Stereochemical differences in fluorocyclopropyl amides enable tuning of Btk inhibition and off-target activity

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Supporting Information

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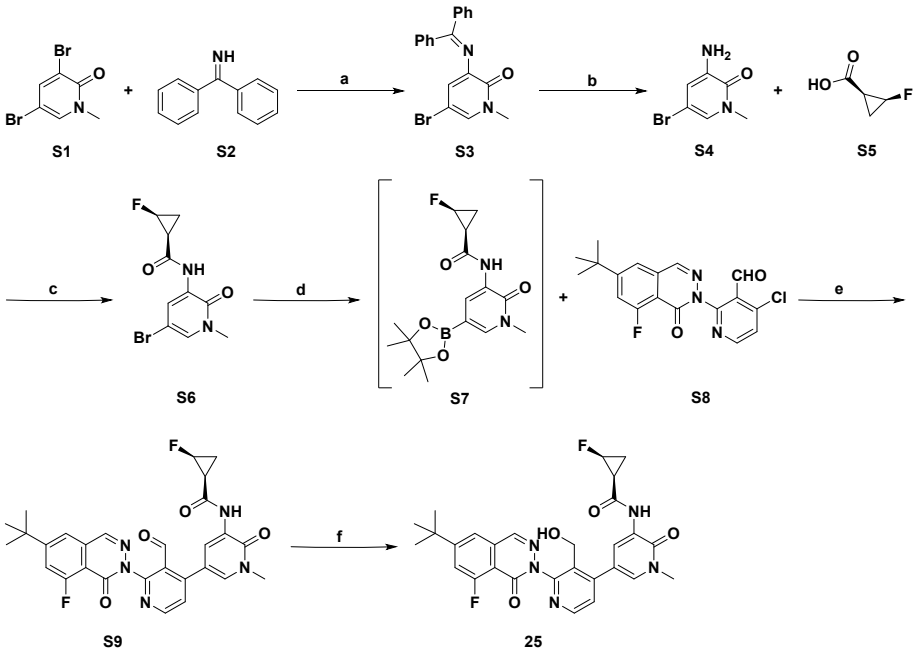
CHEMISTRY

All chemicals were used directly as received from commercial suppliers. ^{1}H NMR spectra were recorded on Bruker Avance 400 or 500 MHz spectrometers. Chemical shifts are expressed in δ ppm referenced to an internal standard, tetramethylsilane (δ = 0 ppm). Abbreviations used in describing peak signals are: br = broad signal, s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet. All final compounds were purified to > 95% by reverse phase high performance liquid chromatography (HPLC), supercritical fluid chromatography (SFC) or normal phase silica gel flash chromatography. The purity was assessed by reverse phase HPLC with a gradient of 5–95% acetonitrile in water (with either acid or base modifier) and monitored by absorption at 254 nm. Low-resolution mass spectra were recorded on liquid chromatography-mass spectrometer in electrospray positive (ES+) mode. HRMS experiments were performed on Dionex LC Ultimate3000 coupled with ThermoScientific Q Exactive orbitrap mass spectrometer using ESI as ionization source and a Phenomenex XB-C18, 1.7mm, 50 × 2.1 mm column with a 0.7 ml / minute flow rate at 40 °C for LC separation. Solvent A is water with 0.1% FA and solvent B is acetonitrile with 0.1% FA. The gradient consisted with 2 - 98% solvent B over 7 min and hold 98%B for 1.5 min following equilibration for 1.0 min. The LC was monitored by absorption at 220nm and 254nm. MS full scan with 10,000 resolution was applied to all experiments.

Scheme S1. Preparation of **25**.[a]



[a]Reagents and conditions: (a) $Pd_2(dba)_3$, XantPhos, Cs_2CO_3, toluene, 120 °C, 18 h, 68.5% yield; (b) 1N HCl, THF, MeOH, RT, 2h, 70.6% yield; (c) HATU, DIPEA, DMF, 60 °C, 83.2% yield;

(d) Pin_2B_2, KOAc, XPhos, $Pd_2(dba)_3$, dioxane, microwave, 100 °C, 30 min (e) $K_3PO_4 \cdot 3H_2O$, 80 °C, microwave, 1 h, 38.6% yield over 2 steps; (f) $NaBH_4$, MeOH, 68.5% yield.

