 (s, 2H), 5.44 (s, 2H), 2.50 (s, 6H), 2.16 (s, 3H).

6-(Benzyloxy)-7-bromopyrazolo[1,5-*a*]pyridine-3-carboxylic acid (SI-16)

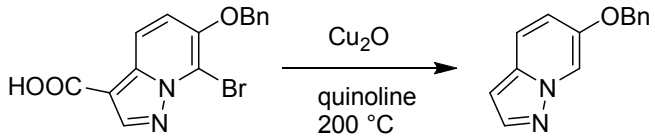


SI-14 **SI-15** **SI-16**

To a solution of **SI-14** (4.91 g, 10.2 mmol) in MeCN was added potassium carbonate (1.70 g, 12.3 mmol) under ice-cooling. After stirring under ice-cooling for 10 min, methyl propiolate (1.40 mL, 15.7 mmol) was added to the mixture. The mixture was gradually warmed to room temperature and stirred at room temperature overnight, then water (50 mL) and AcOEt (50 mL) were poured into the reaction mixture under ice-cooling. The aqueous layer was extracted twice with AcOEt (25 mL), the organic layers were combined and washed with brine (30 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The obtained residue was purified by column chromatography (*n*-hexane/AcOEt = 20/1–4/1) and crude **SI-15** (0.959 g) was obtained. To a solution of crude **SI-15** (100 mg, <0.277 mmol) in THF/MeOH = 2/1 (1.5 mL) was added 4N NaOH aq. (0.277 mL, 1.11 mmol) under ice-cooling. The mixture was gradually warmed to room temperature and stirred at room temperature overnight, then the mixture was acidified with 1N HCl aq. (1.20 mL, 1.20 mmol) under ice-cooling. Water (1 mL) was added and the precipitated solid was filtered, and the filtrate was dried under reduced pressure to give the title compound **SI-16** (60.6 mg, 16% yield for 2 steps).

^{1}H NMR (400 MHz, DMSO-D6) δ: 8.44 (s, 1H), 8.07 (d, J = 9.7 Hz, 1H), 7.78 (d, J = 9.7 Hz, 1H), 7.53–7.48 (m, 2H), 7.44–7.39 (m, 2H), 7.38–7.33 (m, 1H), 5.33 (s, 2H).

6-(Benzyloxy)pyrazolo[1,5-*a*]pyridine (SI-17)

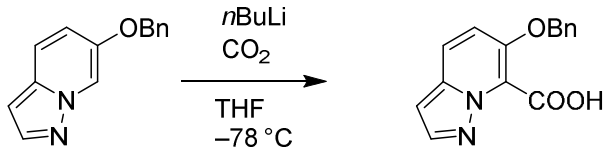


SI-16 **SI-17**

Compound **SI-16** (51.0 mg, 0.147 mmol), copper(I) oxide (22.1 mg, 0.154 mmol), and quinoline (0.25 mL) were mixed in a sealed tube. The mixture was warmed to 200 °C and then stirred for 1 h. After cooling to room temperature, AcOEt (1 mL) and 1N HCl aq. (1 mL) were added. Insoluble material was filtered off, and the filtrate was separated. The organic layer was washed successively with 1N HCl aq. (1 mL) and brine (1 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The obtained residue was purified by preparative TLC (*n*-hexane/AcOEt = 2/1) to give the title compound **SI-17** (19.1 mg, 58% yield).

^{1}H NMR (400 MHz, CDCl3) δ: 8.14 (d, J = 2.2 Hz, 1H), 7.84 (d, J = 2.4 Hz, 1H), 7.47–7.32 (m, 5H), 7.43 (dd, J = 9.5, 0.8 Hz, 1H), 6.99 (dd, J = 9.5, 2.2 Hz, 1H), 6.46 (dd, J = 2.4, 0.8 Hz, 1H), 5.05 (s, 2H).

6-(Benzyloxy)pyrazolo[1,5-*a*]pyridine-7-carboxylic acid (SI-18)

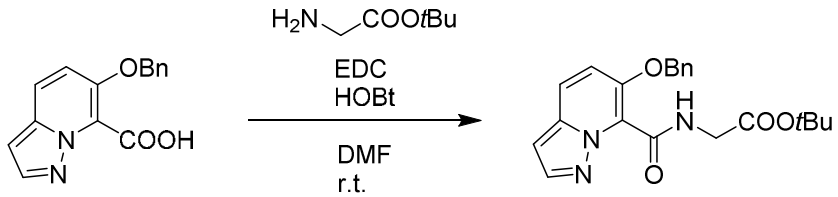


SI-17 **SI-18**

Under an argon stream, to a solution of *n*BuLi (1.66 mol/L in *n*-hexane) (0.0700 mL, 0.116 mmol) in THF (0.7 mL) was added a solution of **SI-17** (23.5 mg, 0.105 mmol) in THF (0.4 mL) at −78 °C. After stirring at −78 °C for 30 min, dry ice was added to the mixture. After stirring at −78 °C for 5 min, 4N HCl in AcOEt (0.0300 mL) was added to the mixture. To the mixture were added successively AcOEt (1 mL), water (1 mL), and 1N HCl aq. (1 mL). The aqueous layer was extracted twice with AcOEt (1 mL), then the organic layers were combined and dried over $MgSO_4$. After filtration, the filtrate was concentrated under reduced pressure to give the title compound **SI-18** (27.8 mg, 99% yield).

^{1}H NMR (400 MHz, DMSO-D6) δ: 11.95 (br s, 1H), 7.95 (d, J = 2.4 Hz, 1H), 7.74 (d, J = 9.7 Hz, 1H), 7.47–7.43 (m, 2H), 7.42–7.36 (m, 2H), 7.40 (d, J = 9.7 Hz, 1H), 7.36–7.30 (m, 1H), 6.65 (d, J = 2.4 Hz, 1H), 5.22 (s, 2H).

***tert*-Butyl (6-(benzyloxy)pyrazolo[1,5-*a*]pyridine-7-carbonyl)glycinate (SI-19)**

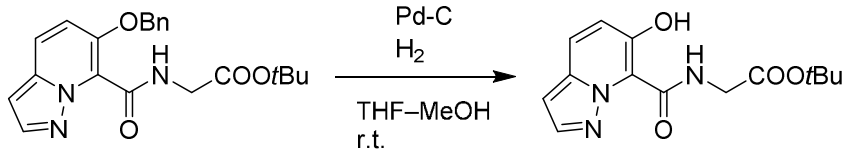


SI-18 **SI-19**

SI-19 was prepared by a method similar to the synthesis of **SI-4**.

^{1}H NMR (400 MHz, CDCl3) δ: 8.90 (t, J = 4.8 Hz, 1H), 7.98 (d, J = 2.4 Hz, 1H), 7.55 (d, J = 9.7 Hz, 1H), 7.50–7.45 (m, 2H), 7.40–7.34 (m, 2H), 7.34–7.29 (m, 1H), 7.08 (d, J = 9.7 Hz, 1H), 6.58 (d, J = 2.4 Hz, 1H), 5.21 (s, 2H), 4.26 (d, J = 4.8 Hz, 2H), 1.51 (s, 9H).

***tert*-Butyl (6-hydroxypyrazolo[1,5-*a*]pyridine-7-carbonyl)glycinate (SI-20)**

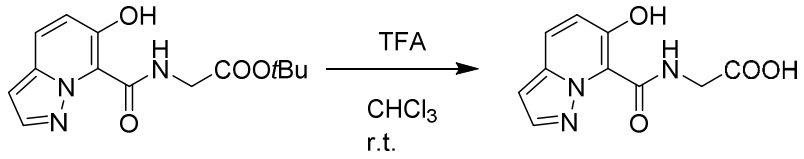


SI-19 **SI-20**

SI-20 was prepared by a method similar to the synthesis of **SI-5**.

^{1}H NMR (400 MHz, CDCl3) δ: 13.41 (s, 1H), 11.43 (t, J = 5.2 Hz, 1H), 7.91 (d, J = 2.4 Hz, 1H), 7.61 (d, J = 9.7 Hz, 1H), 7.02 (d, J = 9.7 Hz, 1H), 6.60 (d, J = 2.4 Hz, 1H), 4.26 (d, J = 5.2 Hz, 2H), 1.53 (s, 9H).

(6-Hydroxypyrazolo[1,5-*a*]pyridine-7-carbonyl)glycine (2)



SI-20 **2**

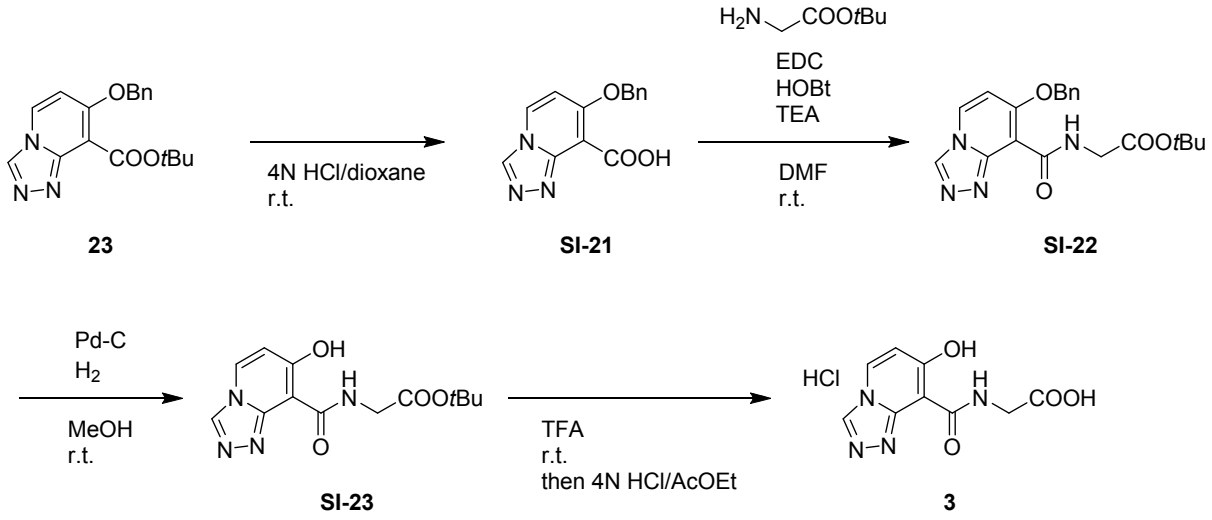
To a solution of **SI-19** (16.0 mg, 0.0549 mmol) in $CHCl_3$ (0.5 mL) was added TFA (0.5 mL) under ice-cooling. After stirring at room temperature for 3 h, the reaction mixture was concentrated under reduced pressure. The obtained residue was slurried in water to give the title compound **2** (8.50 mg, 66% yield).

^{1}H NMR (400 MHz, DMSO-d6) δ: 13.57 (s, 1H), 12.97 (br s, 1H), 11.13 (t, J = 5.6 Hz, 1H), 8.08 (d, J = 2.4 Hz, 1H), 7.97 (d, J = 9.7 Hz, 1H), 7.14 (d, J = 9.7 Hz, 1H), 6.84 (d, J = 2.4 Hz, 1H), 4.25 (d, J = 5.6 Hz, 2H).

HRMS m/z: $[M-H]^-$ calcd for C10H8N3O4, 234.0520; found, 234.0520.

Purity: 98.1%.

