Discovery of 4-Azaindole Inhibitors of TGFβRI as Immuno-oncology Agents

Yong Zhang*‡, Yufen Zhao‡, Andrew J. Tebben, Steven Sheriff, Max Ruzanov, Mark P. Fereshteh, Yi Fan, Jonathan Lippy, Jesse Swanson, Ching-Ping Ho, Barri S. Wautlet, Anne Rose, Karen Parrish, Zheng Yang, Andrew F. Donnell, Liping Zhang, Brian E. Fink, Gregory D. Vite, Karen Augustine-Rauch, Joseph Fargnoli, Robert M. Borzilleri

†Bristol-Myers Squibb Research and Development, PO Box 4000, Princeton, NJ 08543, USA

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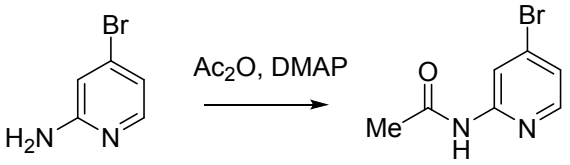
Experimentals

General Methods. All reagents and solvents were purchased from commercial sources and used without further purification. All final products were complex mixtures of amide rotamers at room temperature. ^{1}H and ^{13}C NMR spectra were obtained on a 400 or 500 MHz Bruker instrument with the major conformer reported. Chemical shifts are expressed in units of δ (parts per million, ppm) relative to the solvent peaks ($CDCl_3$ at 7.26 ppm, CD_3OD at 3.31 ppm, DMSO-d_6 at 2.50 ppm). Peak multiplicities are designated as follows: s, singlet; d, doublet; dd, doublet of doublets; t, triplet; q, quartet; br s, broad singlet; m, multiplet. Reaction progress was monitored by LC/MS on a Waters 3100 single quadrupole mass spectrometer interfaced to a Waters Acquity ultra performance liquid chromatograph (UPLC), employing a 2.1 x 50 mm, 1.7 μm, Acquity BEH C18 column using linear gradient elution with H_2O/MeCN mixtures buffered with 0.05% TFA at 0.8 mL/min and UV detection at 220 nm. Electrospray ionization (ESI) high resolution mass spectrometry (HRMS) was obtained on a Thermo Fisher Finnigan LTQ-FT instrument. Flash chromatography was performed using prepacked RediSep® Rf silica gel columns on a CombiFlash® Rf purification system, or RediSep® Rf Gold silica gel columns on a CombiFlash Torrent® purification system. Preparative reverse phase HPLC was performed with a linear gradient elution using H_2O/MeCN mixtures buffered with 0.1% TFA and detection at 220 nm on a SunFire™ C18 10 x 150 mm, 5 μm column. All final compounds were isolated in ≥95% purity as assessed by analytical reverse phase HPLC.

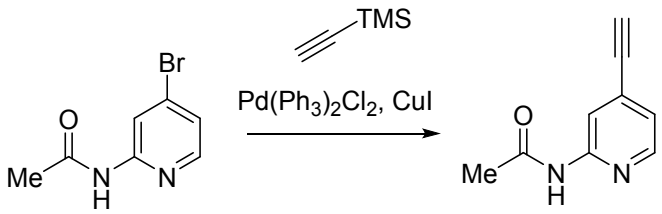
Compound **2a**

Step 1: N-(4-Bromopyridin-2-yl)acetamide



To a mixture of 4-bromopyridin-2-amine (3.8 g, 22 mmol) in Ac_2O (20 mL) was added DMAP (0.054 g, 0.44 mmol). The mixture was heated in a sealed pressure reaction vessel at 140 °C for 1 h, and then cooled to rt. The reaction mixture was poured into ice-water, and the resulting mixture was adjusted to pH = 8.4 with NH_4OH. The resulting precipitate was collected by filtration, washed with water and dried to afford N-(4-bromopyridin-2-yl)acetamide (4.2 g, 88%). HPLC: RT = 0.587 min (H_2O/MeOH with 0.1% TFA, Chromolith SpeedROD, 4.6x50mm, gradient = 4 min, wavelength = 220 nm); MS (ES): calculated for $C_7H_7BrN_2O$ 213.97, found *m/z* = 215/217 $[M+H]^+$; ^1H NMR (400 MHz, Chloroform-d) δ ppm 10.71 (br. s., 1H), 8.32 (d, *J* = 1.8 Hz, 1H), 8.22 (d, *J* = 5.5 Hz, 1H), 7.35 (dd, *J* = 5.3, 1.8 Hz, 1H), 2.11 (s, 3H).

Step 2: N-(4-Ethynylpyridin-2-yl)acetamide

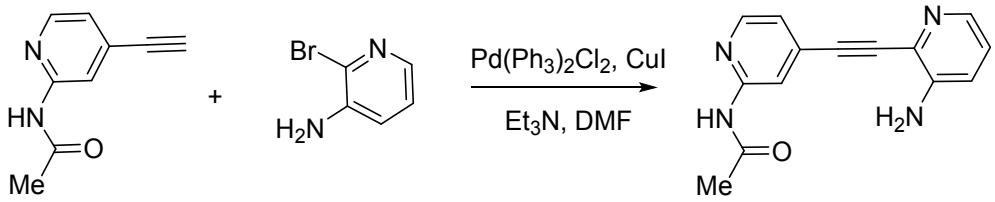


A mixture of N-(4-bromopyridin-2-yl)acetamide (4.2 g, 19.3 mmol), ethynyltrimethylsilane (2.28 g, 23.2 mmol), bis(triphenylphosphine)palladium(II) dichlororide (0.27 g, 0.39 mmol) and copper(I) iodide (0.15 g, 0.77 mmol) in TEA (20 mL, 143 mmol) was purged with nitrogen and heated to 76 °C for 2.5 h, and then cooled to rt. The mixture was filtered through a short silica gel column, eluting with EtOAc. The filtrate was concentrated to give crude N-(4-((trimethylsilyl)ethynyl)pyridin-2-yl)acetamide.

The above solid was dissolved in THF (60 mL), and treated with TBAF (12.6 g, 48.2 mmol) in one portion. The reaction mixture was stirred at room temperature for 2 h and then concentrated to dryness. The residue was treated with water and extracted with EtOAc (3 x). The combined

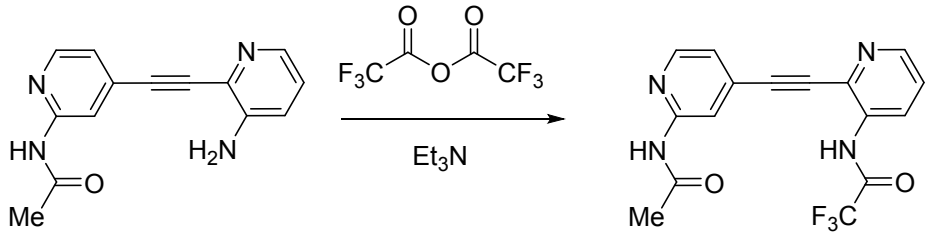
extracts were washed with brine, dried ($MgSO_4$), and then concentrated. The residue was purified by silica gel flash chromatography (12 g column, EtOAc/DCM = 0-100%) to afford N-(4-ethynylpyridin-2-yl)acetamide (2.89 g, 94%) as a light yellow solid. HPLC: RT = 0.43 min (H_2O/MeOH with 0.1% TFA, Chromolith SpeedROD, 4.6x50mm, gradient = 4 min, wavelength = 220 nm); MS (ES): calculated for $C_9H_8N_2O$ 160.06, found *m/z* = 161 $[M+H]^+$; ^1H NMR (400 MHz, Chloroform-d) δ ppm 8.32 (s, 1H), 8.24 (dd, *J*=5.1, 0.7 Hz, 1H), 8.05 (br. s., 1H), 7.11 (dd, *J*=5.1, 1.5 Hz, 1H), 3.30 (s, 1H), 2.23 (s, 3H).