(1*S*,2*S*)-*N*-(2'-(6-(*tert*-butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)-2-fluorocyclopropane-1-carboxamide **25**

Step 1: 5-bromo-3-((diphenylmethylene)amino)-1-methylpyridin-2(1*H*)-one **S3**

To a vessel with screwed cap charged with 3,5-dibromo-1-methyl-pyridin-2-one (4.0 g, 15.0 mmol), Cs_2CO_3 (8.70 g, 45.1 mmol, 3.0 equiv.), tris(dibenzylideneacetone)dipalladium (0) (482 mg, 0.53 mmol, 0.035 equiv.), and XantPhos (870 mg, 1.5 mmol, 0.10 equiv.) was added a solution of diphenylmethanimine (3.27 g, 18.0 mmol, 1.2 equiv.) in toluene (50 mL). The reaction mixture was degassed for 15 min, vacuum purged and backfilled with N_2 (3x). The vessel was capped, and the reaction mixture was stirred at 140 °C for 16h. The reaction mixture was diluted with ethyl acetate and filtered through a pad of Celite. The filtrate was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (SiO_2: ethyl acetate / heptane) to afford 3.78 g of **S3** as yellow solid. Yield: 68.5%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.79 - 7.71 (m 2H), 7.51 – 7.41 (m, 1H), 7.41 – 7.30 (m, 6H), 7.28 – 7.20 (m, 1H), 7.03 (d, J = 2.7 Hz, 1H), 6.64 (d, J = 2.5 Hz, 1H), 3.45 (s, 3H). MS [ES-MS] (ESI+): m/z calcd for $C_{19}H_{16}BrN_2O$ $[M + H]^+$, 367.2; found, 367.

Step 2: 3-amino-5-bromo-1-methylpyridin-2(1*H*)-one **S4**

To a stirred solution of 3-(benzhydrylideneamino)-5-bromo-1-methyl-pyridin-2-one **S3** (1.0 g, 2.72 mmol) dissolved in tetrahydrofuran (13.6 mL) and methanol (2.7 mL) was added 1M HCl (2.0 mL, 3.0 equiv.), and the reaction mixture was stirred at room temperature for 2h. Volatile solvent was then evaporated under reduced pressure, and the crude reaction was basified with sat. $NaHCO_3$ solution until neutral pH and extracted with ethyl acetate (3x). The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (SiO_2: ethyl acetate / heptane) to afford 390.5 mg of **S4** as a white solid. Yield: 70.6 %. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.82 (d, J = 2.3 Hz, 1H), 6.55 (d, J = 2.3 Hz, 1H), 4.34 (br s, 2H), 3.54 (s, 3H). MS [ES-MS] (ESI+): m/z calcd for $C_6H_8BrN_2O$ $[M + H]^+$, 203; found, 203.

Step 3: (1*S*,2*S*)-*N*-(5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)-2-fluorocyclopropane-1-carboxamide **S6**

A mixture of 3-amino-5-bromo-1-methyl-pyridin-2-one **S4** (389 mg, 1.92 mmol,), (1*S*,2*S*)-2-fluorocyclopropanecarboxylic acid **S5** (300 mg, 2.88 mmol, 1.5 equiv.), HATU (1.24 g, 3.26 mmol, 1.7 equiv.), and DIPEA (992 mg, 7.67 mmol, 4.0 equiv.) in DMF (9.6 mL) was stirred at 60 °C under N_2 for 16h. The reaction was quenched with water and extracted with 1:1 v/v ether / ethyl acetate (3x). The combined organic layers were washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (SiO_2: ethyl acetate / heptane) to afford 461 mg of **S6** as a white solid. Yield: 83.2 %. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.63 (br s, 1H), 8.51 (d, J = 2.5 Hz, 1H), 7.13 (d, J = 2.5 Hz, 1H), 4.89 – 4.65 (m, 1H), 3.58 (s, 3H), 1.95 – 1.80 (m, 2H), 1.28 – 1.15 (m, 1H). MS [ES-MS] (ESI+): m/z calcd for $C_{10}H_{11}BrFN_2O_2$ $[M + H]^+$, 289; found, 289.