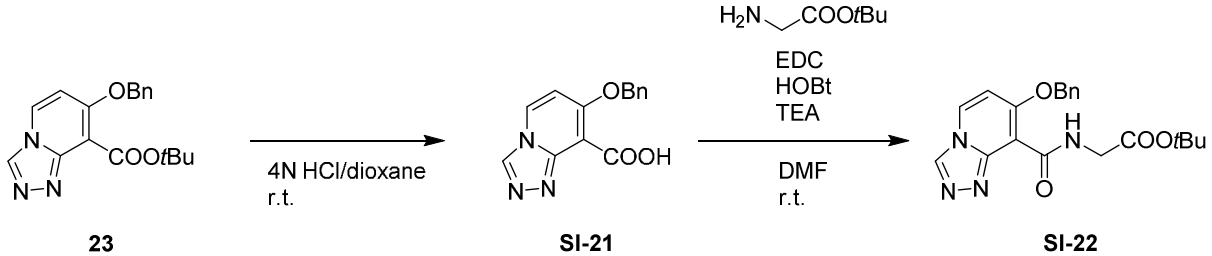
Synthesis of inhibitor 3



23 **SI-21** **SI-22**

SI-23 **3**

***tert*-Butyl (7-(benzyloxy)-[1,2,4]triazolo[4,3-*a*]pyridine-8-carbonyl)glycinate (SI-22)**



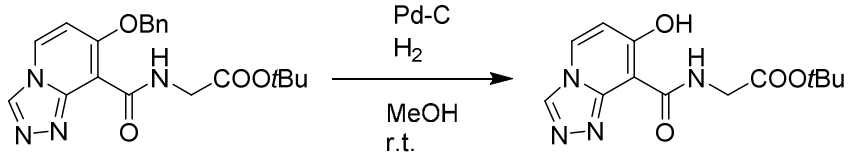
23 **SI-21** **SI-22**

Compound **23** (50.0 mg, 0.154 mmol) and 4N HCl in dioxane (1 mL) were mixed. After stirring at room temperature for 3.5 h, the reaction mixture was concentrated under reduced pressure, then crude **SI-21** was ready for the next reaction. To a solution of crude **SI-21** in DMF (1 mL) were added successively triethylamine (0.021

mL, 0.154 mmol), 1-hydroxybenzotriazole hydrate (35.0 mg, 0.230 mmol) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (44.0 mg, 0.230 mmol) under ice-cooling. After stirring at room temperature for 2 days, saturated $NaHCO_3$ aq. was added to the mixture. The resulting mixture was extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The obtained residue was purified by preparative TLC ($CHCl_3$/MeOH = 9/1) to give the title compound **SI-22** (24.0 mg, 41% yield for 2 steps).

^{1}H NMR (400 MHz, CDCl3) δ: 9.66 (t, J = 5.6 Hz, 1H), 8.67 (s, 1H), 8.10 (d, J = 7.7 Hz, 1H), 7.51–7.46 (m, 2H), 7.39–7.33 (m, 2H), 7.32–7.27 (m, 1H), 6.81 (d, J = 7.7 Hz, 1H), 5.35 (s, 2H), 4.23 (d, J = 5.6 Hz, 2H), 1.51 (s, 9H).

***tert*-Butyl (7-hydroxy-[1,2,4]triazolo[4,3-*a*]pyridine-8-carbonyl)glycinate (SI-23)**

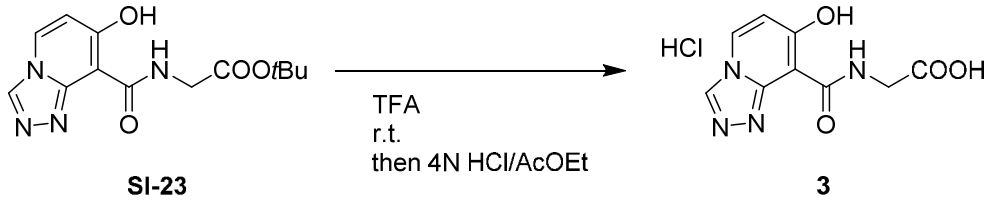


SI-22 **SI-23**

Compound **SI-23** was prepared by a method similar to the synthesis of **SI-5**.

^{1}H NMR (400 MHz, DMSO-d6) δ: 14.45 (br s, 1H), 9.95 (t, J = 5.2 Hz, 1H), 8.63 (s, 1H), 8.03 (d, J = 7.3 Hz, 1H), 6.68 (d, J = 7.3 Hz, 1H), 4.20 (d, J = 5.2 Hz, 2H), 1.51 (s, 9H).

(7-Hydroxy-[1,2,4]triazolo[4,3-*a*]pyridine-8-carbonyl)glycine hydrochloride (3)



SI-23 **3**

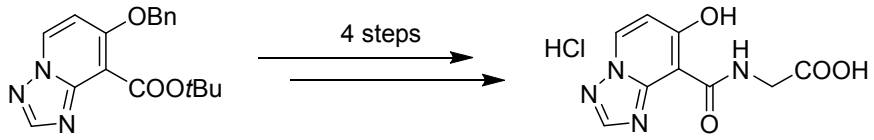
Compound **SI-23** (15.0 mg, 0.0513 mmol) and TFA (1 mL) were mixed. After stirring at room temperature for 1.5 h, the reaction mixture was concentrated under reduced pressure. To the obtained residue were added 4N HCl in AcOEt and diethyl ether, then the precipitated solid was collected by filtration to give the title compound **3** (12.0 mg, 86% yield).

^{1}H NMR (400 MHz, DMSO-d6) δ: 10.09 (s, 1H), 8.99 (s, 1H), 8.33 (d, J = 7.7 Hz, 1H), 6.65 (d, J = 7.7 Hz, 1H), 4.07 (s, 2H).

HRMS m/z: $[M-H]^-$ calcd for C9H7N4O4, 235.0473; found, 235.0471.

Purity: 100.0%.

Synthesis of inhibitor 4



24 **4**

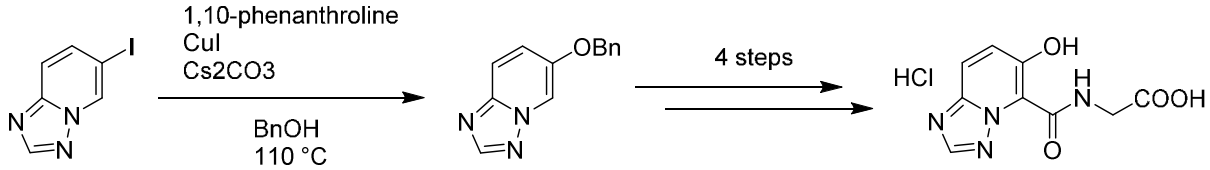
Compound **4** was prepared by a method similar to the synthesis of **3**.

^{1}H NMR (400 MHz, DMSO-d6) δ: 9.87 (t, J = 5.6 Hz, 1H), 8.96 (d, J = 7.7 Hz, 1H), 8.56 (s, 1H), 6.93 (d, J = 7.7 Hz, 1H), 4.22 (d, J = 5.6 Hz, 2H).

HRMS m/z: $[M-H]^-$ calcd for C9H7N4O4, 235.0473; found, 235.0472.

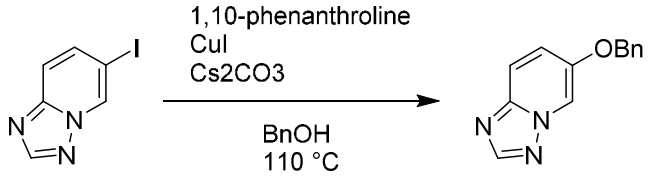
Purity: 100.0%.

Synthesis of inhibitor 5



SI-24 **SI-25** **5**

6-(Benzyloxy)-[1,2,4]triazolo[1,5-*a*]pyridine (SI-25)

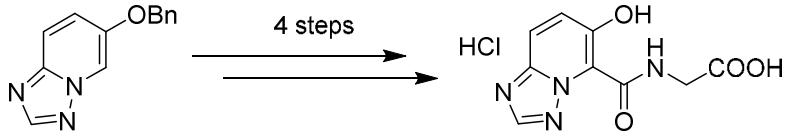


SI-24 **SI-25**

To a mixture of **SI-24** (2.80 g, 11.4 mmol), 1,10-phenanthroline (0.411 g, 2.28 mmol), copper(I) iodide, (0.217 g, 1.14 mmol) and cesium carbonate (7.43 g, 22.8 mmol) was added benzyl alcohol (5.91 mL, 57.1 mmol). The mixture was warmed to 110 °C and then stirred for 15 h. After cooling to room temperature, AcOEt and water were added. The resulting mixture was extracted three times with AcOEt. The organic layer was washed with brine and dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The obtained residue was purified by column chromatography ($CHCl_3$/AcOEt = 85/15–80/20) to give the title compound **SI-25** (860 mg, 33% yield).

^{1}H NMR (400 MHz, DMSO-d6) δ: 8.78 (dd, J = 2.4, 0.8 Hz, 1H), 8.40 (s, 1H), 7.78 (dd, J = 9.7, 0.8 Hz, 1H), 7.52 (dd, J = 9.7, 2.4 Hz, 1H), 7.52–7.48 (m, 2H), 7.45–7.40 (m, 2H), 7.39–7.34 (m, 1H), 5.19 (s, 2H).

(6-Hydroxy-[1,2,4]triazolo[1,5-*a*]pyridine-5-carbonyl)glycine hydrochloride (5)



SI-25 **5**

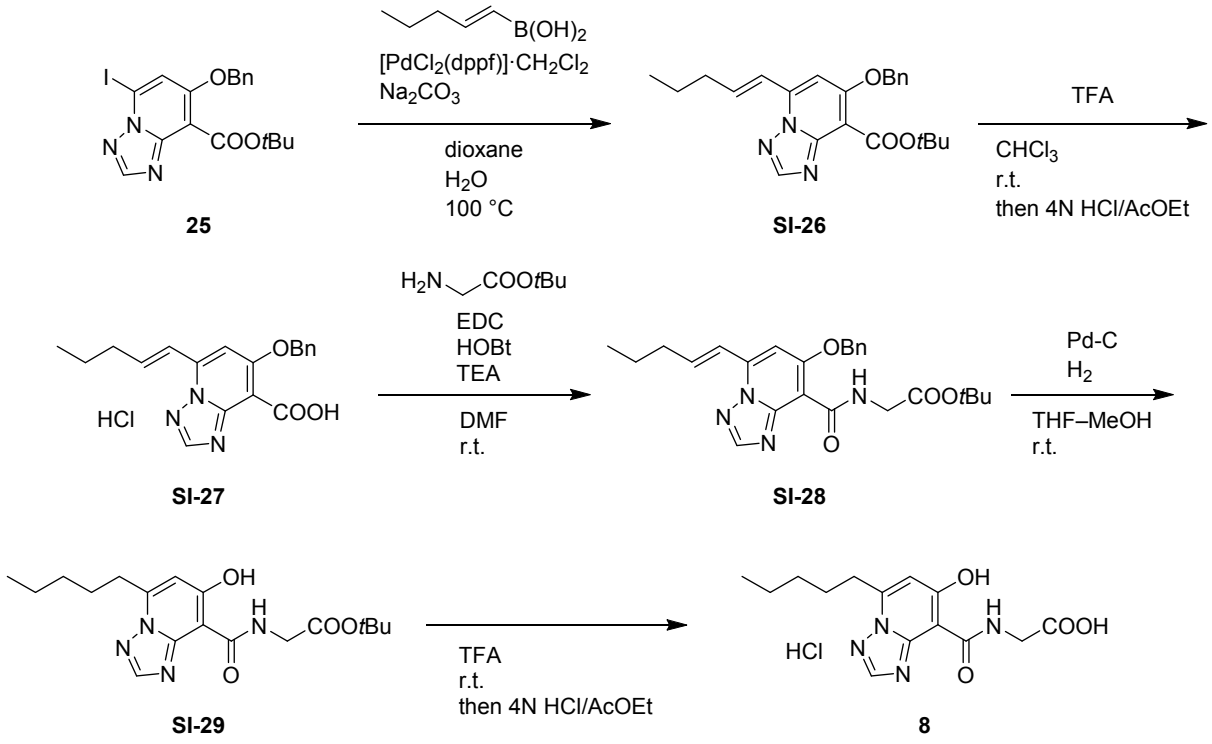
Compound **5** was prepared by the method similar to the synthesis of **2**. In the final step, the hydrochloride salt was formed by the method similar to the synthesis of **3**.

