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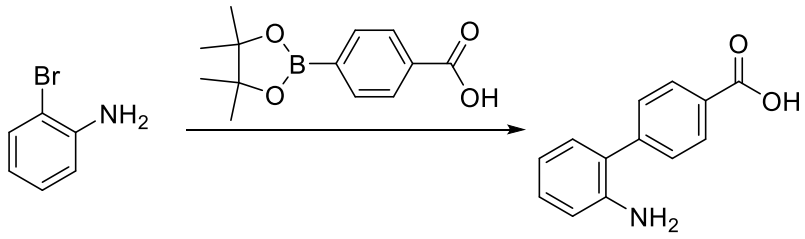
Chemical synthesis of USP7 compounds

General

^{1}H, ^{13}C and ^{19}F nuclear magnetic resonance (NMR) spectra were obtained on either Bruker or Varian spectrometers at 300 or 400 MHz, respectively. Spectra are given in ppm (δ) using the residual peak of the solvent as internal standard (DMSO-d_6: 2.50 ppm (^{1}H) / 39.5 ppm (^{13}C); CD_3OD: 3.31 ppm (1H) or tetramethylsilane (TMS) as internal standards. and coupling constants, J, are reported in Hertz. Mass spectra were collected using a Waters ZQ Single Quad Mass Spectrometer (ion trap electrospray ionization (ESI)). Purity and low-resolution mass spectral data were measured using Waters Acquity i-class ultra-performance liquid chromatography (UPLC) system with Acquity Photo Diode Array Detector, Acquity Evaporative Light Scattering Detector (ELSD) and Waters ZQ Mass Spectrometer. Data was acquired using Waters MassLynx 4.1 software and purity characterized by UV wavelength 220 nm, evaporative light scattering detection (ELSD) and electrospray positive ion (ESI). (Column: Acquity UPLC BEH C18 1.7µm 2.1 X 50 mm; Flow rate 0.6mL/min; Solvent A (95/5/0.1%: 10mM Ammonium Formate/Acetonitrile/Formic Acid), Solvent B (95/5/0.09%: Acetonitrile/Water/Formic Acid); gradient: 5-100% B from 0 to 2mins, hold 100%B to 2.2mins and 5%B at 2.21mins. The absolute configuration of FT671 was assigned by X-Ray crystallography

1. Preparation of FT827

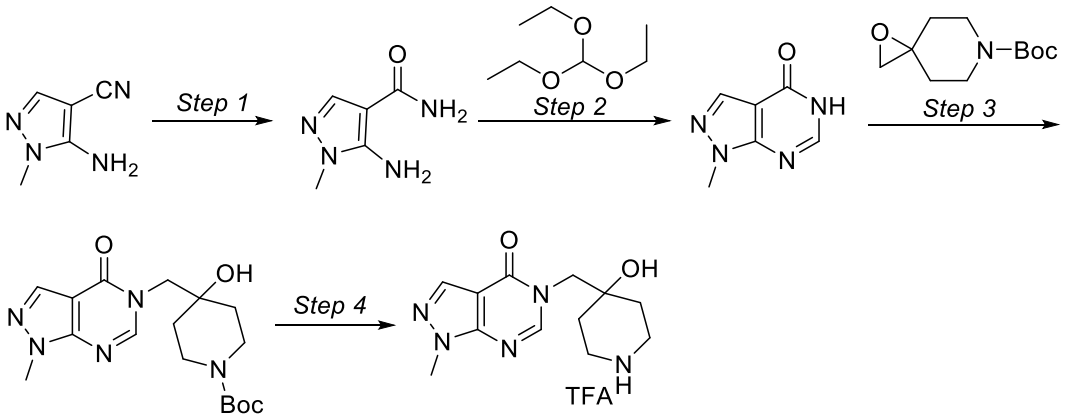
2'-aminobiphenyl-4-carboxylic acid



A 250-mL round-bottom flask fitted with a nitrogen balloon, magnetic stir bar, condenser and thermometer was charged with 2-bromoaniline (2 g, 11.63 mmol), dioxane (40 mL), water (4 mL), K_3PO_4 (7.6 g, 35.80 mmol), 4-(tetramethyl-1,3,2-dioxaborolan-2-yl)benzoic acid (3.2 g, 12.84 mmol) and 1,1'-

bis(diphenylphosphino)ferrocene palladium(II) chloride complex with dichloromethane (0.76 g, 0.94 mmol). The resulting solution was stirred for 10 h at 100 °C under nitrogen. The reaction mixture was cooled to 25 °C. The pH of the mixture was adjusted to 5 with hydrochloric acid (6 N). The product was extracted with dichloromethane (2 x 30 mL). The organic layers were combined, dried over anhydrous sodium sulfate, filtered and concentrated under vacuum to afford 2'-aminobiphenyl-4-carboxylic acid (0.8 g, 32%). LCMS: (ES) *m/z* 214 [M+H].