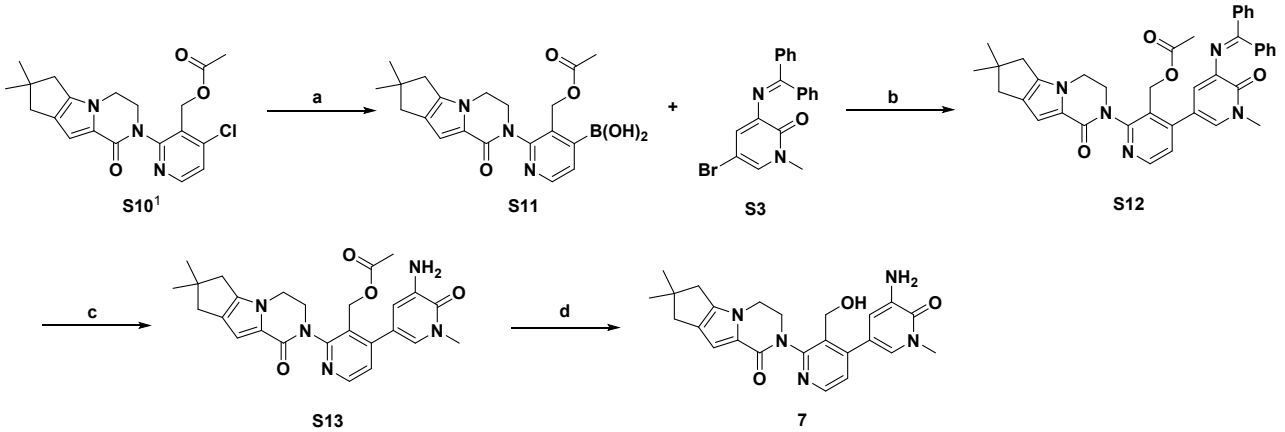
Step 4: (1*S*,2*S*)-*N*-(2'-(6-(*tert*-butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-3'-formyl-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)-2-fluorocyclopropane-1-carboxamide **S9**

A vial was charged with (1*S,*2*S*)-*N*-(5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)-2-fluorocyclopropane-1-carboxamide **S6** (726 mg, 2.51 mmol, 1.0 equiv.), bis(pinacolato)diboron (829 mg, 3.26 mmol, 1.3 equiv.), KOAc (493 mg, 5.02 mmol, 2.0 equiv.), XPhos (122 mg, 0.25 mmol, 0.1 equiv.), and tris(dibenzylideneactone)dipalladium (0) (115 mg, 0.126 mmol, 0.05 equiv.). Degassed 1,4-dioxane (16 mL) was added, and the reaction mixture was vacuum purged / backfilled with N_2 (3x). The vial was capped and the reaction mixture was microwaved at 100 °C for 30 min and cooled to room temperature. To this was added 2-(6-*tert*-butyl-8-fluoro-1-oxo-phthalazin-2-yl)-4-chloro-pyridine-3-carbaldehyde **S8** (904 mg, 2.51 mmol, 1.0 equiv.) followed by a solution of potassium phosphate tribasic (1.37 g, 6.28 mmol, 2.5 equiv.) in water (2.5 mL). The reaction mixture was vacuum purged/ back-filled with N_2 (3x), and the vial was capped. The reaction mixture was then microwaved at 80 °C for 30 min and quenched with EtOAc and filtered. The organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (SiO_2: methanol / ethyl acetate) to afford 516.7 mg of **S9** as a foam. Yield: 38.6 %. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 9.97 (s, 1H), 8.76 (d, J = 5.1 Hz, 1H), 8.69 (s, 1H), 8.60 (d, J = 2.4 Hz, 1H), 8.26 (d, J = 2.5 Hz, 1H), 7.56 – 7.44 (m, 3H), 7.10 (d, J = 2.4 Hz, 1H), 4.90 – 4.67 (m, 1H), 3.65 (s, 3H), 1.95 – 1.86 (m, 2H), 1.40 (s, 9H), 1.29 – 1.15 (m, 1H). MS [ES-MS] (ESI+): m/z calcd for $C_{28}H_{26}F_2N_5O_4$ $[M + H]^+$, 534.2; found, 534.2.

Step 5: (1*S*,2*S*)-*N*-(2'-(6-(*tert*-butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)-2-fluorocyclopropane-1-carboxamide **25**