Step 3: N-(4-((3-Aminopyridin-2-yl)ethynyl)pyridin-2-yl)acetamide



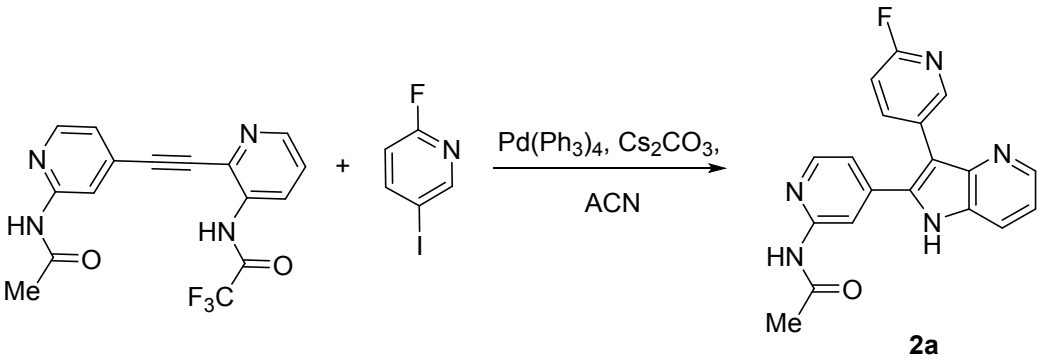
A mixture of 2-bromopyridin-3-amine (3.40 g, 19.7 mmol), N-(4-ethynylpyridin-2-yl)acetamide (3.0 g, 18.7 mmol), bis(triphenylphosphine)palladium(II) dichloride (0.13 g, 0.19 mmol) and copper(I) iodide (0.11 g, 0.56 mmol) in TEA (24 mL) and DMF (8 mL) was purged with N_2, and then heated at 76 °C for 1 h. The reaction mixture was cooled to rt, and then concentrated and the residue was purified by silica gel flash chromatography (40 g column, MeOH/DCM = 0-12%) to afford N-(4-((3-aminopyridin-2-yl)ethynyl)pyridin-2-yl)acetamide (5.5 g) as a yellow solid. HPLC: RT = 0.95 min (H_2O/MeOH with 0.1% TFA, Chromolith SpeedROD, 4.6x50mm, gradient = 4 min, wavelength = 220 nm); MS (ES): calculated for $C_{14}H_{12}N_4O$ 252.1, found *m/z* = 253 $[M+H]^+$; ^1H NMR (400 MHz, Chloroform-d) δ ppm 8.38 (s, 1H), 8.28 (dd, *J* = 5.1, 0.9 Hz, 1H), 8.08 (dd, *J* = 4.5, 1.4 Hz, 1H), 7.96 (br. s., 1H), 7.23 (dd, *J* = 5.2, 1.4 Hz, 1H), 7.16 - 7.10 (m, 1H), 7.09 - 7.04 (m, 1H), 4.34 (br. s., 2H), 2.25 (s, 3H).

Step 4: N-(2-((2-Acetamidopyridin-4-yl)ethynyl)pyridin-3-yl)-2,2,2-trifluoroacetamide



To a suspension of N-(4-((3-aminopyridin-2-yl)ethynyl)pyridin-2-yl)acetamide (335 mg, 1.33 mmol) in DCM (15 mL) was added TEA, and the mixture was cooled to 0 °C. Trifluoroacetic anhydride (0.28 mL, 1.99 mmol) was slowly added. The reaction mixture was stirred at 0 °C for 30 min and then diluted with DCM. The solution was washed with sat.aq.$NaHCO_3$ and brine, dried ($MgSO_4$), and then concentrated. The residue was sonicated with DCM/hexane, and the resulting solid was collected by filtration to afford N-(2-((2-acetamidopyridin-4-yl)ethynyl)pyridin-3-yl)-2,2,2-trifluoroacetamide (412 mg, 71%) as a yellow solid. HPLC: RT = 1.530 min (H_2O/MeOH with 0.1% TFA, Chromolith SpeedROD, 4.6x50mm, gradient = 4 min, wavelength = 220 nm); MS (ES): calculated for $C_{16}H_{11}F_3N_4O_2$ 348.08, found *m/z* = 349 $[M+H]^+$; ^1H NMR (400MHz, Chloroform-d) δ ppm 8.86 - 8.69 (m, 2H), 8.54 (dd, *J* = 4.8, 1.5 Hz, 1H), 8.45 (s, 1H), 8.35 (dd, *J* = 5.1, 0.9 Hz, 1H), 7.99 (br. s., 1H), 7.44 (dd, *J* = 8.5, 4.7 Hz, 1H), 7.21 (dd, *J* = 5.1, 1.3 Hz, 1H), 2.26 (s, 3H).

Step 5. N-{4-[3-(6-Fluoropyridin-3-yl)-1H-pyrrolo[3,2-b]pyridin-2-yl]pyridin-2-yl}acetamide

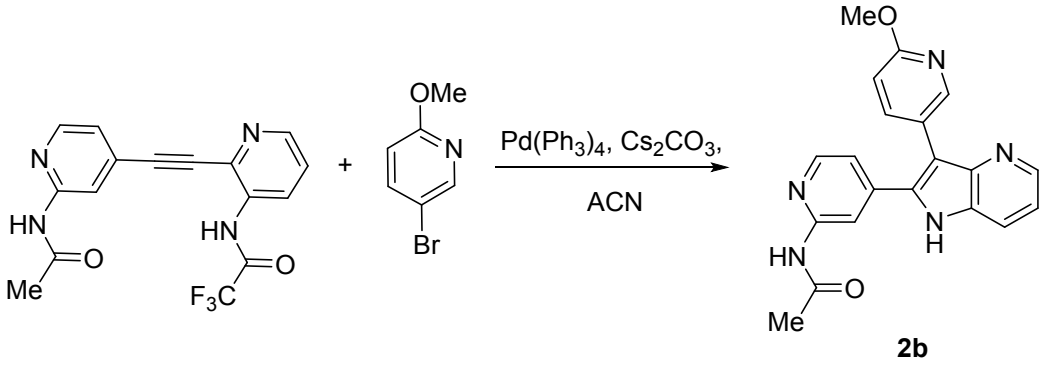


2a

A mixture of N-(2-((2-Acetamidopyridin-4-yl)ethynyl)pyridin-3-yl)-2,2,2-trifluoroacetamide

(40 mg, 0.12 mmol), 2-fluoro-5-iodopyridine (30.7mg, 0.14 mmol), $Pd(Ph_3P)_4$ (6.6 mg, 5.7 μmol) and Cs_2CO_3 (112 mg, 0.35 mmol) in acetonitrile (1 mL) was purged with N_2. The mixture was then heated at 100 °C in a sealed vial for 2 h, and then cooled to room temperature. The solid was filtered off, and rinsed with 10% MeOH/DCM, and the filtrate was concentrated. The residue was dissolved in DMF, and purified by preparative HPLC (Waters XBridge C-18 19 x 200 mm, eluting with 10%-75% aqueous CH_3CN containing 5 mmol NH_4OAc over 20 min, 30 mL/min, wavelength = 254 nm) to give compound **2a** (14.3 mg, 35%) as a white solid. HPLC: RT = 0.58 min (H_2O/ACN with 0.1% TFA, Waters Acquity UPLC BEH C18, 2.1 x 50 mm, 1.7-μm particles, gradient = 3 min, wavelength = 220 nm). MS (ES): calculated for $C_{19}H_{14}FN_5O$ 347.12, found *m/z* = 348 $[M+H]^+$; ^1H NMR (500 MHz, DMSO-d_6) δ ppm 12.18 (s, 1H), 10.61 (s, 1H), 8.44 (d, *J*=4.4 Hz, 1H), 8.36 (d, *J*=5.0 Hz, 1H), 8.29 (s, 2H), 8.16 - 8.05 (m, 1H), 7.91 (d, *J*=8.1 Hz, 1H), 7.34 - 7.21 (m, 2H), 7.17 (d, *J*=5.0 Hz, 1H), 2.09 (s, 3H).

Compound **2b**

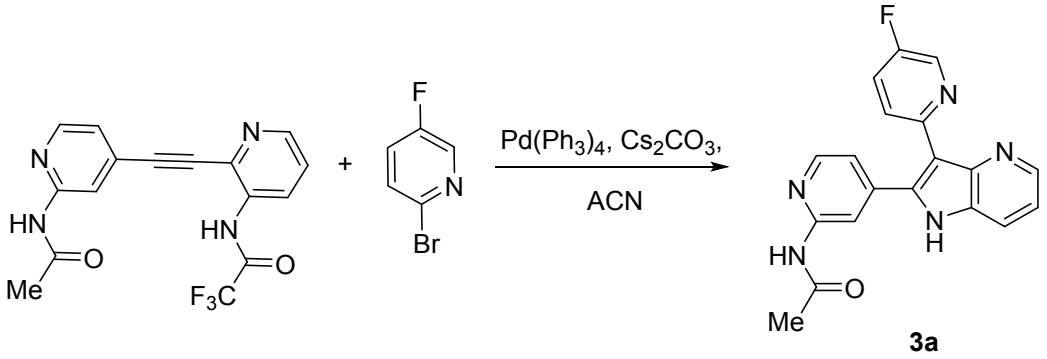


2b

A mixture of N-(2-((2-acetamidopyridin-4-yl)ethynyl)pyridin-3-yl)-2,2,2-trifluoroacetamide (30 mg, 0.09 mmol), 5-bromo-2-methoxypyridine (19.4 mg, 0.1 mmol), $Pd(Ph_3P)_4$ (5 mg, 4.3 μmol) and Cs_2CO_3 (84 mg, 0.26 mmol) in acetonitrile (1 mL) was purged with N_2. The mixture was then heated at 100 °C in a sealed vial for 2 h, and then cooled to room temperature. The solid was filtered off, and rinsed with 10% MeOH/DCM, and the filtrate was concentrated. The residue was dissolved in DMF, and purified via preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid;

Gradient: 0-50% B over 20 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. LCMS (ES): calculated for $C_{20}H_{17}N_5O_2$ 359.14, found *m/z* = 360 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 12.01 (s, 1H), 10.58 (s, 1H), 8.41 (dd, *J* = 4.5, 1.4 Hz, 1H), 8.37 - 8.29 (m, 2H), 8.26 (d, *J* = 2.0 Hz, 1H), 7.87 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.80 (dd, *J* = 8.5, 2.3 Hz, 1H), 7.25 (dd, *J* = 8.3, 4.5 Hz, 1H), 7.13 (dd, *J* = 5.1, 1.5 Hz, 1H), 6.87 (d, *J* = 8.6 Hz, 1H), 2.10 (s, 3H).