5-((4-hydroxypiperidin-4-yl)methyl)-1-methyl-1H-pyrazolo[3,4-d]pyrimidin-4(5H)-one), trifluoroacetic acid salt



Step 1. **5-Amino-1-methyl-1*H*-pyrazole-4-carboxamide**

A 500-mL 3-necked round-bottom flask fitted with a magnetic stir bar and thermometer was charged with sulfuric acid (120 mL), followed 5-amino-1-methyl-1*H*-pyrazole-4-carbonitrile (52 g, 426 mmol) in portions at 0 °C. Upon completion of the addition, the reaction mixture was warmed to room temperature and stirred for 1 h. The reaction quenched with sodium hydroxide (50% wt solution in water) and the pH of the mixture was adjusted to 8. The solids were collected by filtration, washed with water (3 x 50 mL) and dried in an oven to provide 5-amino-1-methyl-*1H*-pyrazole-4-carboxamide (50 g, 84%). LCMS: (ESI) m/z 141 [M+H].

Step 2. **1-Methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4(5H)-one**

A 500-mL 3-necked round-bottom flask fitted with a magnetic stir bar, condenser and thermometer was charged with 5-amino-1-methyl-1*H*-pyrazole-4-carboxamide (Step 1, 50 g, 357 mmol), (diethoxymethoxy)ethane (120 mL) and acetic anhydride (120 mL). The solution was stirred for 1 h at 130 °C in an oil bath. The reaction mixture was allowed to cool to 0 °C. The solids were collected by

filtration, washed with *n*-hexane (3 x 50 mL) and dried in an oven to provide to 1-methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4(5H)-one (51 g, 95%). LCMS: (ESI) m/z 151 [M+H].

Step 3. ***Tert*-butyl4-hydroxy-4-((1-methyl-4-oxo-1H-pyrazolo[3,4-d]pyrimidin-5**

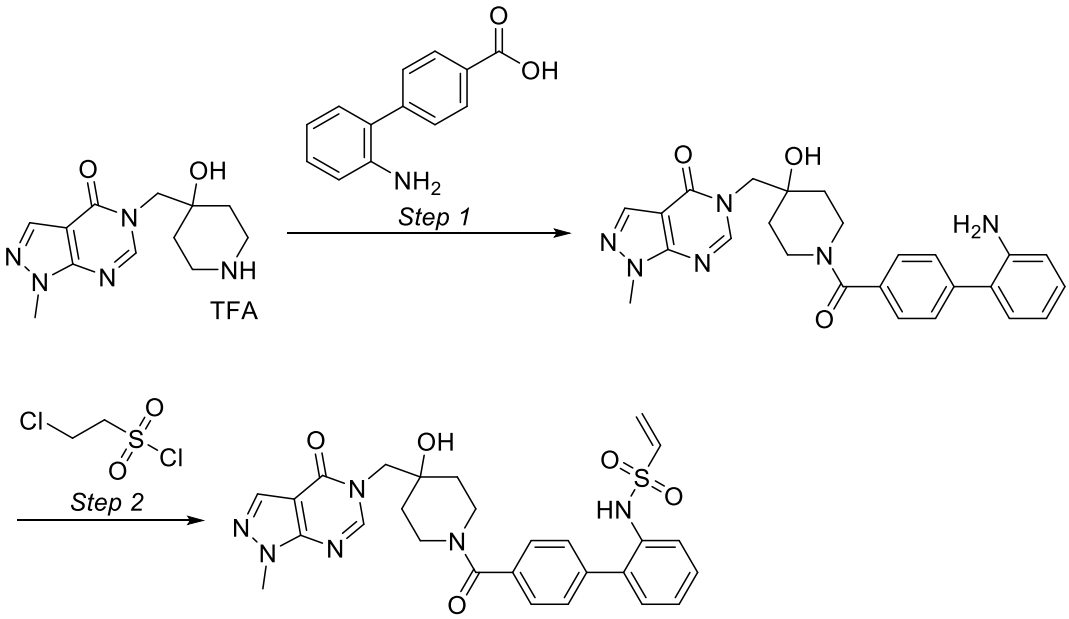
(4*H*)-yl)methyl)piperidine-1-carboxylate