^{1}H NMR (400 MHz, DMSO-D6) δ: 13.29 (br s, 1H), 10.49 (t, J = 5.2 Hz, 1H), 8.68 (s, 1H), 8.09 (d, J = 9.7 Hz, 1H), 7.56 (d, J = 9.7 Hz, 1H), 4.27 (d, J = 5.2 Hz, 2H).

HRMS m/z: $[M-H]^-$ calcd for C9H7N4O4, 235.0473; found, 235.0472.

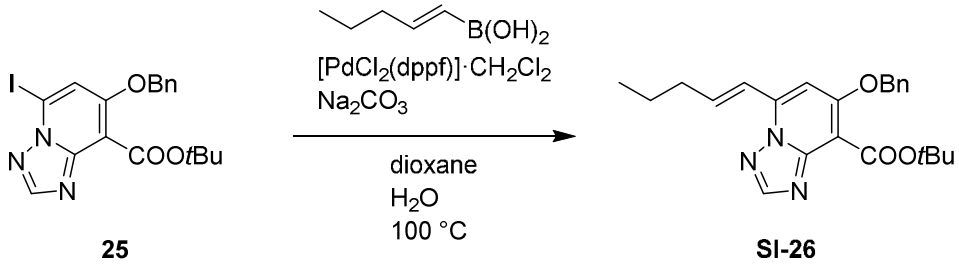
Purity: 100.0%.

Synthesis of inhibitor 8



25 **SI-26** **SI-27** **SI-28** **SI-29** **8**

***tert*-Butyl (*E*)-7-(benzyloxy)-5-(pent-1-en-1-yl)-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxylate (SI-26)**

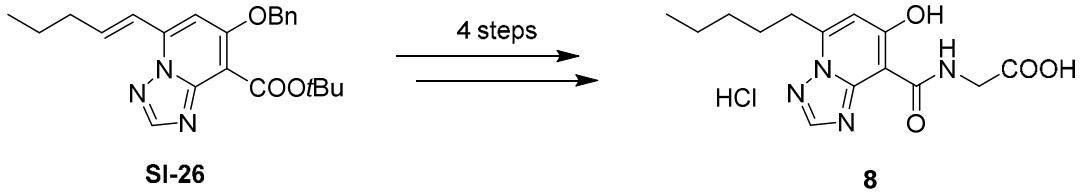


25 **SI-26**

To a solution of **25** (200 mg, 0.443 mmol), (*E*)-pent-1-en-1-ylboronic acid (101 mg, 0.886 mmol) and sodium carbonate (188 mg, 1.77 mmol) in dioxane/water = 5/2 (2.8 mL) was added [1,1'-bis(diphenylphosphino)ferrocene]palladium(II) dichloride dichloromethane complex (1:1) (18.0 mg, 0.0222 mmol). The mixture was warmed to 100 °C and then stirred for 1 h. After cooling to room temperature, brine and water were added. The resulting mixture was extracted three times with AcOEt. The organic layer was washed twice with brine and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The obtained residue was purified by column chromatography (*n*-hexane/AcOEt = 9/1–7/3) to give the title compound **SI-26** (207 mg, quantitative).

^{1}H NMR (400 MHz, DMSO-d6) δ: 8.41 (s, 1H), 7.52 (s, 1H), 7.51–7.46 (m, 2H), 7.45–7.39 (m, 2H), 7.38–7.26 (m, 2H), 7.05–6.99 (m, 1H), 5.41 (s, 2H), 2.40–2.33 (m, 2H), 1.61–1.51 (m, 2H), 1.47 (s, 9H), 0.98 (t, J = 7.5 Hz, 3H).

(7-Hydroxy-5-pentyl-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonyl)glycine hydrochloride (8)



SI-26 **8**

Compound **8** was prepared by a method similar to the synthesis of **3**.

^{1}H NMR (400 MHz, DMSO-D6) δ: 9.83 (t, J = 5.6 Hz, 1H), 8.56 (s, 1H), 6.86 (s, 1H), 4.21 (d, J = 5.6 Hz, 2H), 3.09 (t, J = 7.7 Hz, 2H), 1.78 (tt, J = 7.7, 7.4 Hz, 2H), 1.41–1.30 (m, 4H), 0.88 (t, J = 6.6 Hz, 3H).

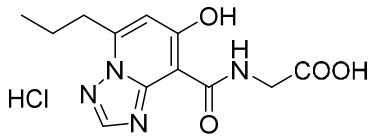
HRMS m/z: $[M-H]^-$ calcd for C14H17N4O4, 305.1255; found, 305.1253.

Purity: 100.0%.

Synthesis of inhibitors 6, 7, 11–15

Compounds **6**, **7**, **11**–**15** were prepared by a method similar to the synthesis of **8**. In these cases, corresponding coupling reagents were used instead of (*E*)-pent-1-en-1-ylboronic acid.

(7-Hydroxy-5-propyl-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonyl)glycine hydrochloride (6)

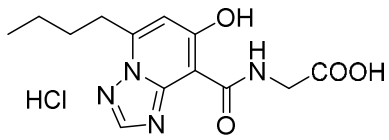


^{1}H NMR (400 MHz, DMSO-d6) δ: 9.84 (t, J = 5.7 Hz, 1H), 8.56 (s, 1H), 6.85 (s, 1H), 4.21 (d, J = 5.7 Hz, 2H), 3.07 (t, J = 7.4 Hz, 2H), 1.80 (tq, J = 7.4, 7.4 Hz, 2H), 0.97 (t, J = 7.4 Hz, 3H).

HRMS m/z: $[M-H]^-$ calcd for C12H13N4O4, 277.0942; found, 277.0940.

Purity: 100.0%.

(5-Butyl-7-hydroxy-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonyl)glycine hydrochloride (7)

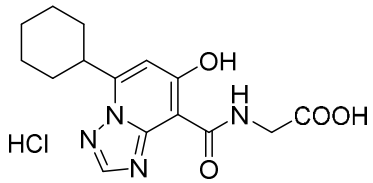


^{1}H NMR (400 MHz, DMSO-D6) δ: 9.84 (t, J = 5.6 Hz, 1H), 8.56 (s, 1H), 6.85 (s, 1H), 4.21 (d, J = 5.6 Hz, 2H), 3.10 (t, J = 7.6 Hz, 2H), 1.76 (tt, J = 7.6, 7.4 Hz, 2H), 1.39 (tq, J = 7.4, 7.4 Hz, 2H), 0.93 (t, J = 7.4 Hz, 3H).

HRMS m/z: $[M-H]^-$ calcd for C13H15N4O4, 291.1099; found, 291.1097.

Purity: 100.0%.

(5-Cyclohexyl-7-hydroxy-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonyl)glycine hydrochloride (11)

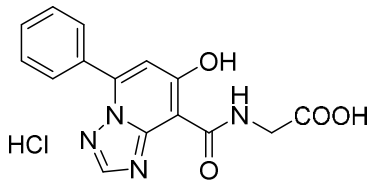


^{1}H NMR (400 MHz, DMSO-d6) δ: 9.83 (t, J = 5.6 Hz, 1H), 8.56 (s, 1H), 6.76 (s, 1H), 4.20 (d, J = 5.6 Hz, 2H), 3.42–3.31 (m, 1H), 2.08–1.98 (m, 2H), 1.87–1.79 (m, 2H), 1.78–1.70 (m, 1H), 1.59–1.36 (m, 4H), 1.35–1.22 (m, 1H).

HRMS m/z: $[M-H]^-$ calcd for C15H17N4O4, 317.1255; found, 317.1254.

Purity: 100.0%.

(7-Hydroxy-5-phenyl-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonyl)glycine hydrochloride (12)

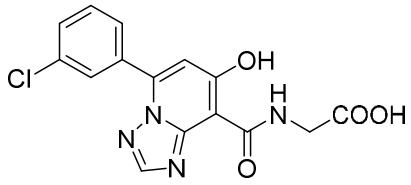


^{1}H NMR (400 MHz, DMSO-d6) δ: 9.98 (t, J = 5.6 Hz, 1H), 8.58 (s, 1H), 8.03–7.98 (m, 2H), 7.63–7.56 (m, 3H), 7.12 (s, 1H), 4.25 (d, J = 5.6 Hz, 2H).

HRMS m/z: [M−H]$^-$ calcd for C15H11N4O4, 311.0786; found, 311.0784.

Purity: 100.0%.

(5-(3-Chlorophenyl)-7-hydroxy-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonyl)glycine (13)

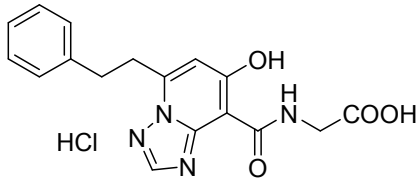


^{1}H NMR (400 MHz, DMSO-d6) δ: 14.37 (br s, 1H), 9.96 (br s, 1H), 8.58 (s, 1H), 8.10 (s, 1H), 7.98–7.91 (m, 1H), 7.70–7.66 (m, 1H), 7.65–7.59 (m, 1H), 7.21 (s, 1H), 4.23 (d, J = 5.6 Hz, 2H).

HRMS m/z: [M−H]$^-$ calcd for C15H10ClN4O4, 345.0396; found, 345.0398.

Purity: 98.6%.

(7-Hydroxy-5-phenethyl-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonyl)glycine hydrochloride (14)

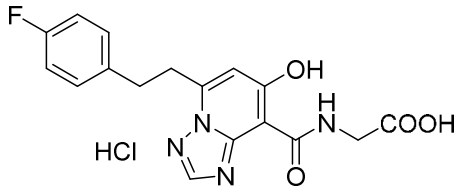


^{1}H NMR (400 MHz, DMSO-D6) δ: 9.85 (t, J = 5.6 Hz, 1H), 8.60 (s, 1H), 7.32–7.18 (m, 5H), 6.81 (s, 1H), 4.21 (d, J = 5.6 Hz, 2H), 3.41 (t, J = 7.7 Hz, 2H), 3.12 (t, J = 7.7 Hz, 2H).

HRMS m/z: [M−H]$^-$ calcd for C17H15N4O4, 339.1099; found, 339.1098.

Purity: 100.0%.