Compound **3a**

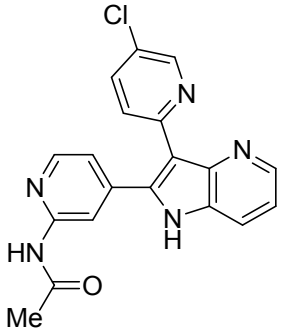


3a

A mixture of N-(2-((2-acetamidopyridin-4-yl)ethynyl)pyridin-3-yl)-2,2,2-trifluoroacetamide (50 mg, 0.14 mmol), 2-bromo-5-fluoropyridine (51 mg, 0.29 mmol), $Pd(Ph_3P)_4$ (8.3 mg, 7.2 µmol) and Cs_2CO_3 (94 mg, 0.29 mmol) in acetonitrile (1.5 mL) was purged with N_2. The mixture was then heated at 110 °C in a sealed vial for 2 h, and then cooled to room temperature. The solid was filtered off, and rinsed with 10% MeOH/DCM, and the filtrate was concentrated. The residue was dissolved in DMF, and purified preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5-µm particles;Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 5-55% B over 20 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. LCMS (ES): calculated for $C_{19}H_{14}FN_5O$ 347.12, found *m/z* = 348 $[M+H]^+$; ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 10.77 (s, 1H), 8.75 - 8.63 (m, 2H), 8.57 (d, *J*=8.4 Hz, 1H), 8.48 (d, *J*=5.4 Hz, 1H), 8.34 (s, 1H), 7.84 - 7.70 (m, 2H), 7.56 (dd, *J*=8.1, 3.7 Hz, 1H), 7.31 (d, *J*=5.0 Hz, 1H), 2.11 (s, 3H).

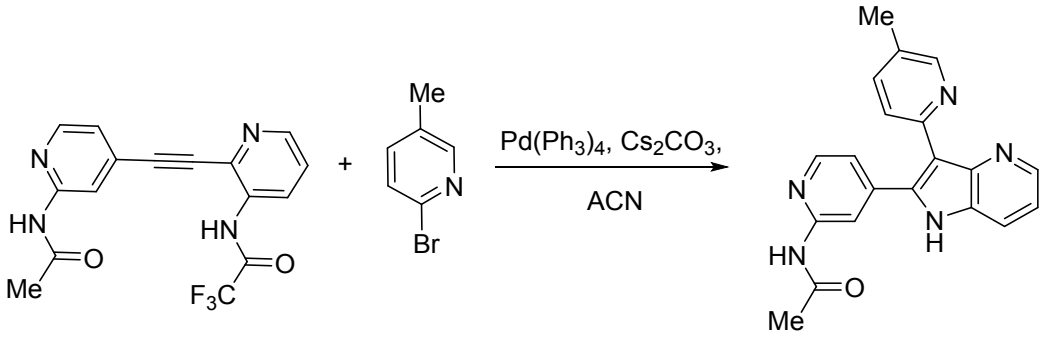
Compound **3b**

N-{4-[3-(5-Chloropyridin-2-yl)-1H-pyrrolo[3,2-b]pyridin-2-yl]pyridin-2-yl}acetamide



A mixture of N-(2-((2-acetamidopyridin-4-yl)ethynyl)pyridin-3-yl)-2,2,2-trifluoroacetamide (40 mg, 0.12 mmol), 2-iodo-5-chloropyridine (33 mg, 0.14 mmol), $Pd(Ph_3P)_4$ (6.6 mg, 5.7 µmol) and Cs_2CO_3 (112 mg, 0.35 mmol) in acetonitrile (1 mL) was purged with N_2. The mixture was then heated at 110 °C in a sealed vial for 2 h, and then cooled to room temperature. The solid was filtered off, and rinsed with 10% MeOH/DCM, and the filtrate was concentrated. The residue was dissolved in DMF, and purified by preparative LC/MS with the following conditions: Waters XBridge C18, 19 x 200 mm, 5-µm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 0-40% B over 25 minutes, then a 5-minute hold at 40% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dired via centrifugal evaporation to give **3b** as a white solid (22.4 mg). HPLC: RT = 0.85 min (H_2O/ACN with 0.1% TFA, Waters Acquity UPLC BEH C18, 2.1 x 50 mm, 1.7-µm particles, gradient = 3 min, wavelength = 220 nm); MS (ES): *m/z* = 364 $[M+H]^+$; ^1H NMR (500 MHz, DMSO-d_6) δ ppm 10.77 (s, 1H), 8.75 - 8.62 (m, 2H), 8.55 - 8.43 (m, 2H), 8.37 (s, 1H), 8.00 - 7.91 (m, 1H), 7.75 - 7.58 (m, 2H), 7.30 (d, *J*=4.4 Hz, 1H), 2.12 (s, 3H).

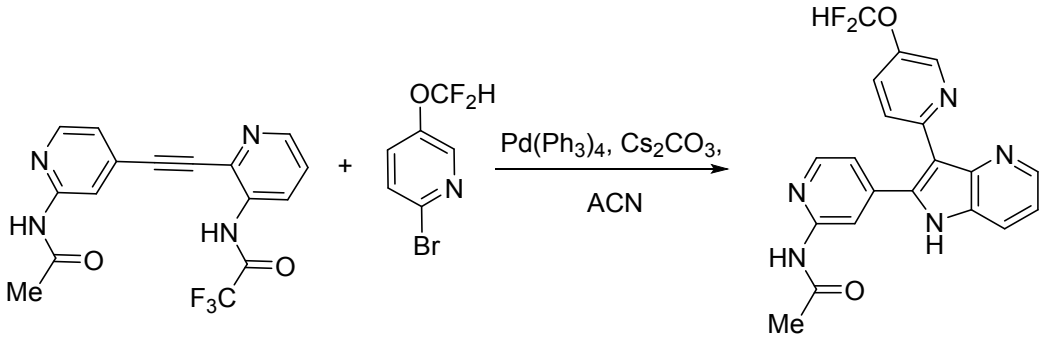
Compound **3c**



3c

A mixture of N-(2-((2-acetamidopyridin-4-yl)ethynyl)pyridin-3-yl)-2,2,2-trifluoroacetamide (30 mg, 0.09 mmol), 2-bromo-5-methylpyridine (22 mg, 0.13 mmol), $Pd(Ph_3P)_4$ (5 mg, 4.3 µmol) and Cs_2CO_3 (84 mg, 0.25 mmol) in acetonitrile (1 mL) was purged with N_2. The mixture was then heated at 110 °C in a sealed vial for 2 h, and then cooled to room temperature. The solid was filtered off, and rinsed with 10% MeOH/DCM, and the filtrate was concentrated. The residue was dissolved in DMF, and purified by preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 250 mm, 5-µm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 0-50% B over 20 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. to give compound **3c** as a white solid (13.7 mg, 46%). LCMS (ES): calculated for $C_{20}H_{17}N_5O$ 343.14, found *m/z* = 344 $[M+H]^+$; ^1H NMR (500 MHz, DMSO-d_6) δ ppm 10.80 (s, 1H), 8.73 - 8.64 (m, 2H), 8.49 (d, *J*=5.0 Hz, 1H), 8.45 (d, *J*=8.1 Hz, 1H), 8.36 (s, 1H), 7.91 (d, *J*=7.7 Hz, 1H), 7.67 (dd, *J*=8.1, 5.4 Hz, 1H), 7.52 (d, *J*=8.4 Hz, 1H), 7.32 (d, *J*=4.0 Hz, 1H), 2.42 (s, 3H), 2.12 (s, 3H).