A 500-mL 3-necked round-bottom flask fitted with a magnetic stir bar, condenser and thermometer was charged with 1-methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4(5H)-one (Step 2, 20 g, 133 mmol), *N,N*-dimethylformamide (150 mL), *tert*-butyl 1-oxa-6-azaspiro[2.5]octane-6-carboxylate (31 g, 146 mmol), Cs_2CO_3 (130 g, 399 mmol). The reaction mixture was stirred for 2 h at 80 °C in an oil bath. After cooling to room temperature, the reaction was quenched with water (300 mL). The product was extracted with ethyl acetate (4 x 200 mL). The combined organic layers were washed with brine (300 mL), dried over anhydrous sodium sulfate, filtered and concentrated under vacuum. The residue was purified by column chromatography eluting with dichloromethane/methanol (10:1, v/v) to yield *tert*-butyl 4-hydroxy-4-((1-methyl-4-oxo-1*H*-pyrazolo[3,4-d]pyrimidin-5(4*H*)-yl)methyl)piperidine-1-carboxylate (30 g, 62%). ^{1}H-NMR (300MHz, $CDCl_3$): δ1.45 (s, 9H), 1.47-1.67 (m, 4H), 3.12-3.19 (m, 2H), 3.96-3.81 (m, 2H), 4.00 (s, 3H), 4.04-4.18 (m, 2H), 8.02 (s, 1H), 8.07 (s, 1H) ppm. ^{13}C-NMR (101 MHz, CHLOROFORM-*d*): δ ppm 28.4, 34.3, 35.1, 55.4, 70.3, 79.7, 105.4, 135.1, 149.7, 151.5, 154.6, 158.7. LCMS: (ESI) m/z 364 [M+H].

Step 4. **5-((4-hydroxypiperidin-4-yl)methyl)-1-methyl-1*H*-pyrazolo [3,4-d]pyrimidin-4(5*H*)-one, trifluoroacetic acid salt**

A 250-mL round-bottom flask fitted with a magnetic stir bar was charged with tert-butyl 4-hydroxy-4-((1-methyl-4-oxo-1H-pyrazolo[3,4-d]pyrimidin-5(4*H*)-yl) methyl)piperidine-1-carboxylate (Step 3, 20 g, 55 mmol), trifluoroacetic acid salt (20 mL) and dichloromethane (150 mL). The solution was stirred for 4 h at room temperature and concentrated under vacuum to give 5-((4-hydroxypiperidin-4-yl)methyl)-1-methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4(5*H*)-one, trifluoroacetic acid salt (21 g). LCMS: (ESI) m/z 264 [M+H].

***N*-(4'-(4-hydroxy-4-((1-methyl-4-oxo-1*H*-pyrazolo[3,4-d] pyrimidin-5(4*H*)-yl)methyl)piperidine-1-carbonyl)biphenyl-2-yl)ethenesulfonamide**



Step 1. **5-((1-(2'-Aminobiphenylcarbonyl)-4-hydroxypiperidin-4-yl)methyl)-1- methyl-1H-pyrazolo[3,4-d]pyrimidin-4(5*H*)-one**

A 100-mL round-bottom flask fitted with a magnetic stir bar was charged with 2'-aminobiphenyl-4-carboxylic acid (400 mg, 1.88 mmol), 5-((4-hydroxypiperidin-4-yl)methyl)-1-methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4(5H)-one, trifluoroacetic acid salt (800 mg, 2.12 mmol) 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate) (857 mg, 2.25 mmol), *N*,*N*-diethylisopropylamine (970 mg, 7.51 mmol) and dichloromethane (30 mL). The resulting solution was stirred for 2 h at 25 °C and then diluted with water (30 mL). The product was extracted with dichloromethane (3 x 30 mL). The combined organic layers were dried over anhydrous sodium sulfate, filtered and concentrated under vacuum. The residue was purified by preparatory thin layer chromatography eluting with ethyl acetate to afford 5-((1-(2'-aminobiphenylcarbonyl)-4-hydroxypiperidin-4-yl)methyl)-1-methyl-1*H*-pyrazolo[3,4-d]pyrimidin-4(5*H*)-one (220 mg, 26%). LCMS: (ESI) m/z 459[M+H].