To ((1*S*,2*S*)-*N*-(2'-(6-(*tert*-butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-3'-formyl-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)-2-fluorocyclopropane-1-carboxamide **S9** (1.04 g, 1.95 mmol) in anhydrous MeOH (49 mL) at 0 °C was added sodium borohydride (185 mg, 4.88 mmol, 2.500 equiv.) portionwise over 30 min. The reaction mixture was stirred at 0 °C for 1h and at RT for 2h, then quenched with water. Volatile solvent was removed under reduced pressure, and the crude reaction was diluted with ethyl acetate. The organic layer was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (SiO_2: MeOH / EtOAc) followed by crystallization from EtOAc / hexane to obtain 716.9 mg of the title compound **25** as a white solid. Yield: 68.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.75 (s, 1H), 8.56 (d, J = 5.2 Hz, 1H), 8.52 (d, J = 2.5 Hz, 1H), 8.41 (d, J = 2.4 Hz, 1H), 7.89 (d, J = 2.0 Hz, 1H), 7.80 – 7.74 (m, 2H), 7.49 (d, J = 5.0 Hz, 1H), 5.01 – 4.78 (m, 2H), 4.40 (t, J = 4.7 Hz, 2H), 3.60 (s, 3H), 2.49 – 2.43 (m, 1H), 1.67 – 1.53 (m, 1H), 1.39 (s, 9H), 1.20 – 1.09 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 166.29 (d), 161.76, 160.02 (d), 159.15, 156.34, 155.59 (d), 153.78, 148.22, 147.98, 137.68, 132.45, 131.98, 131.09, 128.22, 125.34, 123.51, 119.68, 117.00, 116.80, 114.91, 113.86 (d), 73.66, 56.37, 37.61, 35.58, 30.49, 21.40 (d), 10.78 (d). HRMS (ESI+) m/z found MH^+ 536.2099, $C_{28}H_{28}F_2N_5O_4$ requires 536.2104. Chiral t_R = 1.091 min (Chiralpak IB-N, isocratic 45% MeOH w/ 0.1% NH_4OH / CO_2, 4 mL/min flow, 2.5 min at 40 °C, 120 bar). $[\alpha]_D^{20}$ = -12.57 (c = 1.67 mg/mL, MeOH).

Scheme S2. Preparation of **7**.[a]



[a]Reagents and conditions: (a) Pin_2B_2, KOAc, XPhos, $PdCl_2$(dppf), dioxane, 65 °C, 4 h, 65% yield; (b) $PdCl_2$(dppf), NaOAc, $K_3PO_4 \cdot 3H_2O$, ACN, H_2O, 80 °C, 1 h, 47% yield; (c) HCl in dioxane, 0 °C, 0.5h, 60% yield; (d) LiOH, ^{i}PrOH/THF, H_2O, 40°C, 0.5 h, 36% yield.

2-(5-amino-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-2'-yl)-7,7-dimethyl-3,4,7,8-tetrahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-a]pyrazin-1(6*H*)-one **7**

Step 1. Preparation of **S11**.

In a 250-mL single-neck round-bottomed flask equipped with a magnetic stirrer and a reflux condenser was charged with **S10**[1] (4.5 g, 11.6 mmol) [1434051-95-2], bis(pinacolato)diboron (7.38 g, 29.0 mmol, 2.5 equiv,), $PdCl_2$(dppf) (473 mg, 0.58 mmol, 0.05 equiv.), Xphos (470 mg, 1.16 mmol, 0.1 equiv.), potassium acetate (3.41 g., 34.8 mmol, 3.0 equiv.), and dioxane (100 mL). After three cycles of vacuum/argon flush, the reaction mixture was heated at 65 °C for 4h, cooled to room temperature, and filtered. The filtrate was concentrated under reduced pressure to afford crude **S11** as a brown-red liquid (4.0 g, 86.8%). MS (ESI+) m/z found MH^+ 398.3, $C_{20}H_{25}BN_3O_5$ requires 388.2.

Step 2. Preparation of **S12**.

A 100-mL single-neck round-bottomed flask equipped with a magnetic stirrer and a reflux condenser was charged with **S3** (1.0 g, 2.70 mmol), **S11** (1.20 g, 3.00 mmol, 1.1 equiv.), $PdCl_2$(dppf) (122 mg, 0.15 mmol, 0.056 equiv.), NaOAc (460 mg, 5.4 mmol, 2.0 equiv.), $K_3PO_4 \cdot 3H_2O$ (1.27 g, 5.4 mmol, 2.0 equiv.), H_2O (1 mL), and ACN (30 mL). After three cycles of vacuum/argon flush, the reaction mixture was heated at 80°C for 1 h, cooled to room temperature, and filtered. The filtrate was concentrated under reduced pressure, and the crude was purified by column chromatography (SiO_2: 20:1 DCM/MeOH) to afford **S12** as a yellow solid (800 mg, 47%). MS (ESI+) m/z found MH^+ 640.3, $C_{39}H_{38}N_5O_4$ requires 640.3.

Step 3. Preparation of **S13**.