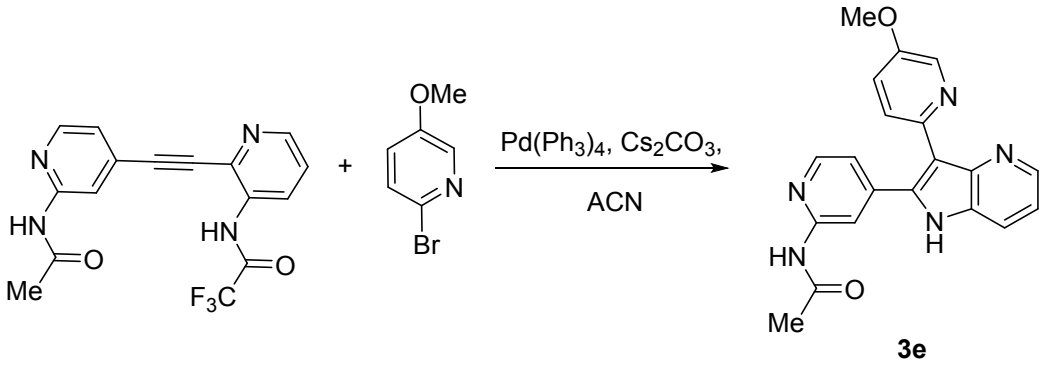
Compound **3d**



3d

A mixture of N-(2-((2-acetamidopyridin-4-yl)ethynyl)pyridin-3-yl)-2,2,2-trifluoroacetamide (50 mg, 0.14 mmol), 2-bromo-4-(difluoromethoxy)pyridine (64 mg, 0.29 mmol), $Pd(Ph_3P)_4$ (8.3 mg, 7.1 μmol) and Cs_2CO_3 (94 mg, 0.29 mmol) in acetonitrile (1 mL) was purged with N_2. The mixture was then heated at 110 °C in a sealed vial for 2 h, and then cooled to room temperature. The solid was filtered off, and rinsed with 10% MeOH/DCM, and the filtrate was concentrated. The residue was dissolved in DMF, and purified preparative LC/MS with the following conditions: Column: XBridge C18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 10-50% B over 19 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. to give compound **3d** as a white solid (9.6 mg, 17%). LCMS (ES): $C_{20}H_{15}F_2N_5O_2$ 359.12, found *m/z* = 396 $[M+H]^+$; ^1H NMR (500 MHz, DMSO-d_6) δ 10.56 (s, 1H), 8.46 (d, *J*=4.0 Hz, 1H), 8.35 (d, *J*=2.4 Hz, 1H), 8.32 - 8.26 (m, 2H), 8.21 (d, *J*=8.7 Hz, 1H), 7.90 (d, *J*=8.1 Hz, 1H), 7.76 (dd, *J*=8.6, 2.6 Hz, 1H), 7.52 - 7.13 (m, 3H), 2.09 (s, 3H).

Compound **3e**

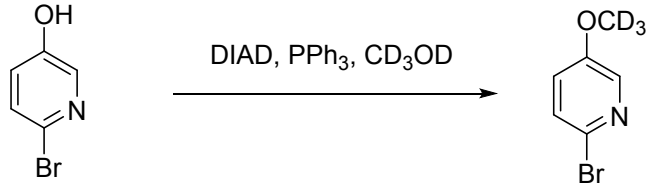


3e

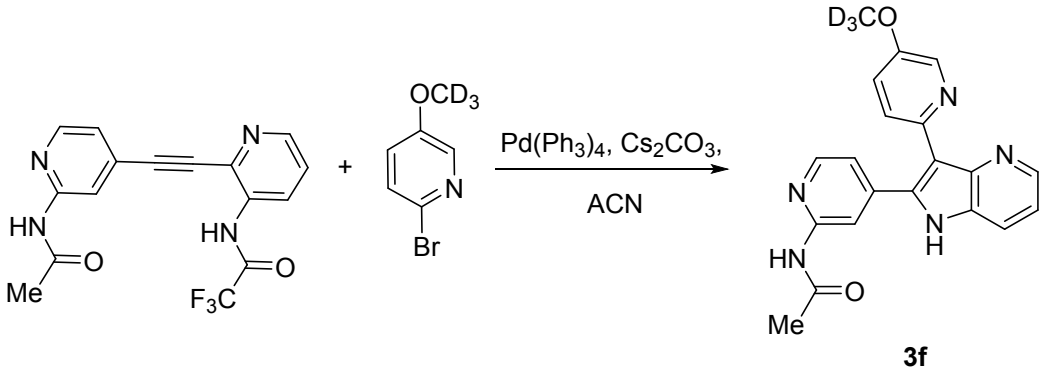
A mixture of N-(2-((2-acetamidopyridin-4-yl)ethynyl)pyridin-3-yl)-2,2,2-trifluoroacetamide (200 mg, 0.57 mmol), 2-bromo-5-methoxypyridine (216 mg, 1.15 mmol), $Pd(Ph_3P)_4$ (33 mg, 29 μmol) and Cs_2CO_3 (374 mg, 1.15 mmol) in acetonitrile (3 mL) was purged with N_2. The mixture was then heated at 110 °C in a sealed vial for 2 h, and then cooled to room temperature. The solid was filtered off, and rinsed with 10% MeOH/DCM, and the filtrate was concentrated. The residue was dissolved in DMF, and purified preparative LC/MS with the following conditions:Column: Luna C18, 21 x 100 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 0-40% B over 19 minutes, then a 5-minute hold at 100% B; Flow:

20 mL/min. to give compound **3e** as a white solid (43.7 mg, 21%). LCMS (ES): calculated for $C_{20}H_{17}N_5O_2$ 359.14, found *m/z* = 360 $[M+H]^+$; ^1H NMR (400 MHz, DMSO-d_6) δ 11.97 (s, 1H), 10.51 (s, 1H), 8.44 (dd, *J*=4.5, 1.4 Hz, 1H), 8.32 (s, 1H), 8.28 (dd, *J*=5.1, 0.7 Hz, 1H), 8.21 - 8.15 (m, 1H), 8.08 - 8.00 (m, 1H), 7.87 (dd, *J*=8.3, 1.4 Hz, 1H), 7.49 (dd, *J*=8.7, 3.0 Hz, 1H), 7.25 (dd, *J*=8.3, 4.5 Hz, 1H), 7.14 (dd, *J*=5.3, 1.5 Hz, 1H), 3.86 (s, 3H), 2.10 (s, 3H).

Compound **3f**



To a solution of 6-bromopyridin-3-ol (10.00 g, 57.5 mmol), methan-d_3-ol-d (12.44 g, 345 mmol), and triphenylphosphine (15.83 g, 60.3 mmol) in dioxane (80 mL) at 0 °C was added dropwise DIAD (11.73 mL, 60.3 mmol). The reaction was stirred at 0 °C for 10 min before warmed to rt and stirred overnight. The reaction was then concentrated and then triturated with DCM (~ 60 mL). The mixture was filtered to remove phosphine oxide by-product. The filtrate was concentrated and purified on ISCO (0-10% MeOH/DCM, 120 g column) to give 2-bromo-5-(methoxy-d_3)pyridine (7.1 g, 64.7 % yield). LCMS (ES): calculated for $C_6H_3D_3BrNO$ 189.98, found *m/z* = 191 $[M+H]^+$; ^1H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J*=3.2 Hz, 1H), 7.39 (dd, *J*=8.7, 0.6 Hz, 1H), 7.12 (dd, *J*=8.7, 3.2 Hz, 1H)



3f

A mixture of N-(2-((2-acetamidopyridin-4-yl)ethynyl)-5-methoxypyridin-3-yl)-2,2,2-trifluoroacetamide (3.5g, 9.25 mmol) and cesium carbonate (6.03 g, 18.50 mmol) was evacuated and backfilled with N_2, then MeCN (61.7 ml) and 2-bromo-5-(methoxy-d_3)pyridine (3.53 g, 18.50 mmol) were added. The mixture was sparged with N_2 for 5 min, then $Pd(Ph_3P)_4$ (0.535 g,

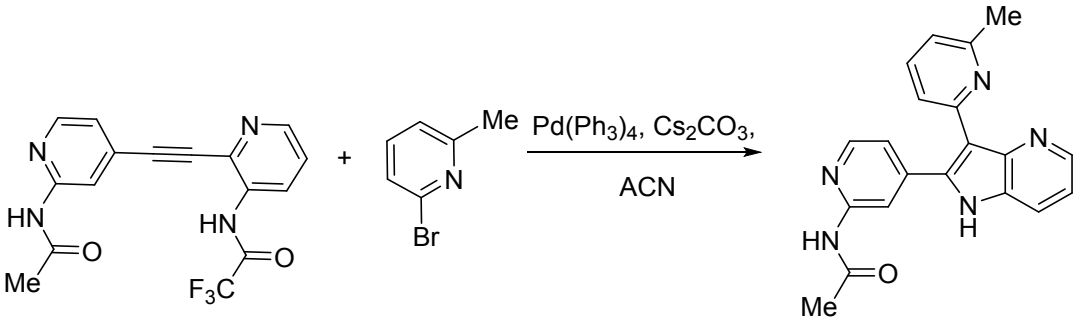
0.463 mmol) was added. The reaction was sparged with N_2 for 1 min, then it was sealed and stirred at 110 °C for 2 h The reaction was cooled to rt. The crude mixture was taken up in 10% MeOH/DCM (100 mL) and then filtered through a pad of Celite, washing with 10% MeOH/DCM. The combined filtrate was concentrated and purified by ISCO (gold column, 220 g, 0-10% MeOH/DCM) to give compound **3f** as a white solid (1.52 g, 4.11 mmol, 44.4 % yield).

LCMS (ES): calculated for $C_{20}H_{14}D_3N_5O_2$ 362.16, found *m/z* = 363 $[M+H]^+$;

^{1}H NMR (400 MHz, DMSO-d_6) δ 11.97 (s, 1H), 10.52 (s, 1H), 8.44 (dd, *J* = 4.5, 1.5 Hz, 1H), 8.32 (s, 1H), 8.28 (dd, *J* = 5.1, 0.6 Hz, 1H), 8.19 (dd, *J* = 3.1, 0.6 Hz, 1H), 8.06 - 8.01 (m, 1H), 7.86 (dd, *J* = 8.2, 1.3 Hz, 1H), 7.49 (dd, *J* = 8.7, 3.1 Hz, 1H), 7.25 (dd, *J* = 8.2, 4.6 Hz, 1H), 7.14 (dd, *J* = 5.3, 1.6 Hz, 1H), 2.10 (s, 3H).