Step 2. ***N*-(4'-(4-hydroxy-4-((1-methyl-4-oxo-1*H*-pyrazolo[3,4-d]pyrimidin-5(4*H*) -yl)methyl)piperidine-1-carbonyl)biphenyl-2-yl)ethenesulfonamide (FT827)**

A 100-mL 3-necked round-bottom flask fitted with a nitrogen balloon, magnetic stir bar and thermometer was charged with 5-((1-(2'-aminobiphenylcarbonyl)-4-hydroxypiperidin-4-yl)methyl)-1-methyl-*1H*-pyrazolo[3,4-d]pyrimidin-4(5H)-one (Step 1, 40 mg, 0.09 mmol), dichloromethane (5 mL) and triethylamine (30.7 mg, 0.30 mmol). A solution of 2-chloroethane-1-sulfonyl chloride (17 mg, 0.10

mmol) in dichloromethane (1 mL) was added in portions at 0 °C. The resulting solution was stirred for 0.5 h at 0 °C and then concentrated under vacuum. The residue was purified by preparatory HPLC* to give *N*-(4'-(4-hydroxy-4-((1-methyl-4-oxo-1*H*-pyrazolo[3,4-d]pyrimidin-5(4*H*)-yl)methyl)piperidine-1-carbonyl)biphenyl-2-yl)ethenesulfonamide as a white solid (11 mg, 21%). *Column: X bridge prep shield RP18 OBD Column 19 x 150 nm, 5 µM Mobile phase A: water (0.05% NH_4HCO_3)/Mobile phase B: acetonitrile. Flow rate: 20 mL/min. Gradient: 16% B to 42% B over 7 min. Detector: 220 and 254 nm (**Supplementary Chemistry Data a**).

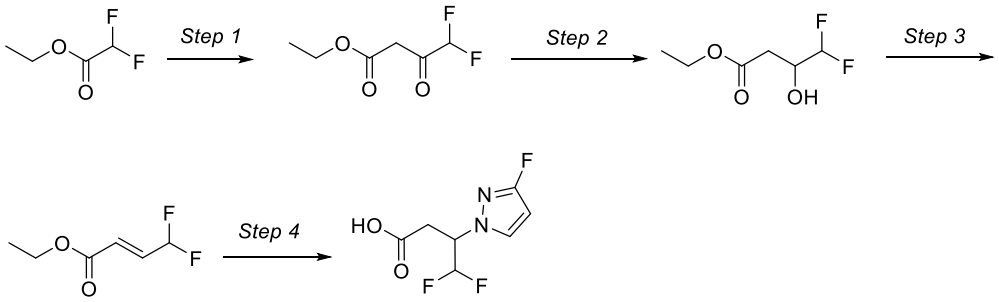
High-resolution mass spectrometry (HRMS): ($C_{24}H_{23}F_4N_7O_3$ – expected $[M+H]^+$ 549.19201, observed $[M+H]^+$ 549.19150 (0.93 ppm) (**Supplementary Chemistry Data b**). Agilent QTOF 6560 LC-MS ESI+ mode. Column: Waters Acquity UPLC BEH C18, 1.7 um, 2.1 x 50 mm. Mobile phase A: 95% Water/5% Acetonitrile with 0.1% Formic Acid in 10 mM Ammonium Formate/Mobile phase B: 95% Acetonitrile/5% Water with 0.09% Formic Acid. Flow rate: 20 mL/min. Column temperature: 35 °C. Gradient: 5-100% B in 2.0 min, hold 100% to 2.2 min. LC Flow Rate: 0.6 mL/min. Detector: 220 nm.

^{1}*H-NMR* (400MHz, CD_3OD) δ 1.46-1.64 (m, 1H), 1.68-1.87 (m, 3H), 3.46-3.49 (m, 2H), 3.68-3.72 (m, 1H), 3.99 (s, 3H), 4.12-4.21 (m, 2H), 4.38-4.41 (m, 1H), 5.88 (d, J = 10.0Hz, 1H), 6.06 (d, J = 16.8Hz, 1H), 6.50-6.54 (m, 1H), 7.31-7.40 (m, 3H), 7.46-7.56 (m, 5H), 8.06 (s, 1H), 8.28 (s, 1H) ppm (**Supplementary Chemistry Data c**).