(5-(4-Fluorophenethyl)-7-hydroxy-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonyl)glycine hydrochloride (15)

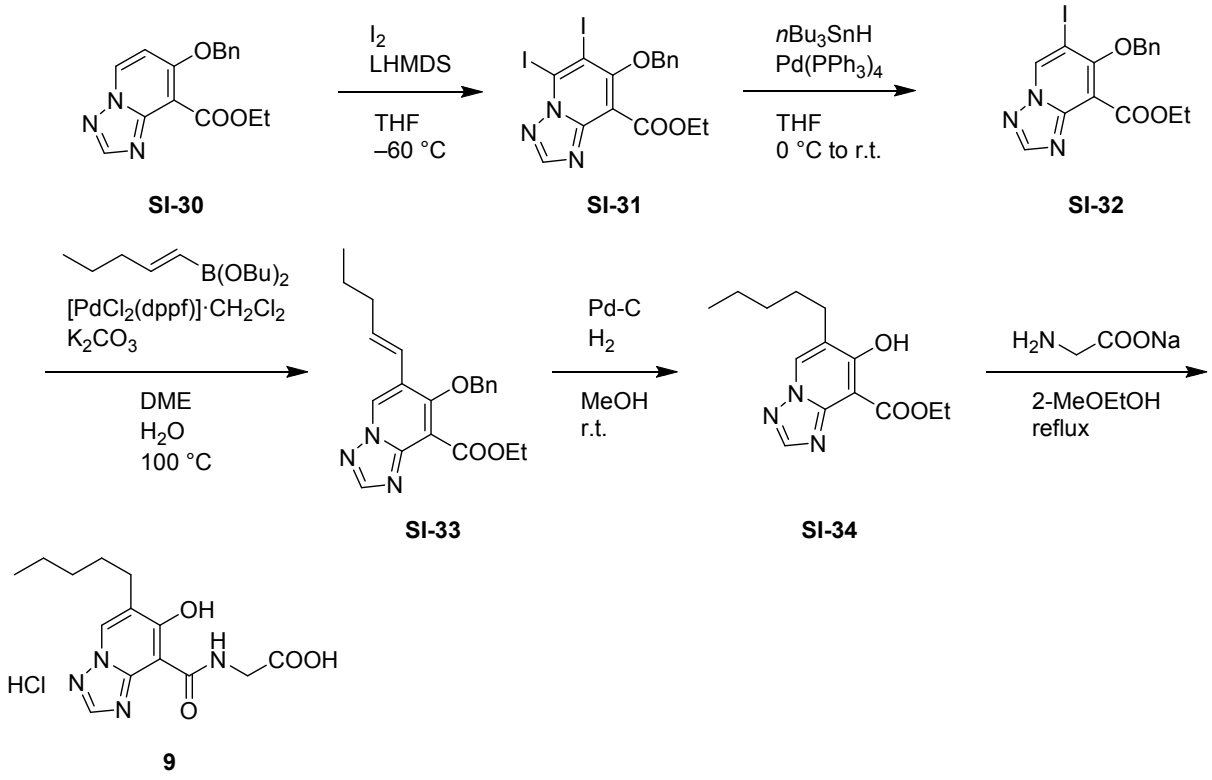


^{1}H NMR (400 MHz, DMSO-d6) δ: 9.85 (t, J = 5.6 Hz, 1H), 8.60 (s, 1H), 7.29–7.25 (m, 2H), 7.15–7.08 (m, 2H), 6.80 (s, 1H), 4.21 (d, J = 5.6 Hz, 2H), 3.39 (t, J = 7.7 Hz, 2H), 3.11 (t, J = 7.7 Hz, 2H).

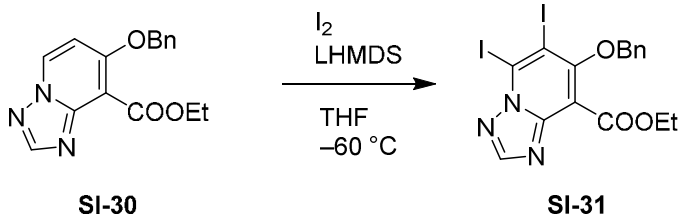
HRMS m/z: [M−H]$^-$ calcd for C17H14FN4O4, 357.1005; found, 357.1003.

Purity: 100.0%.

Synthesis of inhibitor 9



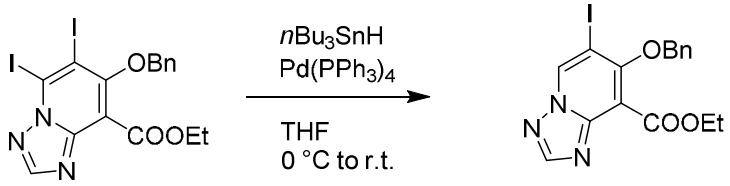
Ethyl 7-(benzyloxy)-5,6-diiodo-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxylate (SI-31)



Under a nitrogen stream, to a solution of **SI-30** (3.98 g, 13.4 mol) (obtained in the same manner as **24**) in THF (40 mL) was added dropwise a solution of iodine (3.4 g, 13.4 mol) in THF (16 mL) under cooling in a dry ice/EtOH bath. To this mixture was added dropwise 1.0 M LHMDS in THF (26.8 mL, 26.8 mol) over 10 min. After stirring under cooling in a dry ice/EtOH bath for 2.5 h, the reaction mixture was poured into a mixture of saturated NH_4Cl aq. (40 mL) and water (40 mL) under ice-cooling. To this mixture was added sodium sulfite (1.7 g), and the organic layer was separated. After the organic layer was concentrated, the obtained residue was combined with the aqueous layer, the mixture was extracted twice with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The obtained residue was purified by column chromatography and recrystallized from heptane/$CHCl_3$ to give the title compound **SI-31** (0.295 g, 4% yield).

^{1}H NMR (400 MHz, CDCl3) δ: 8.33 (s, 1H), 7.56–7.52 (m, 2H), 7.45–7.36 (m, 3H), 5.22 (s, 2H), 4.50 (q, J = 7.1 Hz, 2H), 1.38 (t, J = 7.1 Hz, 3H).

Ethyl 7-(benzyloxy)-6-iodo-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxylate (SI-32)

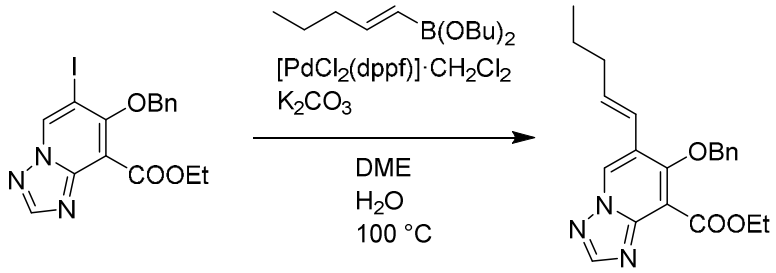


SI-31 **SI-32**

To a solution of **SI-31** (1.31 g, 2.39 mmol) and tetrakis(triphenylphosphine)palladium(0) (0.137g, 0.119 mmol) in THF (15 mL) was added tributyltin hydride (0.70 mL, 2.62 mmol) under ice-cooling. After stirring for 20 min under ice-cooling, the mixture was warmed to room temperature and stirred for 20 min, then concentrated under reduced pressure. The obtained residue was purified by column chromatography (*n*-hexane/AcOEt = 10/0–1/1) to give the title compound **SI-32** (0.440 g, 44%).

^{1}H NMR (400 MHz, CDCl3) δ: 8.99 (s, 1H), 8.31 (s, 1H), 7.56–7.51 (m, 2H), 7.45–7.36 (m, 3H), 5.23 (s, 2H), 4.51 (q, J = 7.2 Hz, 2H), 1.39 (t, J = 7.2 Hz, 3H).

Ethyl (*E*)-7-(benzyloxy)-6-(pent-1-en-1-yl)-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxylate (SI-33)

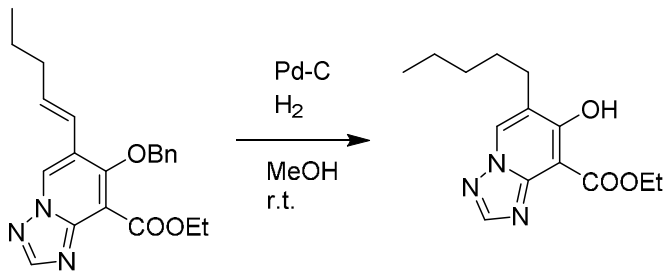


SI-32 **SI-33**

Compound **SI-33** was prepared by a method similar to the synthesis of **SI-26**.

^{1}H NMR (400 MHz, CDCl3) δ: 8.62 (s, 1H), 8.30 (s, 1H), 7.45–7.35 (m, 5H), 6.49 (d, J = 15.9 Hz, 1H), 6.25 (dt, J = 15.9, 6.9 Hz, 1H), 5.12 (s, 2H), 4.50 (q, J = 7.2 Hz, 2H), 2.25–2.17 (m, 2H), 1.55–1.44 (m, 2H), 1.39 (t, J = 7.2 Hz, 3H), 0.95 (t, J = 7.4 Hz, 3H).

Ethyl 7-hydroxy-6-pentyl-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxylate (SI-34)

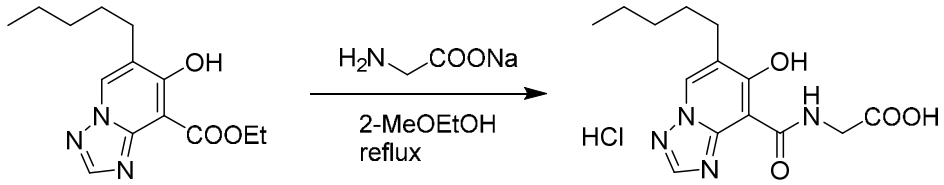


SI-33 **SI-34**

Compound **SI-34** was prepared by a method similar to the synthesis of **SI-5**.

^{1}H NMR (400 MHz, CDCl3) δ: 13.10 (s, 1H), 8.37 (s, 1H), 8.25 (s, 1H), 4.65 (q, J = 7.1 Hz, 2H), 2.69 (t, J = 7.7 Hz, 2H), 1.68 (tt, J = 7.7, 7.4 Hz, 2H), 1.53 (t, J = 7.1 Hz, 3H), 1.41–1.35 (m, 4H), 0.92 (t, J = 7.2 Hz, 3H).

(7-Hydroxy-6-pentyl-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonyl)glycine hydrochloride (9)



SI-34 **9**

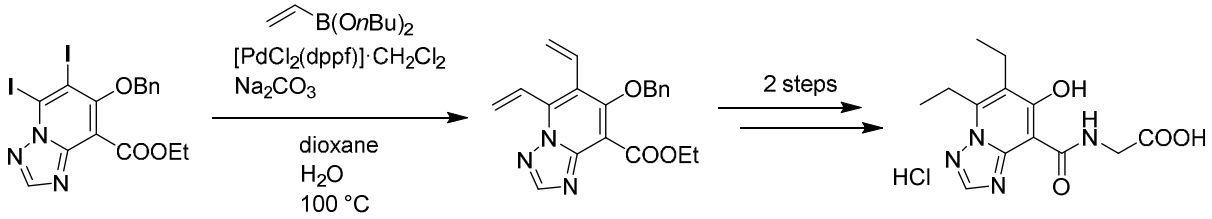
Compound **9** was prepared by a method similar to the synthesis of **1**.

^{1}H NMR (400 MHz, DMSO-d6) δ: 14.80 (br s, 1H), 9.92 (s, 1H), 8.88 (s, 1H), 8.50 (s, 1H), 4.21 (d, J = 5.5 Hz, 2H), 2.62 (t, J = 7.3 Hz, 2H), 1.61 (tt, J = 7.3, 7.4 Hz, 2H), 1.38–1.25 (m, 4H), 0.87 (t, J = 6.9 Hz, 3H).

HRMS m/z: $[M-H]^-$ calcd for C14H17N4O4, 305.1255; found, 305.1253.

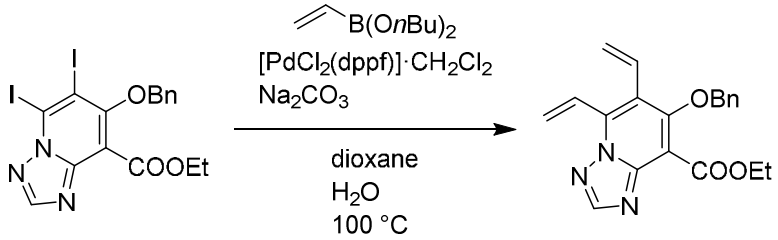
Purity: 100.0%.