A mixture of **S12** (800 mg, 1.25 mmol) in HCl in dioxane (4M, 20 mL) was stirred at 0°C for 0.5 h. The reaction mixture was evaporated *in vacuo*, and the residue was purified by reverse-phase prep-HPLC to afford **S13** as a pale yellow solid (350 mg, 60%). ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.43 (d, J = 5.0 Hz, 1H), 7.09 (d, J = 5.0 Hz, 1H), 6.81 (d, J = 2.0 Hz, 1H), 6.77 (s, 1H), 6.57 (d, J = 2.0 Hz, 1H), 6.24-6.22 (m, 1H), 5.13-5.11 (m, 1H), 4.51-4.47 (m, 1H), 4.36 (s, 2H), 4.24-4.20 (m, 1H), 4.14-4.11 (m, 1H), 4.01-3.98 (m, 1H), 3.62 (s, 3H), 2.55-2.54 (m, 2H),

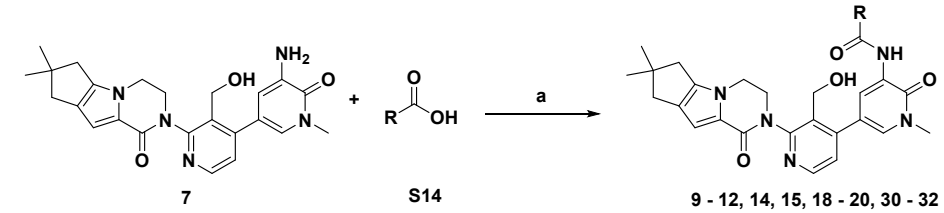
2.49 (s, 2H), 1.81 (s, 3H), 1.26 (s, 6H). MS (ESI+) m/z found MH^+ 476.1, $C_{26}H_{30}N_5O_4$ requires 476.2.

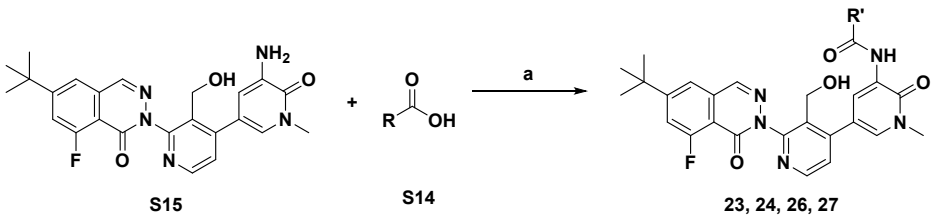
Step 4. Preparation of **7**.

A mixture of **S13** (1.1 g, 2.3 mmol) and LiOH (450 mg, 11.0 mmol, 4.8 equiv.) in ^{i}PrOH/THF (1:1, 10 mL) and H_2O (2.5 mL) was stirred at 40 ^{o}C for 0.5 h. The mixture was evaporated *in vacuo*. The residue was partitioned between EtOAc and water. The combined EtOAc extract was concentrated under reduced pressure. The residue was purified by by column chromatography (SiO_2: 10:1 DCM/MeOH) to afford the title compound **7** as a pale yellow solid (350 mg, 36%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.44 (d, J = 5.0 Hz, 1H), 7.59 (d, J = 2.0 Hz, 1H), 7.21 (d, J = 5.0 Hz, 1H), 6.83 (s, 1H), 6.82 (d, J = 2.0 Hz, 1H), 5.04-5.03(m, 1H), 4.63-4.62 (m, 1H), 4.50-4.48 (m, 1H), 4.30-4.28 (m, 1H), 4.16-4.10 (m, 3H), 3.87-3.85 (m, 1H), 3.65 (s, 3H), 2.57-2.56 (m, 2H), 2.50 (s, 2H), 1.26 (s, 6H). MS (ESI+) m/z found MH^+ 434.2, $C_{24}H_{28}N_5O_3$ requires 434.2.

Amides **9** – **12, 14, 15, 18** – **21, 23**, **24**, **26**, **30** – **32** were prepared in a parallel fashion by HATU coupling of advanced intermediates **7** or **S15** with the corresponding carboxylic acids (Scheme S3). Amides **8** and **13** were prepared from Suzuki coupling of bromide **S16a** or **S16b** with boronic acid **S17**[1] to give the acetylated intermediates **S18a** or **S18b**, respectively, which was then treated with lithium hydroxide in ^{i}PrOH/THF/H_2O to afford the desired alcohols **7** or **13**, respectively (Scheme S4). For compounds **16**, **33**, and **34**, we employed the one-pot borylation / Suzuki coupling methodology described for the synthesis of **25** starting from bromo intermediates **S19**, **S6**, and **S21**[3] to generate the desired alcohols, respectively (Scheme **S5** and **S6**).