^{13}*C-NMR* (101 MHz, CHLOROFORM-*d*): δ ppm 34.3, 55.9, 70.4, 105.4, 119.8, 124.9, 128.0, 128.5, 129.2, 129.4, 130.7, 132.1, 133.4, 135.1, 135.4, 135.8, 139.0, 149.4, 151.5, 158.9, 169.6 (**Supplementary Chemistry Data d,e**).

2. Preparation of FT671

4,4-difluoro-3-(1H-pyrazol-1-yl)butanoic acid



Step 1. **Ethyl 4,4-difluoro-3-oxobutanoate**

A 500-mL round-bottom flask was charged with sodium ethoxide (28.3 g, 416 mmol) and ethanol (80 mL) followed by the addition of a solution of ethyl 2,2-difluoroacetate (43 g, 347 mmol) in ethyl acetate (170 mL) added slowly with stirring at room temperature. The resulting solution was stirred for 2 h at 60 °C. Upon cooling to room temperature, the reaction was quenched by the addition of hydrochloric acid (aqueous 6N, 150 mL) and the pH of the solution was adjusted to 4-5. The resulting solution was extracted with ethyl acetate (3 x 200 mL) and the organic layers were combined and dried over anhydrous sodium sulfate and concentrated under vacuum to afford ethyl 4,4-difluoro-3-oxobutanoate which was used in Step 2 without further purification (29 g). GCMS *m/z* 166.

Step 2. **Ethyl 4,4-difluoro-3-hydroxybutanoate**

A 500-mL round-bottom flask was charged with crude ethyl 4,4-difluoro-3-oxobutanoate (Step 1, 8 g, 96 mmol), toluene (250 mL) and sodium borohydride (2.38 g, 64.63 mmol). The resulting solution was stirred for 2 h at 65 °C. The reaction was then quenched by the addition of water (100 mL). The resulting solution was extracted with ethyl acetate (3 x 50 mL) and the organic layers were combined, washed with brine, dried over anhydrous sodium sulfate and concentrated under vacuum. The residue was purified by column chromatography eluting with ethyl acetate/petroleum ether (1:5 to 1:2 v/v) to afford ethyl 4,4-difluoro-3-hydroxybutanoate (6.8 g, 35% over 2 steps). GCMS *m/z* 168.

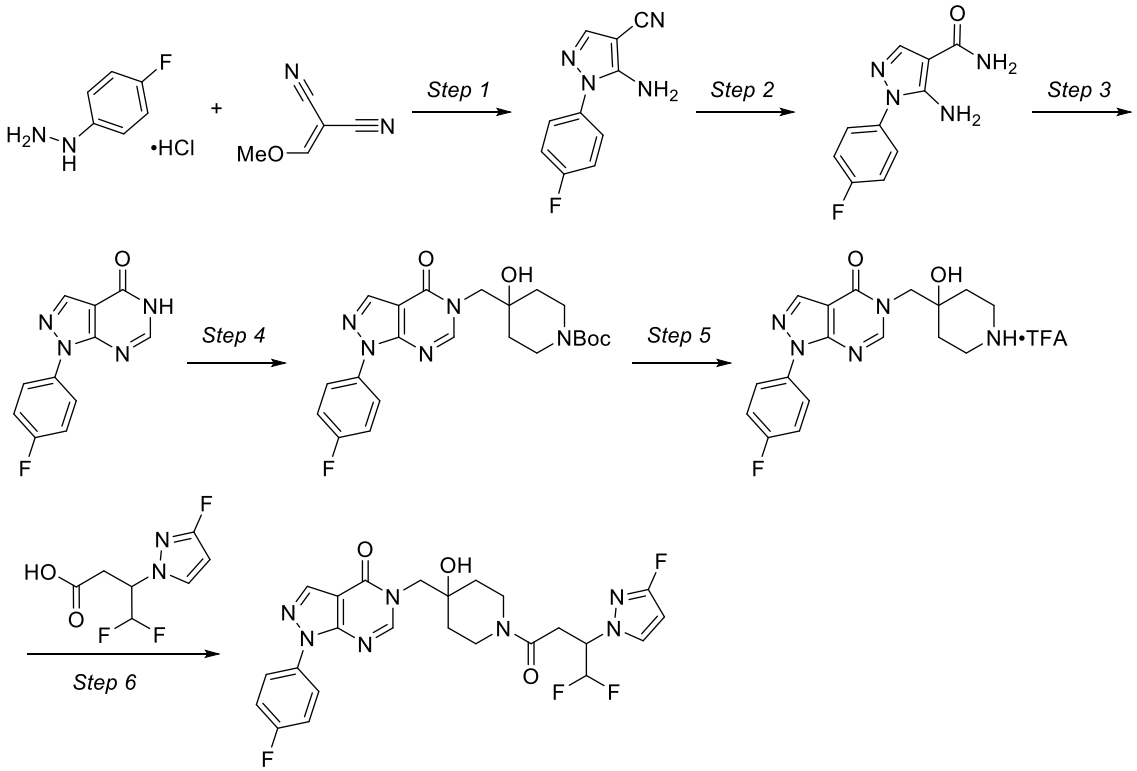
Step 3. ***(E)*-ethyl 4,4-difluorobut-2-enoate**