Synthesis of inhibitor 10



SI-31 **SI-35** **10**

Ethyl 7-(benzyloxy)-5,6-divinyl-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxylate (SI-35)

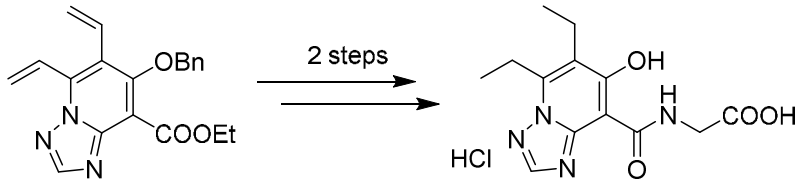


SI-31 **SI-35**

Compound **SI-35** was prepared by a method similar to the synthesis of **SI-26**.

^{1}H NMR (400 MHz, CDCl3) δ: 8.37 (s, 1H), 7.44–7.34 (m, 5H), 7.14 (dd, J = 17.7, 12.1 Hz, 1H), 6.87 (dd, J = 17.7, 1.6 Hz, 1H), 6.77 (dd, J = 17.7, 11.7 Hz, 1H), 6.05 (dd, J = 12.1, 1.6 Hz, 1H), 5.76 (dd, J = 11.7, 1.4 Hz, 1H), 5.73 (dd, J = 17.7, 1.4 Hz, 1H), 5.09 (s, 2H), 4.50 (q, J = 7.1 Hz, 2H), 1.38 (t, J = 7.1 Hz, 3H).

(5,6-Diethyl-7-hydroxy-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonyl)glycine hydrochloride (10)



SI-35 **10**

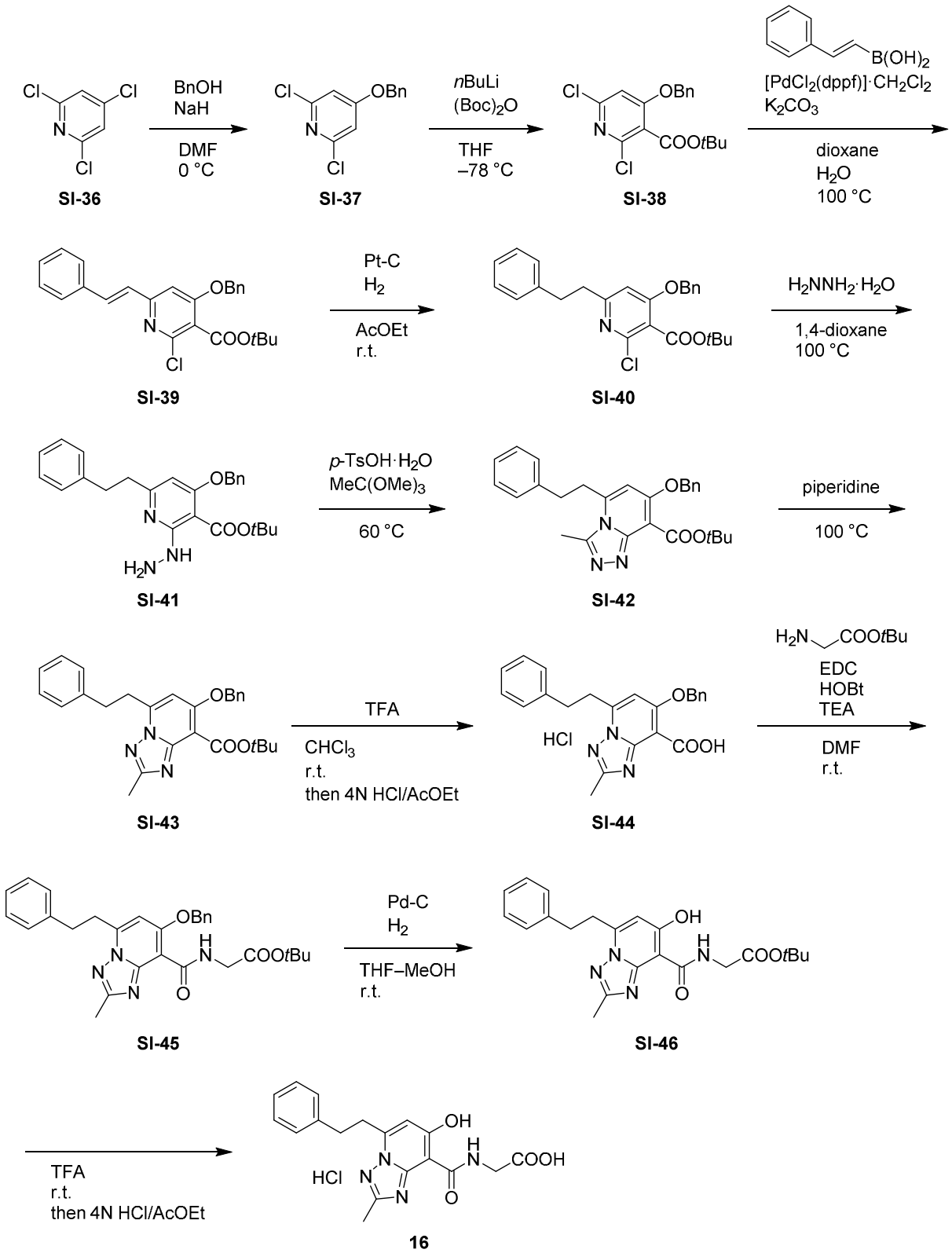
Compound **10** was prepared by a method similar to the synthesis of **9**.

^{1}H NMR (400 MHz, DMSO-d6) δ: 9.95 (t, J = 5.6 Hz, 1H), 8.52 (s, 1H), 4.21 (d, J = 5.6 Hz, 2H), 3.20 (q, J = 7.5 Hz, 2H), 2.72 (q, J = 7.5 Hz, 2H), 1.29 (t, J = 7.5 Hz, 3H), 1.15 (t, J = 7.5 Hz, 3H).

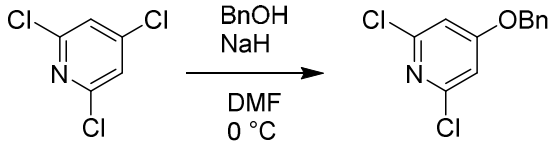
HRMS m/z: $[M-H]^-$ calcd for C13H15N4O4, 291.1099; found, 291.1099.

Purity: 100.0%.

Synthesis of inhibitor 16



4-(Benzyloxy)-2,6-dichloropyridine (SI-37)

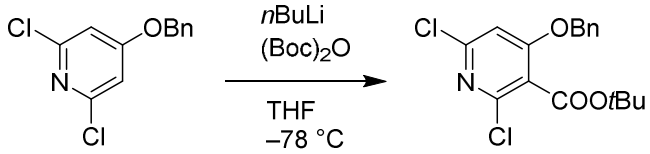


SI-36 **SI-37**

Compound **SI-37** was prepared by a method similar to the synthesis of **22**.

^{1}H NMR (400 MHz, CDCl3) δ: 7.46–7.35 (m, 5H), 6.86 (s, 2H), 5.11 (s, 2H).

***tert*-Butyl 4-(benzyloxy)-2,6-dichloronicotinate (SI-38)**

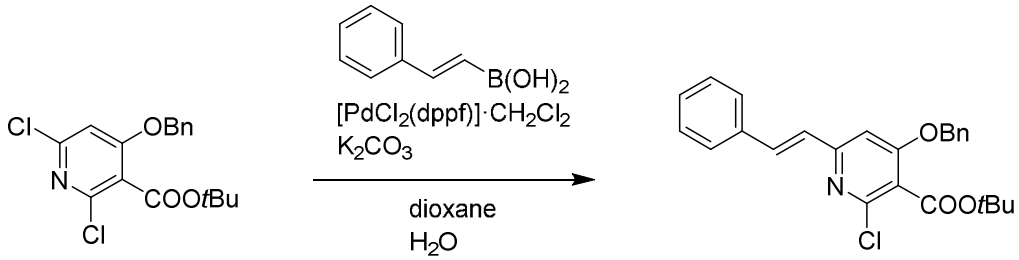


SI-37 **SI-38**

Under a nitrogen stream, to a solution of *n*BuLi (1.65 mol/L in *n*-hexane) (119 mL, 0.197 mol) in THF (250 mL) was added dropwise a solution of **SI-37** (50.0 g, 0.197 mol) in THF (110 mL) at −78 °C. To this mixture was added dropwise a solution of di-*tert*-butyl-dicarbonate (45 mL, 0.197 mol) in THF (100 mL). After stirring at -78 °C for 30 min, water (450 mL) was added to quench the reaction. The mixture was gradually warmed to room temperature, then AcOEt (200 mL) was poured into the reaction mixture. The organic layer was washed successively with water (200 mL) and brine (200 mL). The organic layer was concentrated under reduced pressure and the residue was purified by column chromatography (*n*-hexane/AcOEt = 50/1–11/1). The obtained solid was further slurried in *n*-hexane (100 mL) to give the title compound **SI-38** (15.3 g, 22%).

^{1}H NMR (400 MHz, CDCl3) δ: 7.44–7.33 (m, 5H), 6.87 (s, 1H), 5.16 (s, 2H), 1.52 (s, 9H).

***tert*-Butyl (E)-4-(benzyloxy)-2-chloro-6-styrylnicotinate (SI-39)**



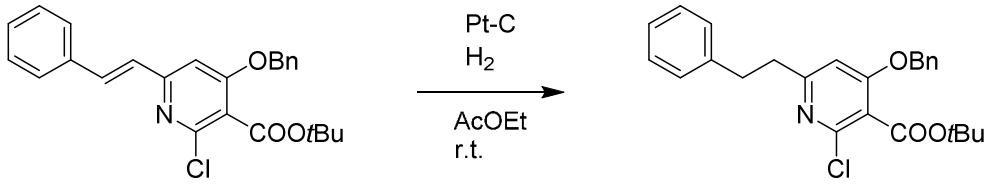
SI-38 **SI-39**

Under a nitrogen stream, to a solution of **SI-38** (15.5 g, 43.8 mmol), (*E*)-styrylboronic acid (7.12 g, 48.1 mmol), [1,1'-bis(diphenylphosphino)ferrocene]palladium(II) dichloride dichloromethane complex (1:1) (1.79 g, 2.19 mmol), and potassium carbonate (18.2 g, 131 mmol) in dioxane (150 mL) was added water (45 mL). The mixture was warmed to 80 °C and then stirred for 1.5 h. (*E*)-Styrylboronic acid (0.640 g, 4.33 mmol) was added and the mixture was stirred for 1.5 h. After cooling to room temperature, brine and water were added. The resulting

mixture was extracted with AcOEt. The organic layer was concentrated under reduced pressure, and the obtained residue was purified by column chromatography (eluent: $CHCl_3$). The obtained solid was further slurried in IPE (100 mL) at 70 °C for 0.5 h to give the title compound **SI-39** (11.5 g, 62%).

^{1}H NMR (400 MHz, CDCl3) δ: 7.65 (d, J = 16.1 Hz, 1H), 7.59–7.53 (m, 2H), 7.43–7.29 (m, 8H), 7.01 (d, J = 16.1 Hz, 1H), 5.21 (s, 2H), 1.55 (s, 9H).

***tert*-Butyl 4-(benzyloxy)-2-chloro-6-phenethylnicotinate (SI-40)**

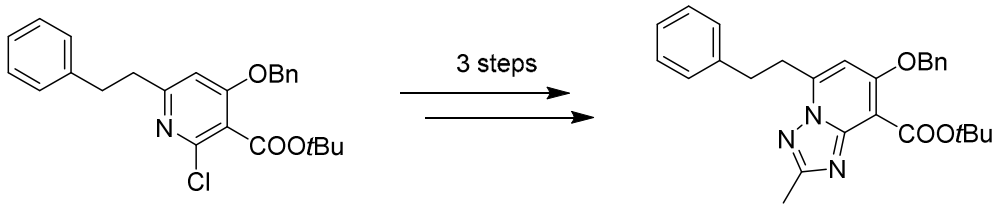


SI-39 **SI-40**

To a solution of **SI-39** (11.5 g, 27.2 mmol) in AcOEt (120 mL) was added 2% platinum carbon (2.5 g), and the mixture was stirred under a hydrogen atmosphere (3.8 kgf/cm^2) for 23 h. The reaction mixture was filtered through celite, and concentrated under reduced pressure to give the title compound **SI-40** (11.5 g, 100% yield).