Scheme S3. Preparation of **9** – **12**, **14**, **15**, **18** – **20**, **23**, **24**, **26**, **27**, **30** – **32**.[a]





[a] Reagents and conditions: (a) HATU, DIPEA, DMF, 50 °C, 18h, 11.5 – 54% yield.

Representative library synthesis: Preparation of **9** – **12**, **14**, **15**, **17** – **20**, **23**, **24**, **26, 27, 30 – 32**.

N-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)acetamide **9**

A screw-capped vial was charged with 2-(5-amino-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-2'-yl)-7,7-dimethyl-3,4,7,8-tetrahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-a]pyrazin-1(6*H*)-one **7** (50 mg, 0.115 mmol), acetic acid (9.0 mg, 0.345 mmol, 1.3 equiv.), HATU (66.5 mg, 0.173 mmol, 1.5 equiv.), DIPEA (45.2 mg, 0.346 mmol, 3 equiv.), and DMF (1 mL). The capped was sealed, and the reaction mixture was stirred at 50 °C for 18h. Solvent was concentrated under reduced pressure, and the crude was purified by reverse phase HPLC to give 15.4 mg (28.1% yield) of **9** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.37 (s, 1H), 8.47 (d, J = 5.0 Hz, 1H), 8.43 (d, J = 2.4 Hz, 1H), 7.72 (d, J = 2.5 Hz, 1H), 7.29 (d, J = 5.1 Hz, 1H), 6.55 (s, 1H), 4.92 (t, J = 5.3 Hz, 1H), 4.42 – 4.36 (m, 2H), 4.29 – 4.14 (m, 3H), 3.90 – 3.80 (m, 1H), 3.58 (s, 3H), 2.57 (d, J = 7.5 Hz, 2H), 2.43 (s, 2H), 2.14 (s, 3H), 1.22 (s, 6H). HRMS (ESI+) m/z found MH^+ 476.2290, $C_{26}H_{30}N_5O_4$ requires 476.2292.

The following compounds were prepared using the procedure described for **9** and replacing acetic acid with the appropriate carboxylic acids:

N-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)propionamide **10**: Yield 40.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 1H), 8.47 (d, J = 5.0 Hz, 1H), 8.45 (d, J = 2.4 Hz, 1H), 7.73 (d, J = 2.4 Hz, 1H), 7.30 (d, J = 5.1 Hz, 1H), 6.56 (s, 1H), 4.96 – 4.89 (m, 1H), 4.47 – 4.33 (m, 2H), 4.30 – 4.14 (m, 3H), 3.90 – 3.81 (m, 1H), 3.58 (s, 3H), 2.57 (d, J = 7.3 Hz, 2H), 2.48 (q, J = 7.5 Hz, 2H), 2.43 (s, 2H), 1.22 (s, 6H), 1.05 (t, J = 7.5 Hz, 3H). HRMS (ESI+) m/z found MH^+ 490.2446, $C_{27}H_{32}N_5O_4$ requires 490.2449.

N-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)butyramide **11**: Yield 41.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.27 (s, 1H), 8.47 (d, J = 5.0 Hz, 1H), 8.45 (d, J = 2.3 Hz, 1H), 7.73 (d, J = 2.3 Hz, 1H), 7.30 (d, J = 5.1 Hz, 1H), 6.56 (s, 1H), 4.97 – 4.92 (m, 1H), 4.47 – 4.33 (m, 2H), 4.30 – 4.12 (m, 3H), 3.90 – 3.80 (m, 1H), 3.58 (s, 3H), 2.64 – 2.52 (m, 2H), 2.45 (d, J = 7.3 Hz, 1H), 2.42 (s, 3H), 1.58 (h, J = 7.3 Hz, 2H), 1.22 (s, 6H), 0.89 (t, J = 7.3 Hz, 3H).

^{13}C NMR (101 MHz, DMSO) δ 172.31, 159.22, 156.41, 154.66, 147.88, 147.74, 140.90, 132.06, 130.65, 128.04, 125.77, 125.56, 123.47, 123.41, 115.64, 108.86, 57.20, 47.79, 45.30, 41.95, 40.71, 38.96, 37.99, 37.59, 30.16, 30.05, 18.53, 13.54. HRMS (ESI+) m/z found MH^+ 504.2601, $C_{28}H_{34}N_5O_4$ requires 504.2605.