^{13}C NMR (101 MHz, DMSO-d_6) δ 169.7, 154.3, 152.8, 148.0, 145.4, 145.0, 144.1, 142.5, 137.1, 136.6, 129.6, 125.9, 121.0, 119.5, 118.4, 115.0, 113.0, 24.4

Compound **3g**

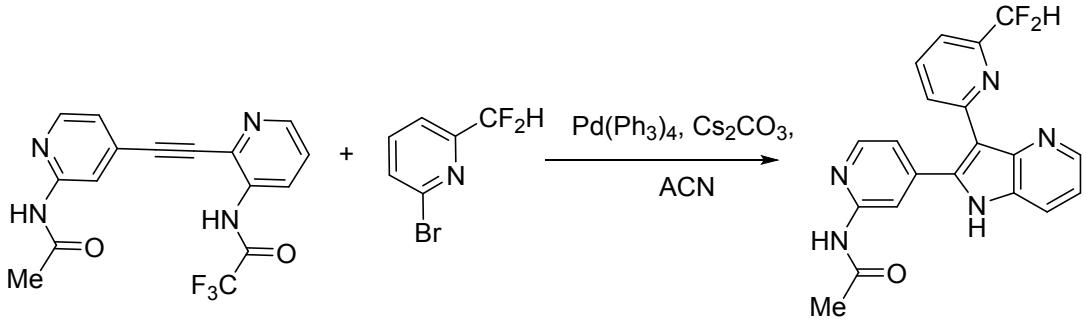


3g

A mixture of N-(2-((2-acetamidopyridin-4-yl)ethynyl)pyridin-3-yl)-2,2,2-trifluoroacetamide (40 mg, 0.11 mmol), 2-bromo-6-methylpyridine (23.7 mg, 0.14 mmol), $Pd(Ph_3P)_4$ (6.6 mg, 5.7 µmol) and Cs_2CO_3 (112 mg, 0.34 mmol) in acetonitrile (1 mL) was purged with N_2. The mixture was then heated at 110 °C in a sealed vial for 2 h, and then cooled to room temperature. The solid was filtered off, and rinsed with 10% MeOH/DCM, and the filtrate was concentrated. The residue was dissolved in DMF, and purified preparative LC/MS with the following conditions:Column: YMC-Pac C18, 30 x 100 mm, 5-µm particles; Mobile Phase A: 5:95 acetonitrile: water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile: water with 10-mM ammonium acetate; Gradient: 10-70% B over 20 minutes, then a 5-minute hold at

100% B; Flow: 20 mL/min. to give compound **3g** as a white solid (12.5 mg, 31%). LCMS (ES): calculated for $C_{20}H_{17}N_5O$ 343.14, found *m/z* = 344 ^{1}H NMR (400MHz, Methanol-d_4) d 8.39 (dd, J=4.6, 1.3 Hz, 1H), 8.31 - 8.21 (m, 2H), 7.97 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.83 (t, *J* = 7.7 Hz, 1H), 7.50 (d, *J* = 7.7 Hz, 1H), 7.32 (dt, *J* = 8.4, 4.2 Hz, 2H), 7.13 (dd, *J* = 5.2, 1.7 Hz, 1H), 2.53 (s, 3H), 2.17 (s, 3H).

Compound **3h**



3h

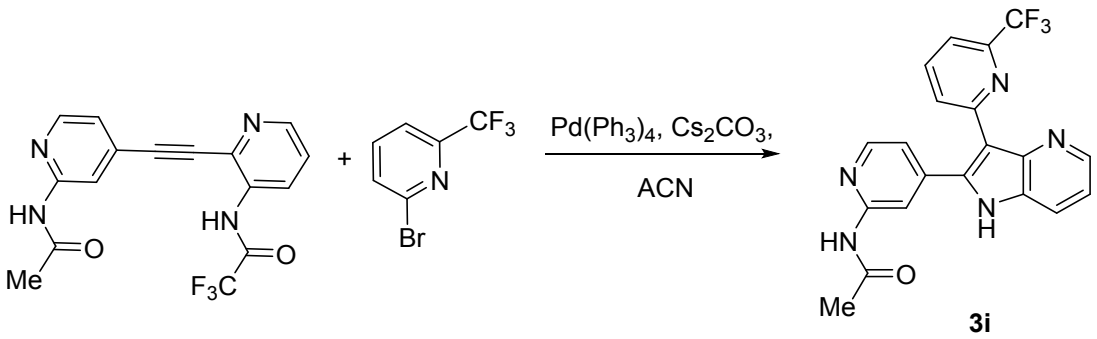
A mixture of N-(2-((2-acetamidopyridin-4-yl)ethynyl)-5-methoxypyridin-3-yl)-2,2,2-trifluoroacetamide (300 mg, 0.86 mmol) and cesium carbonate (561 mg, 1.7 mmol) was evacuated and backfilled with N_2, then MeCN (8.6 ml) and 2-bromo-6-(difluoromethyl)pyridine (358 mg, 1.7 mmol) were added. The mixture was sparged with N_2 for 5 min, then $Pd(Ph_3P)_4$ (50 mg, 0.04 mmol) was added. The reaction was sparged with N_2 for 1 min, then it was sealed and stirred at 110 °C for 2 h The reaction was cooled to rt. The crude mixture was taken up in 10% MeOH/DCM (100 mL) and then filtered through a pad of Celite, washing with 10% MeOH/DCM. The combined filtrate was concentrated and purified by ISCO (gold column, 220 g , 0-8% MeOH/DCM) to give the monohydrate of compound **3h** as a white solid (189 mg, 57 % yield).

LCMS (ES): calculated for $C_{20}H_{15}F_2N_5O$ 379.12, found *m/z* = 380 [M+H];

^{1}H NMR (400 MHz, DMSO-d_6) δ 12.20 (br s, 1H), 10.51 (s, 1H), 8.48 (dd, *J* = 4.5, 1.5 Hz, 1H), 8.40 (dd, *J* = 8.0, 0.9 Hz, 1H), 8.36 (s, 1H), 8.30 (dd, *J* = 5.2, 0.7 Hz, 1H), 8.06 (t, *J*=7.8 Hz, 1H), 7.90 (dd, *J* = 8.3, 1.4 Hz, 1H), 7.53 (d, *J* = 7.6 Hz, 1H), 7.28 (dd, *J* = 8.2, 4.5 Hz, 1H), 7.24 (dd, *J* = 5.1, 1.6 Hz, 1H), 6.65 (t, *J* = 55.1 Hz, 1H), 2.08 (s, 3H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 169.7, 153.2, 152.6, 151.9, 151.7, 151.5, 147.9, 144.8, 144.4, 142.1, 138.2, 138.1, 129.7, 127.0, 119.8, 118.6, 118.0, 116.2, 114.3, 113.6, 113.5, 112.4.
Water content determined by Karl-Fisher titration: 4.51%
Elemental analysis: %C/H/N calculated for $C_{20}H_{17}F_2N_5O_2$ (**3h** monohydrate) 60.47/4.31/17.63. Measured 60.54/4.27/17.54

Compound **3i**



3i

A mixture of N-(2-((2-acetamidopyridin-4-yl)ethynyl)pyridin-3-yl)-2,2,2-trifluoroacetamide (35 mg, 0.1 mmol), 2-bromo-6-(trifluoromethyl)pyridine (27.3 mg, 0.12 mmol), $Pd(Ph_3P)_4$ (5.8 mg, 5. μmol) and Cs_2CO_3 (98 mg, 0.3 mmol) in acetonitrile (1 mL) was purged with N_2. The mixture was then heated at 110 °C in a sealed vial for 2 h, and then cooled to room temperature. The solid was filtered off, and rinsed with 10% MeOH/DCM, and the filtrate was concentrated. The residue was dissolved in DMF, and purified preparative LC/MS with the following conditions: Column: Waters XBridge C18, 19 x 200 mm, 5-μm particles; Mobile Phase A: 5:95 acetonitrile: water with 0.1% trifluoroacetic acid; Mobile Phase B: 95:5 acetonitrile: water with 0.1% trifluoroacetic acid; Gradient: 0-100% B over 20 minutes, then a 3-minute hold at 100% B; Flow: 20 mL/min.. to give compound **3i** as a white solid (21 mg, 53%). LCMS (ES): calculated for $C_{20}H_{14}F_3N_5O$ 397.12, found *m/z* = 398. ^{1}H NMR (500 MHz, DMSO-d_6) δ 10.63 (s, 1H), 8.62 (br d, *J* = 4.4 Hz, 1H), 8.41 - 8.32 (m, 2H), 8.28 - 8.22 (m, 2H), 8.17 (t, *J* = 7.7 Hz, 1H), 7.80 (d, *J* = 7.7 Hz, 1H), 7.53 (br dd, *J* = 8.1, 5.0 Hz, 1H), 7.26 (br d, *J* = 4.4 Hz, 1H), 2.08 (s, 3H)