^{1}H NMR (400 MHz, CDCl3) δ: 7.42–7.32 (m, 5H), 7.32–7.26 (m, 2H), 7.24–7.16 (m, 3H), 6.55 (s, 1H), 5.05 (s, 2H), 3.04–2.99 (m, 4H), 1.55 (s, 9H).

***tert*-Butyl 7-(benzyloxy)-2-methyl-5-phenethyl-[1,2,4]triazolo[1,5-*a*]pyridine-8-carboxylate (SI-43)**

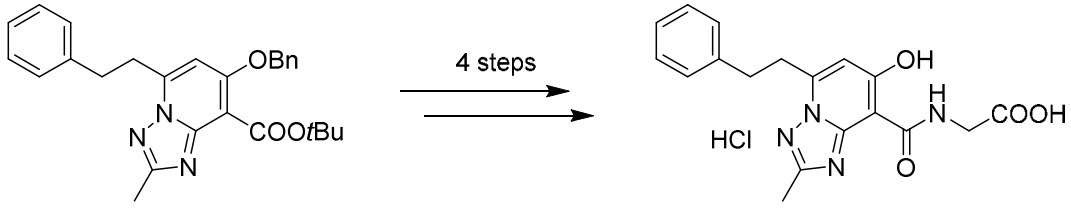


SI-40 **SI-43**

Compound **SI-43** was prepared by a method similar to the synthesis of **24**.

^{1}H NMR (400 MHz, MeOH-d4) δ: 7.45–7.33 (m, 5H), 7.28–7.22 (m, 2H), 7.20–7.14 (m, 3H), 6.83 (s, 1H), 5.16 (s, 2H), 3.42 (t, J = 7.7 Hz, 2H), 3.13 (t, J = 7.7 Hz, 2H), 2.52 (s, 3H), 1.49 (s, 9H).

(7-Hydroxy-2-methyl-5-phenethyl-[1,2,4]triazolo[1,5-*a*]pyridine-8-carbonyl)glycine hydrochloride (16)



SI-43 **16**

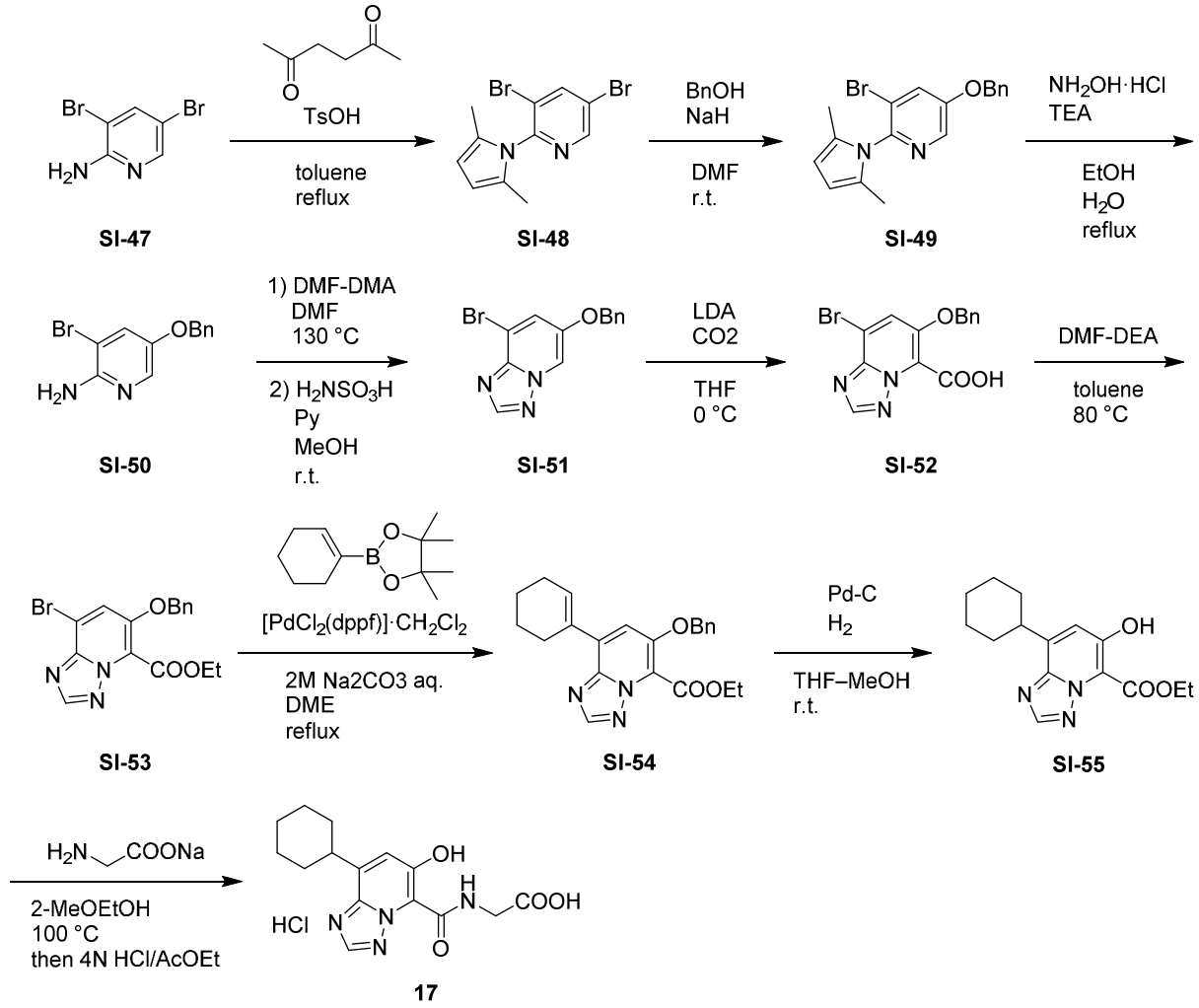
Compound **16** was prepared by a method similar to the synthesis of **3**.

^{1}H NMR (400 MHz, DMSO-d6) δ: 9.81 (t, J = 5.7 Hz, 1H), 7.32–7.18 (m, 5H), 6.69 (s, 1H), 4.19 (d, J = 5.7 Hz, 2H), 3.35 (t, J = 7.8 Hz, 2H), 3.10 (t, J = 7.8 Hz, 2H), 2.52 (s, 3H).

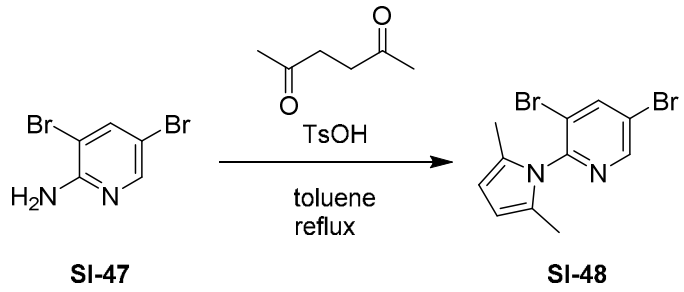
HRMS m/z: $[M-H]^-$ calcd for C18H17N4O4, 353.1255; found, 353.1255.

Purity: 100.0%.

Synthesis of inhibitor 17



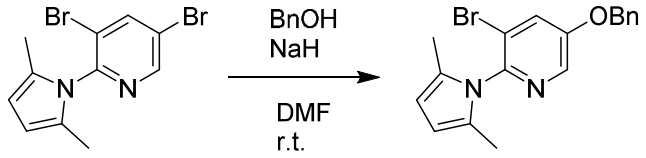
3,5-Dibromo-2-(2,5-dimethyl-1*H*-pyrrol-1-yl)pyridine (SI-48)



Compound **SI-47** (10.0 g, 39.7 mmol), 1,5-hexanedione (5.16 mL, 44.0 mmol), *p*-toluenesulfonic acid monohydrate (0.533 g, 2.80 mmol) and toluene (50 mL) were mixed, and then stirred under reflux for 2.5 h. After cooling to room temperature, toluene, AcOEt and saturated $NaHCO_3$ aq. were added. The organic layer was washed successively with saturated $NaHCO_3$ aq., water, and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give the title compound **SI-48** (13.0 g, 98% yield).

^{1}H NMR (400 MHz, CDCl3) δ: 8.62 (d, J = 2.2 Hz, 1H), 8.22 (d, J = 2.2 Hz, 1H), 5.92 (s, 2H), 2.00 (s, 6H).

5-(Benzyloxy)-3-bromo-2-(2,5-dimethyl-1*H*-pyrrol-1-yl)pyridine (SI-49)

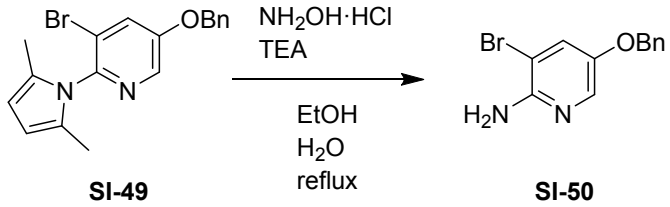


SI-48 **SI-49**

Under a nitrogen stream, to a solution of NaH (60% oil suspension) (1.84 g, 46.0 mmol) in DMF (10 mL) was added benzyl alcohol (4.8 mL, 46.3 mmol) at 0 °C. After stirring at room temperature for 1 h, a solution of **SI-48** (12.8 g, 38.8 mmol) in DMF (10 mL) was added to the reaction mixture at 0 °C. The mixture was gradually warmed to room temperature and stirred overnight, then acetic acid (0.66 mL) and AcOEt (50 mL) were poured into the reaction mixture at 0 °C. The mixture was filtered through celite, and concentrated under reduced pressure. The obtained residue was slurried in *n*-hexane/AcOEt/water = 6/1/10 (85 mL) to give the title compound **SI-49** (8.53g, 62% yield).

^{1}H NMR (400 MHz, CDCl3) δ: 8.32 (d, J = 2.8 Hz, 1H), 7.64 (d, J = 2.8 Hz, 1H), 7.48–7.37 (m, 5H), 5.89 (s, 2H), 5.16 (s, 2H), 1.99 (s, 6H).

5-(Benzyloxy)-3-bromopyridin-2-amine (SI-50)

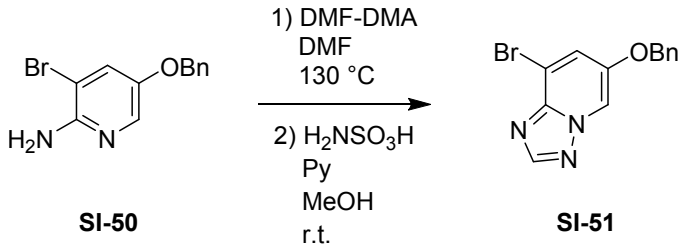


SI-49 **SI-50**

Compound **SI-49** (8.00 g, 22 mmol), hydroxylamine hydrochloride (32.0 g, 460 mmol), triethylamine (6.70 mL, 47 mmol) and EtOH/water = 2/1 (240 mL) were mixed, and then stirred under reflux for 2 days. After cooling to room temperature, the resulting mixture was extracted three times with AcOEt (200 mL, 50 mL, 50 mL). The organic layer was washed successively with water and brine, and dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The obtained residue was purified by column chromatography (*n*-hexane/AcOEt = 3/1) to give the title compound **SI-50** (5.03 g, 80% yield).