N-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)isobutyramide **12**: Yield 11.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.23 (s, 1H), 8.47 (d, J = 5.0 Hz, 1H), 8.44 (d, J = 2.4 Hz, 1H), 7.74 (d, J = 2.4 Hz, 1H), 7.30 (d, J = 5.0 Hz, 1H), 6.56 (s, 1H), 4.98 – 4.90 (m, 1H), 4.49 – 4.33 (m, 2H), 4.30 – 4.11 (m, 3H), 3.89 – 3.81 (m, 1H), 3.58 (s, 3H), 2.95 – 2.84 (m, 1H), 2.60 – 2.56 (m, 2H), 2.43 (s, 2H), 1.22 (s, 6H), 1.08 (d, J = 6.8 Hz, 6H). HRMS (ESI+) m/z found MH^+ 504.2602, $C_{28}H_{34}N_5O_4$ requires 504.2605.

2-Cyclopropyl-*N*-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)acetamide **14**: Yield 29.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.27 (s, 1H), 8.51 – 8.44 (m, 2H), 7.73 (d, J = 2.4 Hz, 1H), 7.30 (d, J = 5.0 Hz, 1H), 6.56 (s, 1H), 4.97 – 4.87 (m, 1H), 4.47 – 4.33 (m, 2H), 4.32 – 4.13 (m, 3H), 3.85 – 3.65 (m, 1H), 3.59 (s, 3H), 2.57 (d, J = 7.5 Hz, 2H), 2.43 (s, 2H), 2.37 (d, J = 7.1 Hz, 2H), 1.22 (s, 6H), 1.08 – 0.94 (m, 1H), 0.56 – 0.46 (m, 2H), 0.26 – 0.17 (m, 2H). HRMS (ESI+) m/z found MH^+ 516.2599, $C_{29}H_{34}N_5O_4$ requires 516.2605.

N-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)oxetane-3-carboxamide **15**: Yield 12.0%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.52 (s, 1H), 8.53 (d, J = 2.5 Hz, 1H), 8.48 (d, J = 5.0 Hz, 1H), 7.76 (d, J = 2.5 Hz, 1H), 7.32 (d, J = 5.0 Hz, 1H), 6.60 – 6.55 (m, 2H), 4.94 (t, J = 5.3 Hz, 1H), 4.70 – 4.62 (m, 3H), 4.46 – 4.38 (m, 2H), 4.30 – 4.16 (m, 3H), 3.87 (s, 1H), 3.58 (s, 3H), 2.58 (d, J = 7.1 Hz, 2H), 2.43 (s, 3H), 1.22 (s, 6H). HRMS (ESI+) m/z found MH^+ 518.2395, $C_{28}H_{32}N_5O_5$ requires 518.2398.

(*S*)-*N*-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)tetrahydrofuran-2-carboxamide **17**: Yield 35.0%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.39 (s, 1H), 8.48 (d, J = 5.0 Hz, 1H), 8.46 (d, J = 2.5 Hz, 1H), 7.76 (d, J = 2.5 Hz, 1H), 7.31 (d, J = 5.0 Hz, 1H), 6.56 (s, 1H), 4.95 (t, J = 5.3 Hz, 1H), 4.47 (dd, J = 8.5, 5.6 Hz, 1H), 4.34 – 4.33 (m, 2H), 4.31 – 4.14 (m, 3H), 4.00 – 3.81 (m, 3H), 3.59 (s, 3H), 2.57 (d, J = 7.6 Hz, 2H), 2.42 (s, 2H), 2.29 – 2.18 (m, 1H), 2.04 –

1.77 (m, 3H), 1.22 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 171.82, 159.23, 156.28, 154.62, 147.93, 147.54, 140.92, 132.46, 130.71, 126.85, 125.74, 125.58, 123.51, 122.36, 115.87, 108.88, 77.97, 69.05, 57.17, 47.79, 45.31, 41.97, 40.72, 38.97, 37.57, 30.16, 30.06, 29.69, 25.09. HRMS (ESI+) m/z found MH^+ 532.2550, $C_{29}H_{34}N_5O_5$ requires 532.2554

N-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)-1-fluorocyclopropane-1-carboxamide **18**: Yield 46.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.24 (d, J = 3.9 Hz, 1H), 8.48 (d, J = 5.0 Hz, 1H), 8.42 (d, J = 2.4 Hz, 1H), 7.82 (d, J = 2.4 Hz, 1H), 7.32 (d, J = 5.0 Hz, 1H), 6.55 (s, 1H), 4.94 (t, J = 5.3 Hz, 1H), 4.46 – 4.34 (m, 2H), 4.30 – 4.14 (m, 3H), 3.85 (d, J = 11.1 Hz, 1H), 3.62 (s, 3H), 2.57 (d, J = 7.3 Hz, 2H), 2.43 (s, 2H), 1.56 – 1.45 (m, 2H), 1.39 – 1.31 (m, 2H), 1.22 (s, 6H). HRMS (ESI+) m/z found MH^+ 520.2351, $C_{28}H_{31}FN_5O_4$ requires 520.2360.