Biological assays

HTRF Assays

Assays were conducted in 1536-well plates and 2 mL reactions were prepared from addition of HIS-TGFβRI T204D kinase domain or HIS-TGFβRII WT kinase domain, anti-HIS detection antibody, a labeled small molecule probe (K_d = <100 nM; k_{off} = <0.001 s^{-1}) and test compounds in assay buffer (20 mM HEPES pH 7.4, 10 mM $MgCl_2$, 0.015% Brij35, 4 mM DTT, and 0.05 mg/mL BSA). The reaction was incubated for 1 h at room temperature and the HTRF signal was measured on an Envision plate reader (Ex: 340 nm; Em: 520 nm/495 nm). Inhibition data were calculated by comparison to no enzyme control reactions for 100% inhibition and vehicle-only reactions for 0% inhibition. The final concentration of reagents in the assay were 1 nM HIS-TGFβRI T204D or HIS-TGFβRII WT, 0.2 nM anti-HIS detection antibody, labeled small molecule probe (at K_d) and 0.5% DMSO. Dose response curves were generated to determine the concentration required inhibiting 50% of kinase activity (IC_{50}). Compounds were dissolved at 10 mM in dimethyl sulfoxide (DMSO) and evaluated at eleven concentrations. IC_{50} values were derived by non-linear regression analysis.

MINK TSMAD Assay

Mv1Lu mink lung epithelial cells were plated 4000 cells/well in RPMI + 10% FBS in 384 well PDL coated plate overnight and then starved in RPMI + 0.1% FBS for 20 h. The cells were echo dispensed with compounds and incubated for 1 h. 2.5 ng/mL TGFβ was applied to stimulate the cells for 15 min. The reaction was stopped with 4% formaldehyde fixation buffer for 20 min. The cells were permeabilized with 0.1% triton, and stained for DNA and total SMAD. The plate was read using Cellomics ArrayScan Health Profiling BioApplication, 10× objectives. The data was analyzed in Toolset and Curvemaster. The background was from unstimulated cells and the total was the 2.5 ng/mL TGFβ stimulation without compound inhibition.

TGFβ stimulated PSMAD inhibition assay in primary human T cells

Primary human T cells were starved by centrifuging and resuspending in RPMI media (Corning Cellgro, Cat# 10-040-CM) + 0.5% FBS (Sigma, Cat# F4135-500mls). Cells were incubated overnight at 37 °C. Compounds were diluted from 5 mM, 1:3 in DMSO (Sigma, Cat # D2650), as a 10 point series in v bottom plates (Nunc, Cat # 442581). 400 µl of RPMI + 0.5% FBS were added to each well of a 1 mL deep well block (Axygen, Cat# PDW11CS). 2 µl of the compound in DMSO were transferred to the media in the deep well blocks (1:200) and mixed. 80 µl, in duplicate rows, of the compound + media solution were added to 96 well RB plates (Corning Costar, Cat # 3799). Starved cells were counted, centrifuged, and cell pellets were re-suspended at 1.5×10^6 cells/mL with RPMI + 0.5% FBS. 100 µl of cells (1.5×10^5 cells/well) were added to each well of the plate, then incubated for 1 h at 37 °C. Stock of TGFβ (rh TGFβ-R&D Systems, Cat # 240-B/CF; 33.3 µg/mL) was diluted 33,000× to 1 ng/mL in RPMI + 0.5% FBS (10×). Each well of columns 1–11 was stimulated with 20 µl/well of 1.0 ng/mL (10×) TGFβ. (0.1 ng/mL Final). 20 µl of RPMI + 0.5% FBS were added to each well of column 12. Plates were incubated at 37 °C for 90 min, centrifuged, flicked off media, and the cells were lysed with 50 µl 0.2% Triton X-100 (Sigma, Cat# 93443-5001) in PBS (Corning Cellgro, Cat# 21-040-CM) containing protease inhibitors (Roche, Cat# 11-836-153-001) and phosphatase inhibitors (Roche, Cat# 04-906-837-001). PSMAD AlphaLisa was run according to manufacturer instructions (Surefire PSMAD Alpha Screen-Perkin Elmer, Cat# TGRSM3S10K; Protein A Alphalisa Acceptor Beads-Perkin Elmer, Cat# AL101M; Streptavidin Donor Beads-Perkin Elmer, Cat# 6760002); incubated overnight at room temperature and read on appropriate Alpha screen reader.

FoxP3 Regulation in Human Treg Cells

Naïve cells (CD4+, CD45RA+, CD25-depleted) were obtained from AllCells. Treg cells were prepared as follows. Plates were coated overnight at 4°C with 1µg/mL anti-CD3 (BD Biosciences) and 5 µg/mL anti-CD28 (Beckman Coulter, Indianapolis, IN). Naïve cells (50,000 cells/well) were seeded in 80 µL Iscove's Modified Dulbecco's Medium plus 10% FBS at and activated for 5 days with the plate-bound anti-CD3 and anti-CD28, and 5 µL 20X TGFβ (5 ng/mL), 5 µL 20X IL2 (50 U/mL) and 10 µL 10X compound (100 µL total volume) at 37°C, 5% CO2. After 5 days activation, the plate was centrifuged at 4°C for 5 minutes at 1000 rpm. The wells were washed with 1 mL of FACS buffer. The supernatant was removed and the cells were resuspended in 100 µL staining reagent (anti-CD4-FITC/anti-CD25-PE) and gently mixed. The cells were then

incubated for 20 minutes at room temperature in the dark. The cells were washed with cold FACS buffer and resuspended in 200 μL of freshly prepared Fixation/Permeabilization working solution (1X from 4X concentrate using diluent). Samples were incubated at room temperature for 30 minutes to 18 hours. Cells were washed with 200 μL 1X Permeabilization Buffer twice (1:10 in water). Washed cells were resuspended in 50 μL staining reagent (anti-Foxp3-eFluor660 in 1X Permeabilization Buffer) and mixed. Finally, cells were incubated for 30 minutes at room temperature in the dark, washed with 200 μL 1X Permeabilization Buffer twice, and resuspended in 100 μL FACS buffer for FACS analysis.

***Mouse pharmacokinetics studies**:*

Compounds 3f and 3h were administered in a 100% polyethylene glycol 400 (PEG-400) solution to Balb/C mice. Measurements of 3f were made at 1, 2, 4, 7 and 24 hours post dose and measurements of 3h were made at 1, 4, and 24 hours post dose (n=3 per time point).

In Vivo efficacy study

All animal procedures were approved by the Bristol-Myers Squibb (BMS) Institutional Animal Care and Use Committee. The animal care and use program at BMS is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International (AAALAC).

Animal Models: Tumored syngeneic mouse models were utilized for the evaluation of efficacy response to treatment therapy. Tumor response was determined by measurement of tumors with a digital caliper twice weekly, until the tumors reached a predetermined "target" size of 1000mm^3. Tumor volume (mm^3) was estimated from the formula: Tumor volume = 1/2 (length x (width) e^2).Tumor response end-point was determined by the number of cured animals per group. A mouse was considered cured when no mass larger than 0mm^3 was present at the site of tumor implant after the number of days post-treatment had elapsed, equivalent to > 10 x tumor volume doubling time (TVDT).

Treated animals were checked daily for treatment related toxicity/mortality. Body weights (BW) were collected on each group of animals before the initiation of treatment (Wt1) and then again

following the last treatment dose (Wt2). The difference in body weight (Wt2-Wt1) provides a measure of treatment-related toxicity. Additional weights were recorded at each measurement date to monitor toxicity.

Preparation of Tumor cells: The mouse adenocarcinoma tumor cell line MC38 was maintained in 10% fetal bovine serum (FBS, Invitrogen) and Roswell Park Memorial Institute (RPMI) 1640 Medium (Gibco) in T75 flasks. Cells were grown to subconfluency and passaged two times per week simply by rinsing with DPBS (Dulbecco's Phosphate-Buffered Saline, Gibco), allowing cells to sit for a few minutes and tapping the flask. MC38 cell passage ratios ranged from 1:16 to -1:20 depending on timing and confluency.

For *in-vivo* implantation, cells were rinsed with DPBS and then collected in ice-cold HBSS (Hank's Balanced Salt Solution, Gibco) in 50 mL conical tubes on ice. Tubes were spun at 1300 rpm for 10 minutes, the supernatant carefully removed, and the pellets washed with HBSS and spun again. Pellets were resuspended in approximate implant volumes of HBSS. The cell concentration was measured using a Moxi-Z (Orflo) and adjusted to the final concentration with HBSS. Cell viability was measured using trypan blue exclusion on a Countess II (Life Technologies).