^{1}H NMR (400 MHz, CDCl3) δ: 7.83 (d, J = 2.6 Hz, 1H), 7.41 (d, J = 2.6 Hz, 1H), 7.40–7.31 (m, 5H), 5.00 (s, 2H), 4.59 (br s, 2H).

6-(Benzyloxy)-8-bromo-[1,2,4]triazolo[1,5-*a*]pyridine (SI-51)

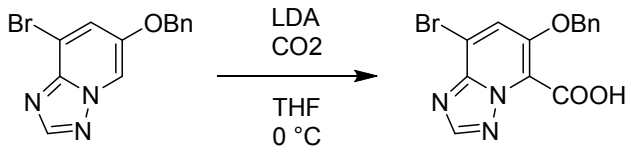


SI-50 **SI-51**

To a solution of **SI-50** (7.25 g, 26.0 mmol) in DMF (11 mL) was added *N*,*N*-dimethylformamide dimethyl acetal (11 mL, 77.6 mmol) at room temperature. After stirring at 130 °C for 15 min, the reaction mixture was concentrated under reduced pressure. The obtained residue was dissolved in MeOH (58 mL). To the solution were added successively pyridine (4.2 mL, 51.9 mmol) and sulfamic acid (4.1 g, 36.3 mmol) under ice-cooling. The mixture was gradually warmed to room temperature and stirred overnight, then water (29 mL) and saturated $NaHCO_3$ aq. (58 mL) were poured dropwise into the reaction mixture under ice-cooling. The precipitated solid was collected by filtration to give the title compound **SI-51** (6.13 g, 78% yield).

^{1}H NMR (400 MHz, CDCl3) δ: 8.29 (s, 1H), 8.19 (d, J = 2.4 Hz, 1H), 7.66 (d, J = 2.4 Hz, 1H), 7.44–7.36 (m, 5H), 5.09 (s, 2H).

6-(Benzyloxy)-8-bromo-[1,2,4]triazolo[1,5-*a*]pyridine-5-carboxylic acid (SI-52)

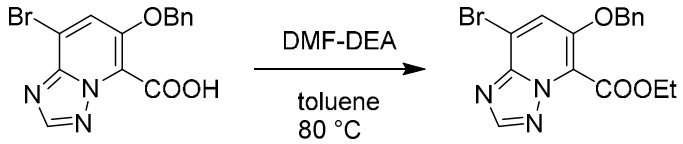


SI-51 **SI-52**

Under a nitrogen stream, to a solution of **SI-51** (10 g, 32.9 mmol) in THF (150 mL) was added dropwise lithium diisopropylamide (2.0 M in THF/heptane/ethylbenzene) (33 mL, 66 mmol) at −78 °C. After stirring at −78 °C for 1 h, CO_2 gas was bubbled through the reaction mixture for 30 min. To the reaction mixture was added water/THF = 1/10 (13.2 mL) at −78 °C. The mixture was gradually warmed to room temperature, then water (200 mL) was poured dropwise into the reaction mixture. The aqueous layer was washed three times with AcOEt (150 mL, 100 mL, 100 mL), and the organic layers were combined and extracted three times with 1N NaOH aq. (100 mL). The aqueous layers were combined and acidified with 6N HCl aq. (75 mL). The precipitated solid was collected by filtration to give the title compound **SI-52** (5.67 g, 49% yield).

^{1}H NMR (400 MHz, DMSO-d6) δ: 8.56 (s, 1H), 8.37 (s, 1H), 7.49–7.33 (m, 5H), 5.33 (s, 2H).

Ethyl 6-(benzyloxy)-8-bromo-[1,2,4]triazolo[1,5-*a*]pyridine-5-carboxylate (SI-53)

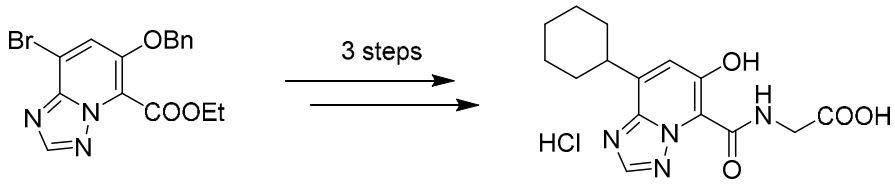


SI-52 **SI-53**

To a solution of **SI-52** (5.68 g, 17.9 mmol) in toluene (57 mL) was added *N*,*N*-dimethylformamide diethyl acetal (4.5 mL, 26.3 mmol) in three portions at 80 °C. After completion of the reaction was confirmed by TLC, the reaction mixture was concentrated under reduced pressure. The obtained residue was purified by column chromatography ($CHCl_3$/AcOEt = 10/1–4/1) to give the title compound **SI-53** (4.68 g, 69% yield).

^{1}H NMR (400 MHz, CDCl3) δ: 8.38 (s, 1H), 7.72 (s, 1H), 7.43–7.35 (m, 5H), 5.21 (s, 2H), 4.53 (q, J = 7.1 Hz, 2H), 1.39 (t, J = 7.1 Hz, 3H).

(8-Cyclohexyl-6-hydroxy-[1,2,4]triazolo[1,5-*a*]pyridine-5-carbonyl)glycine hydrochloride (17)



SI-53 **17**

Compound **17** was prepared by a method similar to the synthesis of **9**.

^{1}H NMR (400 MHz, DMSO-d6) δ: 13.29 (br s, 1H), 10.42 (t, J = 5.6 Hz, 1H), 8.63 (s, 1H), 7.33 (s, 1H), 4.25 (d, J = 5.6 Hz, 2H), 3.23–3.13 (m, 1H), 1.98–1.89 (m, 2H), 1.88–1.79 (m, 2H), 1.79–1.71 (m, 1H), 1.68–1.56 (m, 2H), 1.50–1.37 (m, 2H), 1.35–1.25 (m, 1H).

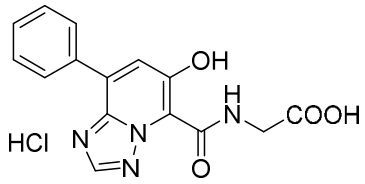
HRMS m/z: $[M-H]^-$ calcd for C15H17N4O4, 317.1255; found, 317.1253.

Purity: 100.0%.

Synthesis of inhibitors 18–20

Compounds **18**–**20** were prepared by a method similar to the synthesis of **17**. In these cases, corresponding coupling reagents were used instead of 2-(cyclohex-1-en-1-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane.

(6-Hydroxy-8-phenyl-[1,2,4]triazolo[1,5-*a*]pyridine-5-carbonyl)glycine hydrochloride (18)

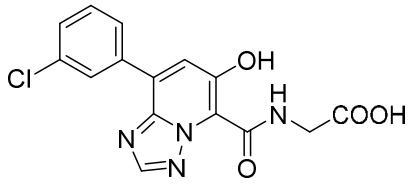


^{1}H NMR (400 MHz, DMSO-d6) δ: 13.36 (s, 1H), 10.53 (t, J = 5.4 Hz, 1H), 8.74 (s, 1H), 8.24–8.19 (m, 2H), 7.80 (s, 1H), 7.60–7.52 (m, 3H), 4.28 (d, J = 5.4 Hz, 2H).

HRMS m/z: $[M-H]^-$ calcd for C15H11N4O4, 311.0786; found, 311.0784.

Purity: 100.0%.

(8-(3-Chlorophenyl)-6-hydroxy-[1,2,4]triazolo[1,5-*a*]pyridine-5-carbonyl)glycine (19)

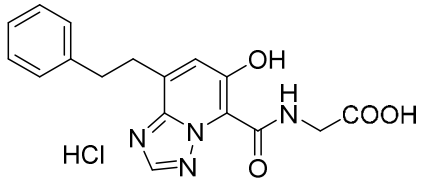


^{1}H NMR (400 MHz, DMSO-d6) δ: 13.36 (br s, 1H), 13.07 (br s, 1H), 10.54 (t, J = 5.6 Hz, 1H), 8.76 (s, 1H), 8.39–8.37 (m, 1H), 8.21–8.15 (m, 1H), 7.92 (s, 1H), 7.64–7.58 (m, 2H), 4.28 (d, J = 5.6 Hz, 2H).

HRMS m/z: $[M-H]^-$ calcd for C15H10ClN4O4, 345.0396; found, 345.0399.

Purity: 100.0%.

(6-Hydroxy-8-phenethyl-[1,2,4]triazolo[1,5-*a*]pyridine-5-carbonyl)glycine (20)



^{1}H NMR (400 MHz, DMSO-d6) δ: 13.25 (br s, 1H), 10.41 (t, J = 5.6 Hz, 1H), 8.66 (s, 1H), 7.35 (s, 1H), 7.30–7.15 (m, 5H), 4.24 (d, J = 5.6 Hz, 2H), 3.27 (t, J = 7.8 Hz, 2H), 3.08 (t, J = 7.8 Hz, 2H).

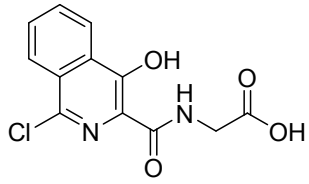
HRMS m/z: $[M-H]^-$ calcd for C17H15N4O4, 339.1099; found, 339.1097.

Purity: 100.0%.

Synthesis of FG-2216 and FG-4592

FG-2216 and **FG-4592** were prepared by previously reported procedures.[5]

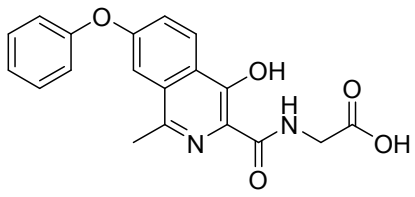
(1-Chloro-4-hydroxyisoquinoline-3-carbonyl)glycine (FG-2216)



FG-2216

^{1}H NMR (400 MHz, DMSO-d6) δ: 13.67 (s, 1H), 12.84 (s, 1H), 9.22 (t, J = 6.2 Hz, 1H), 8.39–8.33 (m, 1H), 8.33–8.28 (m, 1H), 8.04–7.97 (m, 2H), 4.04 (d, J = 6.2 Hz, 2H).
HRMS m/z: $[M-H]^-$ calcd for C12H8ClN2O4, 279.0178; found, 279.0176.
Purity: 100.0%.

(4-Hydroxy-1-methyl-7-phenoxyisoquinoline-3-carbonyl)glycine (FG-4592)



FG-4592

^{1}H NMR (400 MHz, DMSO-d6) δ: 13.32 (s, 1H), 12.81 (br s, 1H), 9.11 (t, J = 6.2 Hz, 1H), 8.31 (d, J = 9.0 Hz, 1H), 7.63 (d, J = 2.4 Hz, 1H), 7.54 (dd, J = 9.0, 2.4 Hz, 1H), 7.51–7.46 (m, 2H), 7.29–7.24 (m, 1H), 7.21–7.16 (m, 2H), 4.05 (d, J = 6.2 Hz, 2H), 2.71 (s, 3H).
HRMS m/z: $[M-H]^-$ calcd for C19H15N2O5, 351.0986; found, 351.0984.
Purity: 97.0%.

3. Modeling study

Maestro from Schrödinger Suite 2016 was used for graphical displays. Molecular Electrostatic Potential (MEP) maps for all the molecules were calculated on B3LYP/6-31G** optimized geometries of molecules using Jaguar from Schrödinger Suite 2016. The three-dimensional isosurface of the MEP was represented as the electrostatic potential superimposed onto a surface of constant electron density (we used 0.001 e/au^3). Conventionally, the deepest red color represents the most electronegative potential, whereas the deepest blue indicates the most positive potential in the molecule.