A 25-mL round-bottom flask was charged with ethyl 4,4-difluoro-3-hydroxybutanoate (Step 2, 5g, 48.16 mmol) and phosphorus pentoxide (3.4 g, 23.62 mmol). The resulting solution was

stirred for 2 h at 70 °C in an oil bath. The mixture was purified by distillation under reduced pressure (1 mm Hg) and the fraction was collected at 100 °C to give (E)-ethyl 4,4-difluorobut-2-enoate as a colorless oil (1.8 g, 25%). GCMS *m/z* 150.

Step 4. **4,4-difluoro-3-(3-fluoro-1*H*-pyrazol-1-yl)butanoic acid**

A 100-mL round-bottom flask was charged with 3-fluoro-1*H*-pyrazole (102 mg, 1.50 mmol) and tetrahydrofuran (20 mL) followed by the addition of sodium hydride (52 mg, 2.17 mmol) at 0 °C. The resulting solution was stirred 30 min at 0 °C before adding (*E*)-ethyl 4,4-difluorobut-2-enoate (Step 3, 150 mg, 1.00 mmol). The resulting solution was stirred 16 h at room temperature. The reaction was quenched by the addition of 10 mL of water and extracted with ethyl acetate (30 mL). The pH of the aqueous layer was adjusted to 4-5 with aqueous hydrochloric acid (aqueous, 6 N) and extracted with dichloromethane (2 x 30 mL). The organic layers were combined, dried over anhydrous sodium sulfate and concentrated under vacuum to give 4,4-difluoro-3-(3-fluoro-1*H*-pyrazol-1-yl)butanoic acid (90 mg) as a yellow oil which was used without further purification. LCMS: (ESI) *m/z* 191 [M+H].



Step 1. **5-Amino-1-(4-fluorophenyl)-1*H*-pyrazole-4-carbonitrile**

2-(Methoxymethylene)malononitrile (1.00 g, 9.25 mmol), (4-fluorophenyl) hydrazine hydrochloride (1.40 g, 8.61 mmol), triethylamine (1.66 g, 16.4 mmol) and ethanol (50 mL) were added to a 100-mL round-bottom flask fitted with a magnetic stir bar and condenser. The resulting solution was heated at reflux for 16 h. The resulting mixture was concentrated under vacuum. The residue was purified by column chromatography eluting with dichloromethane/methanol (10:1 v/v) to give 5-amino-1-(4-fluorophenyl)-1*H*-pyrazole-4-carbonitrile (1.00 g, 57 %). LCMS: (ESI) *m/z* 203 [M+H].

Step 2. **5-Amino-1-(4-fluorophenyl)-1*H*-pyrazole-4-carboxamide**

5-Amino-1-(4-fluorophenyl)-1*H*-pyrazole-4-carboxamide (Step 1, 900 mg, 4.45 mmol) was added dropwise to sulfuric acid (10 mL) at 0 ºC. The resulting solution was stirred for 2 h at 25 ºC. The pH of the solution was adjusted to 8 by the addition of 10% aqueous sodium carbonate. The resulting mixture was extracted with dichloromethane (4 x 50 mL) and the organic layers were combined, dried over anhydrous sodium sulfate, filtered and concentrated under vacuum to provide 5-amino-1-(4-fluorophenyl)-1*H*-pyrazole-4-carboxamide which was used in Step 3 without further purification. LCMS: (ESI) *m/z* 221 [M+H].