Tumor implantation: For study MC38-103, female, C57Bl/6 mice, obtained from Charles River Laboratories were received in house at age 6-8 weeks and acclimated for 3-7 days. At the time of tumor implantation (day 0), mice were given a subcutaneous injection of 0.1 mL of MC38 cells at a concentration of $1x10^7$ cells/ml using a 1mL tuberculin syringe with a 25 gauge needle, implanted into the right flank. Tumors grew to a pre-determined size, ~96mm at which time animals were randomized into various treatment and control groups with like mean and median tumor values, where n=9 per group on day 5 (days post implant). Treatment was initiated on the same day.

Study Termination: Treatment groups were terminated when mean tumor weight reached target size of $1000mm^3$ at any measurement. If the mean tumor volume never reached target size, the treatment group was terminated when the remaining animals had stagnant tumor change for a period of >10 x TVDT.

crystal structure data collection and refinement.

Data collection and structure refinement followed that of Tebben *et al*. (2016) with all data sets collected at the Advanced Photon Source Beamline 17-ID.

Tebben, A. J., Ruzanov, M. Gao, M., Xie, D., Kiefer, S. E., Yan, C., Newitt, J. A., Zhang, L., Kim, K. Lu, H., Kopcho, L. M., Sheriff, S. (2016). The Structure of TGFβR2 Kinase Domain Both Apo and in Complex with Inhibitors and Comparison with TGFβR1 Kinase Domain. *Acta Crystallogr. Sect. D. Struct. Biol.* **72**, 658-674. (http://dx.doi.org/10.1107/S2059798316003624).

Table S1. Crystallographic Statistics for complexes of TGFβRI or TGFβRII with various inhibitors.

Compound	**1**	**2b**	**2b**	**3e**
Protein	TGFβRI-T204D	TGFβRI-WT	TGFβRII-6M	TGFβRI-T204D
Resolution range (Å)	23.13-1.58 (1.62-1.58)	20.80-1.98 (2.09-1.98)	40.99-1.57 (1.61-1.57)	22.41-1.75 (1.81-1.75)
Completeness (%)	99.3 (99.3)	99.9 (99.9)	99.7 (99.7)	99.9 (99.9)
σ cutoff	0	0	0	0
No. of reflections: working set	38430 (2569)	19544 (2794)	48883 (3487)	29014 (2807)
No. of reflections: test set	2030 (138)	987 (140)	2530 (189)	1529 (148)
Final Rwork	0.165 (0.190)	0.169 (0.177)	0.187 (0.213)	0.168 (0.198)
Final Rfree	0.190 (0.244)	0.210 (0.247)	0.203 (0.267)	0.188 (0.257)
No. of non-H atoms				
Protein	2421	2408	2275	2406
Ion (MG)	-	-	1	
GOL	12	-	6	6
Ligand	27	27	27	27
Water	246	156	218	203
Total	2706	2591	2527	2642
R.m.s. deviations				
Bonds (Å)	0.010	0.010	0.010	0.010
Angles (o)	1.0	1.0	1.0	1.0
Average B factors (Å^2)				

Protein	19.1	27.2	29.5	25.0
Ion (MG)	-	-	41.2	
GOL	37.1	-	46.1	47.4
Ligand	11.6	20.0	19.1	18.2
Water	28.3	32.2	38.8	32.4
Ramachandran plot*				
Most favored	92.3	91.6	91.6	92.7
Allowed	7.7	8.4	8.0	7.3
Disallowed	0.0	0.0	0.0	0.0

Numbers in parentheses represent the highest resolution shell

*as defined in Laskowski, R.A.; MacArthur, M.W.; Moss, D.S.; Thornton, J.M. *PROCHECK*: a program to check the stereochemical quality of protein structures. *J. Appl. Crystallogr*. **1993**, *26*, 283–291.

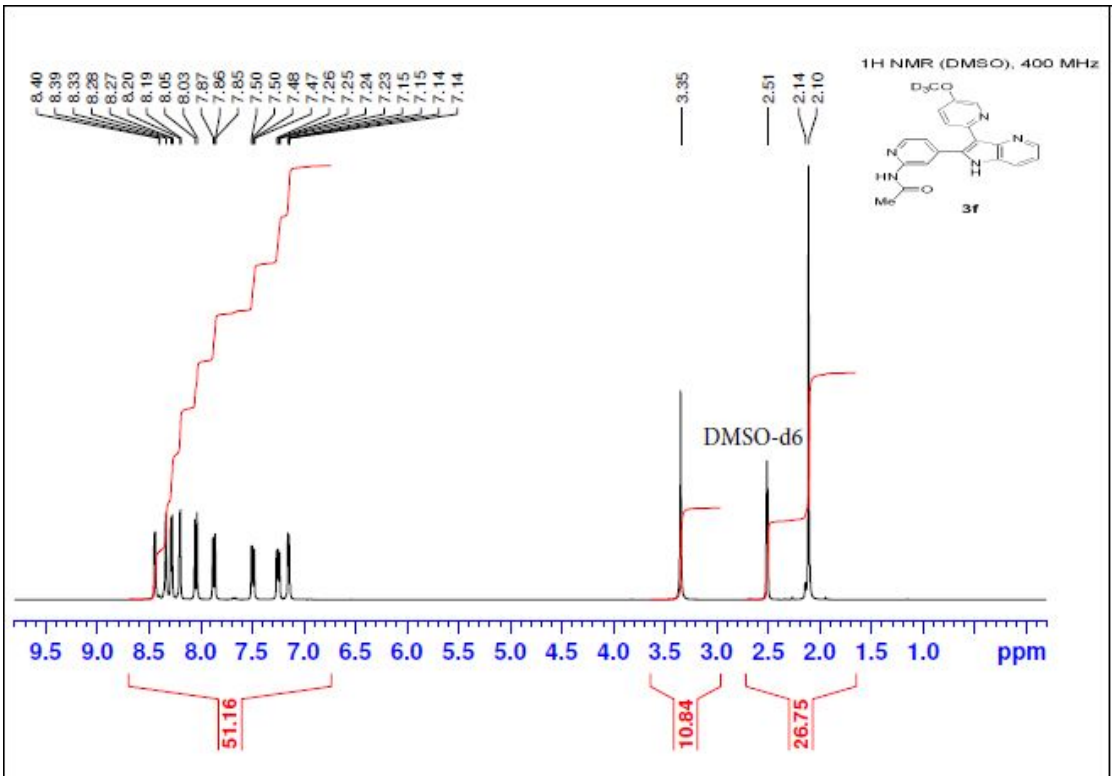
Table S2. Profiling data for TGFβ inhibitors

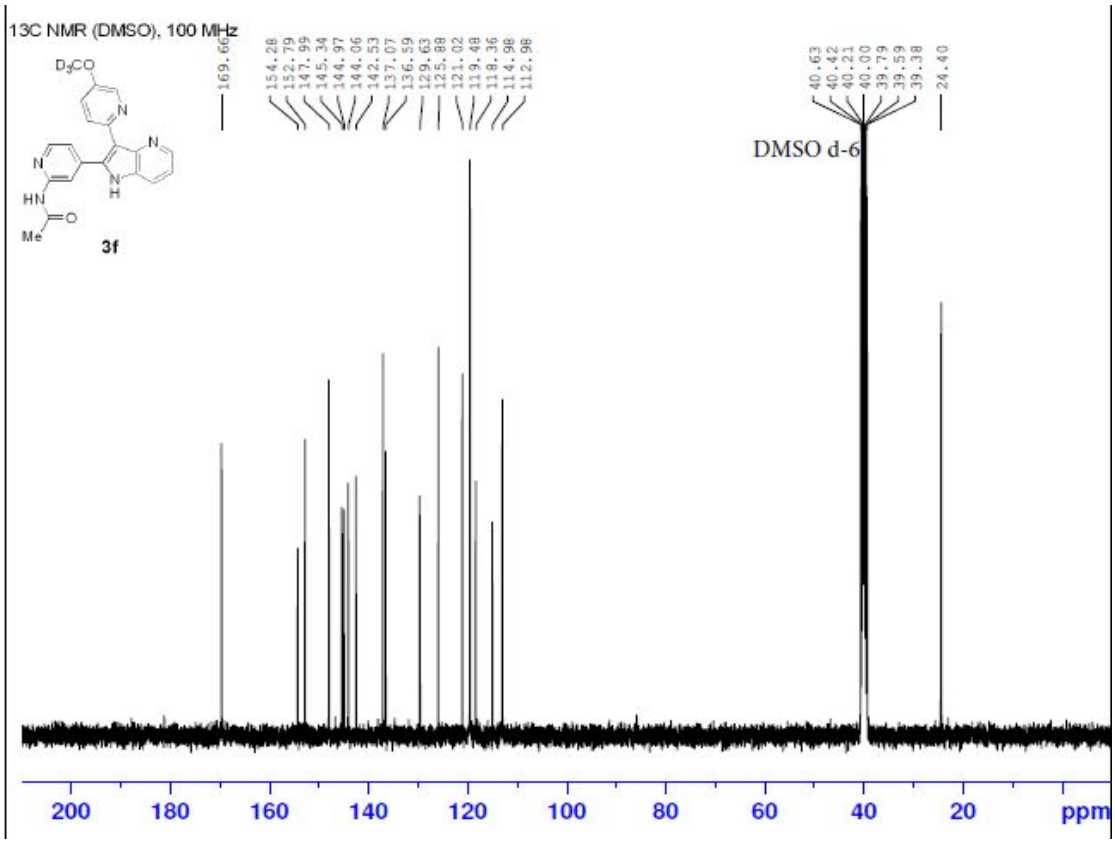
compd	Kinase selectivity No. of kinases with IC_{50} < 1 uM/ No. of kinases tested	Met Stab (h, r, m; % rem)	PAMPA (pH 7.4, nm/sec)	CYP inhibition (1A2, 2C9, 2C19, 2D6, 3A4, 2C8)
1	81/240	93/77/99	8.0	NA
2a	29/235	71/87/40	289	>20 uM except 1A2 = 11 μM
3e	12/240	96/95/72	218	>20 uM except 3A4 = 4.5 μM
3f	4/244	96/89/74	272	>20 uM
3g	31/216	87/90/74	208	>20 uM
3h	13/246	60/85/66	393	>20 uM