4. Biological assay

Enzyme assay

Recombinant proteins of human HIF-PHD2 and VBC complex (a complex of human von Hippel-Lindau protein with a GST-tag, human Elongin B with a Flag-tag and human Elongin C with a His-tag) were prepared by Japan Tobacco Inc. (Osaka, Japan). The enzyme reaction was performed at room temperature for 10 min with 1 nM human HIF-PHD2, 2 μM 2-oxoglutarate, 30 nM HIF-1α peptide (biotin-DLDLEMLAPYIPMDDDFQL, Sigma-Aldrich Japan K.K.), 0.5 mM ascorbic acid, 0.25 mM $FeSO_4$, 120 mM NaCl, 0.2 mM 3-[(3-cholamidopropyl) dimethylammonio] propanesulfonate (CHAPS), 0.1% bovine serum albumin, 50 mM tris-HCl (pH 7.5) and test compound (1% DMSO); an EDTA solution was added to stop the enzyme reaction. Then the potassium fluoride solution containing human VBC complex, anti-GST-cryptate and streptavidin-XL$^{ent!}$

(CIS bio international) were added. The fluorescence intensity was measured at 620 nm for the energy donor excited at a wavelength of 320 nm and at 665 nm for the luminescent reagent using an HTRF® microplate reader (K-101, Kyoritsu Radio Co., Ltd.) to calculate the fluorescence intensity ratio.

EPO production in Hep3B cells

Human Hep3B cells were purchased from American Type Culture Collection and cultured in Eagle-MEM containing 10% fetal bovine serum, 100 units/mL penicillin and 100 μg/mL streptomycin in a CO_2 incubator (37 °C, 5% CO_2). These cells were inoculated into 96-well flat-bottomed plates and on the next day, each test compound was added at appropriate concentrations for the assessment of EPO production. The culture supernatants were collected at 24 hours after the addition of each of the test compounds. A hypoxic condition was established and the EPO concentration of this condition was defined as 100% when the EC_{50} was calculated. The EPO concentration in culture supernatants was measured by human EPO ELISA kit (Stemcell Technologies Inc.).

EPO production in normal mouse and rat

All procedures related to the use of animals in this manuscript were reviewed and approved by the Institutional Animal Care and Use Committee of Japan Tobacco Inc., Central Pharmaceutical Research Institute. Male balb/c mice and CD (SD) rats (Charles River Japan) were orally administered a single dose of 10 mg/kg [0.5% methyl cellulose (MC) suspension] of each of the test compounds, and eight hours after the administration, the plasma samples were collected. The murine and rat plasma EPO concentrations were measured by ELISA kit (R&D systems Inc.) or RIA kit (LSI Medience Corporation), respectively.

Erythropoiesis-stimulating effect in normal rat

The vehicle solution (0.5% MC) or test compound suspension at appropriate doses were administered orally to the male CD (SD) rats once daily for 28 days. Blood were collected from each rat to measure the hemoglobin concentrations using a hematology analyzer (ADVIA® 120, Siemens Healthcare K.K.).

These assay data are summarized in table S1 and table S2 with standard deviations.

Table S1. biological assay data[a]

Compd	IC_{50}[b]	EC_{50}[b]	Plasma EPO (10 mg/kg, po)[c]	
	PHD2 (μM)	Hep3B (μM)	mouse (pg/ml)	rat (pg/ml)
FG-2216	7.5 ± 0.44	79 ± 10	-	-
FG-4592	2.4 ± 0.19	19 ± 1.0	-	-
1	16 ± 2.3	>30	-	-
2	6.5 ± 0.21	>30	-	-
3	1.2 ± 0.083	>30	-	-
4	0.82 ± 0.10	>30	-	-
5	0.65 ± 0.17	>30	-	-
6	0.73 ± 0.050	14 ± 1.3	<300	-
7	0.45 ± 0.047	5.8 ± 0.77	3700 ± 2900	2200 ± 360
8	0.85 ± 0.15	4.2 ± 0.37	2300 ± 1300	11000 ± 1800
9	2.5 ± 0.31	9.3 ± 1.7	<330	-
10	7.1 ± 0.52	21 ± 1.5	1100 ± 660	-
11	0.73 ± 0.21	4.7 ± 0.56	540 ± 110	-
12	0.21 ± 0.018	12 ± 0.35	<320	-
13	0.12 ± 0.011	4.5 ± 0.35	>15000	7900 ± 4600
14	0.22 ± 0.068	5.7 ± 0.86	6500 ± 6400	17000 ± 3000
15	0.29 ± 0.031	5.8 ± 0.66	1000 ± 660	-
16	1.6 ± 0.13	15 ± 0.092	<260	-
17	0.72 ± 0.080	17 ± 2.9	<450	-
18	0.19 ± 0.034	14 ± 1.5	<260	-
19	0.11 ± 0.012	5.8 ± 0.55	<265	-
20	0.37 ± 0.068	13 ± 0.28	<320	-

[a]Mean value ± SD. [b]Mean value determined from four replicates. [c]Mean value determined from three replicates.

Table S2. Hemoglobin levels in erythropoiesis stimulation test [a]

Compd	Hemoglobin (g/dL)						
	Day						
	1	4	8	11	15	22	29
vehicle	16.1 ± 0.677	16.2 ± 0.351	15.8 ± 0.410	16.1 ± 0.775	16.3 ± 0.559	16.3 ± 0.727	15.0 ± 0.735
7 (3 mg/kg)	15.9 ± 0.582	16.0 ± 0.641	15.9 ± 0.669	16.1 ± 0.777	16.1 ± 0.864	16.0 ± 1.16	16.2 ± 0.869
7 (10 mg/kg)	15.8 ± 0.704	16.4 ± 0.905	16.9 ± 0.310	17.7 ± 0.657	18.8 ± 0.822	20.0 ± 1.66	21.1 ± 1.22
14 (1 mg/kg)	16.0 ± 0.675	16.3 ± 0.957	16.1 ± 0.980	16.5 ± 0.805	17.1 ± 0.967	16.8 ± 0.836	16.7 ± 0.561
14 (3 mg/kg)	16.0 ± 0.751	16.4 ± 0.650	17.3 ± 0.455	18.0 ± 0.641	18.0 ± 0.836	18.9 ± 1.45	20.0 ± 1.03

[a]Mean value ± SD.

5. Pharmacokinetics

Rat PK (IV, PO)

Male CD(SD) rats (Charles River Laboratories (Japan)) were intravenously or orally administered a single dose of JTZ-951 at 0.3 mg/kg (60% dimethylsulfoxide solution) or 1.0 mg/kg (0.5% methylcellulose), respectively. After the administration, the plasma samples were collected over a period of 24 h. The time-course of the plasma concentrations of JTZ-951 was analyzed by non-compartmental analysis and the pharmacokinetic parameters were calculated.

Metabolic stability in liver microsomes

14C-JTZ-951 (final concentration: 10 μmol/L) was incubated in the presence of pooled liver microsomes (protein concentration: 1 mg protein/mL) prepared from male rats (SD rats: 400 animals), male dogs (beagles: eight animals), male monkeys (cynomolgus monkeys: 10 animals) and male and female humans (25 males and 25 females) at 37 °C for two hours in the presence of nicotinamide adenine dinucleotide phosphate (NADPH). After the reaction, the amounts of JTZ-951 in the samples were determined by Radio-HPLC.

***In vitro* Caco-2 Permeability study**

A sample of the test compound (final concentrations: 25 μmol/L) was added to the apical side of Caco-2 cell monolayers, and incubated at 37 °C for 2 h. After incubation, the transported amounts of test compound were measured by liquid chromatography/tandem mass spectrometry (LC/MS/MS). Apparent permeability coefficients (Papp) were calculated from the transported amounts.

6. Others

CLogP

The CLogP data were calculated using DAYLIGHT software (DAYLIGHT Chemical Information Systems, Inc., version 4.95).

Solubility study

Test compound solutions were placed in 96 well plates, and DMSO was removed with a centrifugal evaporator for 2 h. FeSSIF solvent was added to each well. The plates were mixed at 2500 rpm at room temperature for 4 h. Incubation samples were filtered twice with 96 well filtration plates. An aliquot of the second filtrate and acetonitrile were mixed, and injected into the LC/MS to quantify the amount of each compound in the filtrates.

CYP inhibition assay

JTZ-951 (final concentrations: 0, 1, 3, 10, 30, and 100 μmol/L) and the model substrates for each CYP isoform (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, and 3A4/5 [testosterone and midazolam]) were incubated with human liver microsomes prepared from male and female humans (male: 31 subjects, female: 19 subjects) at 37 °C for the designated time in the presence of NADPH. After incubation, the metabolites of the model substrates were analyzed by LC/MS/MS and the metabolic rates were calculated to evaluate the inhibitory potential of JTZ-951.

hERG inhibition assay

The hERG current was measured by the whole cell patch clamp method. hERG-transfected HEK293 cells were cultured in MEM solution containing 10% fetal bovine serum, 1 mmol/L MEM sodium pyruvate solution, 0.1 mmol/L MEM non-essential amino acid solution, 100 U/mL penicillin, 100 μg/mL streptomycin, and 400 μg/mL geneticin. Cells on the cover slip were set in the measurement chamber and the chamber was superfused with the external solution containing (in mM): 137 NaCl, 4 KCl, 1 $MgCl_2 \cdot 6H_2O$, 1.8 $CaCl_2 \cdot 2H_2O$, 10 HEPES and 10 glucose (pH 7.4), maintained at 24 ± 2 °C. The hERG current was measured with a glass electrode (resistance: 2 to 6 MΩ) filled with the internal solution containing (in mM): 130 KCl, 1 $MgCl_2 \cdot 6H_2O$, 5 EGTA, 10 HEPES and 5 MgATP (pH 7.2), through a patch clamp amplifier (EPC-10, HEKA Elektronik). The cell membrane voltage was held at –80 mV by the patch clamp software (PULSE, HEKA Elektronik) with the amplifier. A test pulse consisting of +20 mV for 1.5 seconds and –40 mV for 1.5 seconds was applied with intervals of 15 seconds. The currents before and 11 minutes after initiation of the treatment with the vehicle and test article were analyzed.

7. References

1) CAUTION: Explosions have been reported involving this reagent and so it is prepared immediately prior to use and that it should not be stored.
2) Nishigaya, Y.; Umei, K.; Yamamoto, E.; Kohno, Y.; Seto, S. Direct access to 2-aminopyrazolo[1,5-*a*]pyridines via *N*-amination/cyclization reactions of 2-pyridineacetonitriles. *Tetrahedron Lett*. **2014**, *55*, 5963–5966.

3) Ning, R. Y. *Chem. Eng. News*, **1973**, *51*, 36

4) *O*-(Mesitylsulfonyl)hydroxylamine was prepared as a dichloromethane solution by the following method. To a solution of ethyl *N*-(mesitylsulfonyl)oxyacetimidate (6.50 g, 22.8 mmol) in dioxane (6.5 mL) was added dropwise 70% perchloric acid aq. (2.00 mL, 23.3 mmol) under ice-cooling. After stirring under ice-cooling for 30 min, the progress of the reaction was checked by TLC. Since the starting material remained, 70% perchloric acid aq. (0.4 mL) was added. After stirring under ice-cooling for 10 min, the reaction mixture was poured into ice-water (26 mL). The precipitated solid was filtered and washed with water, then the filtrate was dissolved in cold dichloromethane (26 mL). After water was removed using a separating funnel, the organic layer was dried over Na_2SO_4.

5) Arend, M. P.; Flippin, L. A.; Guenzler-Pukall, V.; Ho, W.-B.; Turtle, E. D.; Du, X. Nitrogen-containing heteroaryl compounds and their use in increasing endogenous erythropoietin. WO2004108681A1, 2004; Caplus AN 2004:1080867.