Step 3. **1-(4-Fluorophenyl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one**

5-Amino-1-(4-fluorophenyl)-1*H*-pyrazole-4-carboxamide (Step 2, 1.00 g, 4.5 mmol), triethyl orthoformate (20 mL) and acetic anhydride (20 mL) were added to a 100-mL round-bottom flask fitted with a magnetic stir bar and condenser. The resulting solution was heated at reflux for 1 h and then concentrated under vacuum. The solids were collected and washed with hexane (3 x 20 mL) to afford 1-(4-fluorophenyl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one which was used in Step 4 without further purification. LCMS: (ESI) *m/z* 231 [M+H].

Step 4. ***tert*-Butyl 4-((1-(4-fluorophenyl)-4-oxo-1,4-dihydro-5*H*-pyrazolo[3,4-*d*]pyrimidin-5-yl)methyl)-4-hydroxypiperidine-1-carboxylate**

1-(4-Fluorophenyl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one (Step 3, 1.00 g, 4.3 mmol), *tert*-butyl 1-oxa-6-azaspiro[2.5]octane-6-carboxylate (926 mg, 4.34 mmol), cesium carbonate (4.30 g, 13.2 mmol) and DMF (50 mL) were added to a 100-mL round-bottom flask fitted with a magnetic stir bar and thermometer. The resulting solution was stirred for 5 h at 80 ºC. The

resulting solution was diluted with water (50 mL). The mixture was extracted with methyl tert-butyl ether (5 x 20 mL) and the organic layers were combined, dried over anhydrous sodium sulfate, filtered and concentrated under vacuum. The residue was purified by column chromatography eluting with dichloromethane/methanol (50:1 v/v) to give *tert*-butyl 4-((1-(4-fluorophenyl)-4-oxo-1,4-dihydro-5*H*-pyrazolo[3,4-*d*]pyrimidin-5-yl)methyl)-4-hydroxypiperidine-1-carboxylate **(**500 mg, 26%). LCMS: (ESI) *m/z* 444 [M+H].

Step 5. **1-(4-Fluorophenyl)-5-((4-hydroxypiperidin-4-yl)methyl)-1,5-dihydro-4*H*-pyrazolo [3,4-*d*]pyrimidin-4-one trifluoroacetic acid salt**

tert-Butyl 4-((1-(4-fluorophenyl)-4-oxo-1,4-dihydro-5*H*-pyrazolo[3,4-*d*]pyrimidin-5-yl)methyl)-4-hydroxypiperidine-1-carboxylate (Step 4, 500 mg, 1.13 mmol), dichloromethane (30 mL) and trifluoroacetic acid (3 mL) were added to a 50-mL round-bottom flask fitted with a magnetic stir bar and condenser. The resulting solution was stirred for 2 h at 25 ºC. The resulting mixture was concentrated under vacuum to give 1-(4-fluorophenyl)-5-((4-hydroxypiperidin-4-yl)methyl)-1,5-dihydro-4*H*-pyrazolo[3,4-*d*]pyrimidin-4-one trifluoroacetic acid salt which was used in Step 6 without further purification. LCMS: (ESI) *m/z* 344 [M+H].

Step 6. **5-((1-(4,4-difluoro-3-(3-fluoro-1H-pyrazol-1-yl)butanoyl)-4-hydroxypiperidin-4-yl)methyl)-1-(4-fluorophenyl)-1,5-dihydro-4H-pyrazolo[3,4-d]pyrimidin-4-one (FT671)**

To a 250-mL round-bottom flask was added 4,4-difluoro-3-(5-fluoro-1*H*-pyrazol-1-yl) butanoic acid (2.0 g, 9.61 mmol), *N,N*-dimethylformamide (40 mL), *N*-(3-Dimethylaminopropyl)-*N*'-ethylcarbodiimide hydrochloride (2.4 g, 12.5 mmol), Hydroxybenzotriazole (1.7 g, 12.6 mmol), *N,N*-diethylisopropylamine (3.7 g, 28.6 mmol), 1-(4-fluorophenyl)-5-((4-hydroxypiperidin-4-yl)methyl)-1,5-dihydro-4*H*-pyrazolo[3,4-d]pyrimidin-4-one, trifluoroacetic acid salt (Step 5, 4.4 g, 9.6 mmol). The resulting solution was stirred for 1 h at 22 °C. The reaction was poured into 200 mL of water and the solution was extracted with 3 x100 mL of ethyl acetate. The organic layers were combined and dried over anhydrous sodium sulfate. The solids were removed by filtration and the solution was concentrated under vacuum. The residue was filtered initially with a silica gel column eluting with ethyl acetate/petroleum ether (7:10). The collected fractions were combined and concentrated under vacuum. The material was further purified by