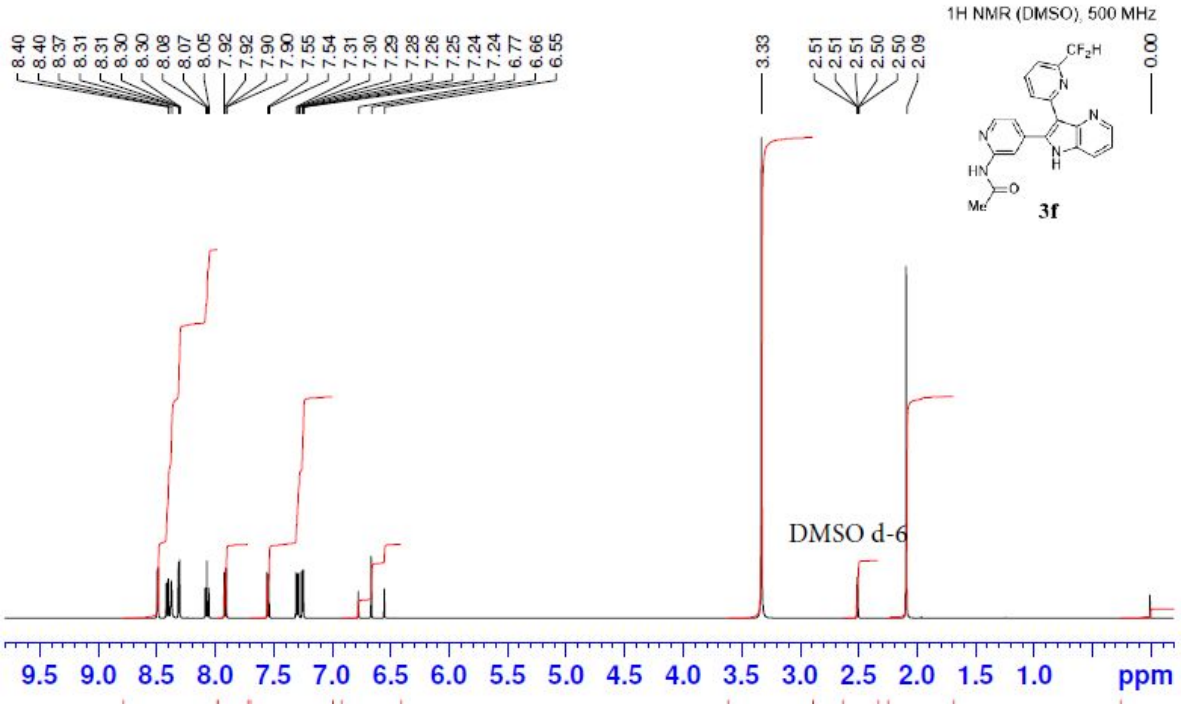
Table S3. Biochemical and cellular activities for TGFβ inhibitors

compd	TGFβRI IC_{50} (μM) (replicates)	TGFβRII IC_{50} (μM) (replicates)	MINK SMAD transloation IC_{50} (μM) (replicates)
2a	0.022 ± 0.02 (2)	0.007 ± 0.001 (2)	1.8 ± 0.12 (2)
2b	0.0026 ± 0.0019 (6)	0.035 ± 0.015 (6)	0.31 ± 0.13 (11)
3a	0.0026 ± 0.0013 (2)	0.39 (1)	0.32 ± 0.02 (2)
3b	0.002 ± 0.02 (2)	14 (2)	0.90 ± 0.22 (3)
3c	0.005 ± 0.001 (2)	>15 (2)	NA
3d	0.011 (1)	14 (1)	1.2 (1)
3e	0.006 ± 0.004 (3)	>15 (3)	0.79 ± 1.0 (3)
3f	0.0016 ± 0.0005 (2)	7.6 ± 1.3 (2)	0.46 ± 0.02 (3)
3g	0.0019 ± 0.00005 (2)	0.73 (1)	0.76 ± 0.29 (2)
3h	0.0008 ± 0.0001 (4)	4.3 ± 2.3 (4)	0.26 ± 0.16 (4)

NMR spectrum of selected compounds






1H NMR (DMSO), 500 MHz
8.40
8.40
8.37
8.31
8.31
8.30
8.30
8.08
8.07
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7.92
7.92
7.90
7.90
7.55
7.54
7.31
7.30
7.29
7.28
7.26
7.25
7.24
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6.77
6.66
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2.51
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2.09
0.00
CF2H
N
N
HN
O
Me
H
3f
DMSO d-6
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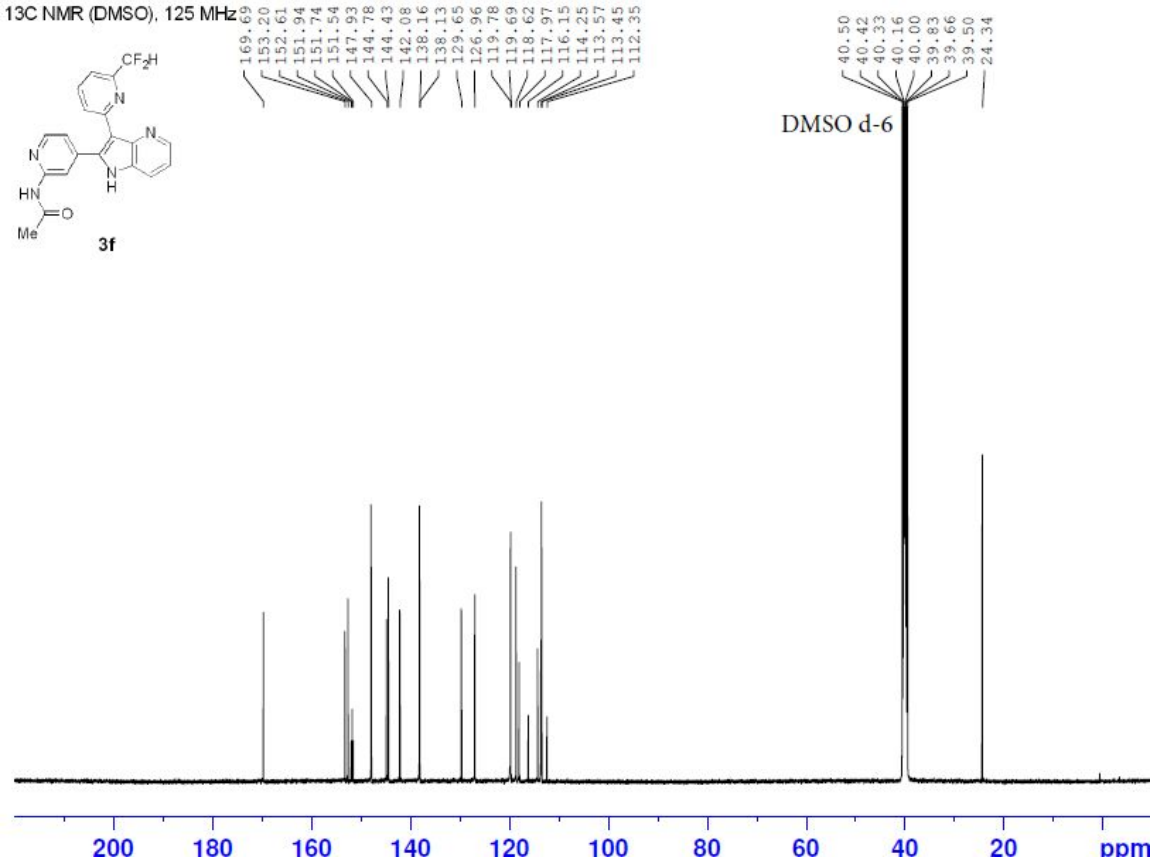
13C NMR (DMSO), 125 MHz
169.69
153.20
152.61
151.94
151.74
151.54
147.93
144.78
144.43
142.08
138.16
138.13
129.65
126.96
119.78
119.69
118.62
117.97
116.15
114.25
113.57
113.45
112.35
40.50
40.42
40.33
40.16
40.00
39.83
39.66
39.50
24.34
CF2H
N
N
HN
O
Me
H
3f
DMSO d-6
200 180 160 140 120 100 80 60 40 20 ppm