Flash-Prep-HPLC with the following conditions (IntelFlash-1): Reverse-phase column; mobile phase, water (NH_4HCO_3, 10mmol/L)/acetonitrile = 10% increasing to water (NH_4HCO_3, 10 mmol/L)/acetonitrile = 70% within 50 min; Detector, UV 254 nm. The collected fractions were concentrated under vacuum to give 5-((1-(4,4-difluoro-3-(3-fluoro-1*H*-pyrazol-1-yl)butanoyl)-4-hydroxypiperidin-4-yl)methyl)-1-(4-fluorophenyl)-1,5-dihydro-4*H*-pyrazolo[3,4-d]pyrimidin-4-one (7 g, 91%).

High-resolution mass spectrometry (HRMS): ($C_{24}H_{23}F_4N_7O_3$ – expected $[M+H]^+$ 534.18768, observed $[M+H]^+$ 534.187770 (0.17 ppm). Agilent QTOF 6560 ESI+ mode. Column: Waters Acquity UPLC BEH C18, 1.7 um, 2.1 x 50 mm. Mobile phase A: 95% Water/5% Acetonitrile with 0.1% Formic Acid /Mobile phase B: 95% Acetonitrile/5% Water with 0.085% Formic Acid. Flow rate: 20 mL/min. Column temperature: 35 °C. Gradient: 5-100% B in 2.0 min, hold 100% to 2.2 min. LC Flow Rate: 0.6 mL/min. UV Wavelength: 220 nm (**Supplementary Chemistry Data f**).

^{1}H NMR: (300 MHz, DMSO-d_6) δ 8.47 – 8.30 (m, 2H), 8.08 (dd, *J* = 9.0, 4.8 Hz, 2H), 7.81 (q, *J* = 2.8 Hz, 1H), 7.44 (t, *J* = 8.8 Hz, 2H), 6.26 (td, *J* = 55.0, 3.6 Hz, 1H), 6.00-5.90 (m, 1H), 4.97 (s, 2H), 4.15 – 3.96 (m, 3H), 3.70 (d, *J* = 13.6 Hz, 1H), 3.30 – 3.16 (m, 2H), 2.91 (ddd, *J* = 16.7, 11.7, 4.2 Hz, 2H), 1.72 – 1.27 (m, 4H) ppm (**Supplementary Chemistry Data g**).

^{19}F NMR (376 MHz, DMSO-d_6) δ -131.12 (br s, 1 F) -127.80 - -126.20 (m, 1 F) -125.84 - - 123.99 (m, 1 F) -114.84 (br d, J=2.63 Hz, 1 F) ppm (**Supplementary Chemistry Data h**).

^{13}C NMR (101 MHz, DMSO-d_6) δ 30.8, 34.4, 34.5, 35.1, 37.7, 53.5, 60.0, 69.5, 90.0, 107.1, 115.1, 116.7, 124.1, 134.9, 136.7, 151.5, 152.2, 152.8, 159.8, 162.2, 164.5, 166.4 ppm (**Supplementary Chemistry Data i**).

FT671 chiral purification: Preparative supercritical fluid column (SFC) chromatography, (R,R)-WHELK-O1-Kromasil, 5cm x 25cm (5μM; mobile phase, CO_2 (50%), isopropylalcohol:acetonitrile = 2:1(50%); detector: UV 220nm.

First eluting isomer (FT671): 5-([1-[(3*S*)-4,4-difluoro-3-(3-fluoro-1*H*-pyrazol-1-yl)butanoyl]-4-hydroxypiperidin-4-yl]methyl)-1-(4-fluorophenyl)-1*H*,4*H*,5*H*-pyrazolo[3,4-d]pyrimidin-4-one (retention time: 5.78 min) (3.0 g) (**Supplementary Chemistry Data j**).

Second eluting isomer: 5-([1-[(3*R*)-4,4-difluoro-3-(3-fluoro-1*H*-pyrazol-1-yl)butanoyl]-4-hydroxypiperidin-4-yl]methyl)-1-(4-fluorophenyl)-1*H*,4*H*,5*H*-pyrazolo[3,4-d]pyrimidin-4-one (retention time: 7.74 min) (3.1 g) (**Supplementary Chemistry Data k**).