(1*S*,2*S*)-*N*-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)-2-fluorocyclopropane-1-carboxamide **19**: Yield 53.9%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (s, 1H), 8.47 (d, J = 5.0 Hz, 1H), 8.43 (d, J = 2.4 Hz, 1H), 7.74 (d, J = 2.4 Hz, 1H), 7.30 (d, J = 5.1 Hz, 1H), 6.56 (s, 1H), 4.93 (t, J = 5.3 Hz, 2H), 5.01 – 4.72 (m, 1H), 4.48 – 4.36 (m, 2H), 4.25 – 4.14 (m, 3H), 3.85 (d, J = 10.1 Hz, 1H), 3.60 (s, 3H), 2.57 (d, J = 7.2 Hz, 2H), 2.43 (s, 2H), 1.67 – 1.52 (m, 1H), 1.22 (s, 6H), 1.20 – 1.08 (m, 1H). MS (ESI+) m/z found MH^+ found 520.2, $C_{28}H_{31}FN_5O_4$ requires 520.2. Chiral t_R = 0.579 min (Chiralpak IB-N, isocratic 45% MeOH w/ 0.1% NH_4OH / CO_2, 4 mL/min flow, 2.5 min at 40 °C, 120 bar).

(1*R*,2*R*)-N-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)-2-fluorocyclopropane-1-carboxamide **20**: Yield 48.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (s, 1H), 8.47 (d, J = 5.1 Hz, 1H), 8.43 (d, J = 2.4 Hz, 1H), 7.74 (d, J = 2.4 Hz, 1H), 7.30 (d, J = 5.0 Hz, 1H), 6.56 (s, 1H), 4.93 (t, J = 5.3 Hz, 2H), 5.04 – 4.71 (m, 1H), 4.45 – 4.36 (m, 2H), 4.25 – 4.15 (m, 3H), 3.85 (d, J = 10.5 Hz, 1H), 3.60 (s, 3H), 2.57 (d, J = 7.2 Hz, 2H), 2.43 (s, 2H), 1.66 – 1.54 (m, 1H), 1.22 (s, 6H), 1.21 – 1.08 (m, 1H). HRMS (ESI+) m/z found MH^+ 520.2348, $C_{28}H_{31}FN_5O_4$ requires 520.2355. Chiral t_R = 0.704 min (Chiralpak IB-N, isocratic 45% MeOH w/ 0.1% NH_4OH / CO_2, 4 mL/min flow, 2.5 min at 40 °C, 120 bar).

N-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)-1-

hydroxycyclopropane-1-carboxamide **30**: Yield 4.1% ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.76 (s, 1H), 8.49 – 8.45 (m, 2H), 7.75 (d, J = 2.4 Hz, 1H), 7.31 (d, J = 5.1 Hz, 1H), 6.86 (s, 1H), 6.55 (s, 1H), 4.94 – 4.89 (m, 1H), 4.45 – 4.36 (m, 2H), 4.22 - 4.15 (m, 2H), 3.89 - 3.82 (m, 1H), 3.61 (s, 3H), 2.57 (d, J = 7.2 Hz, 1H), 2.43 (s, 2H), 1.24 (s, 1H), 1.22 (s, 6H), 1.16 (q, J = 3.9, 3.5 Hz, 2H), 1.02 (d, J = 3.4 Hz, 2H), 0.95 (d, J = 6.5 Hz, 1H). MS (ESI+) m/z found MH$^+$ 518.2, $C_{28}H_{32}N_5O_5$ requires 518.2.

N-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)-2-morpholinoacetamide **31**: Yield 32.2%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.96 (s, 1H), 8.48 (d, J = 5.0 Hz, 1H), 8.45 (d, J = 2.4 Hz, 1H), 7.74 (d, J = 2.4 Hz, 1H), 7.30 (d, J = 5.1 Hz, 1H), 6.55 (s, 1H), 4.93 (br s, 1H), 4.46 – 4.34 (m, 2H), 4.28 – 4.15 (m, 3H), 3.89 – 3.81 (m, 1H), 3.69 – 3.63 (m, 4H), 3.60 (s, 3H), 3.27 (s, 2H), 3.17 (s, 2H), 2.61 – 2.52 (m, 6H), 1.22 (s, 6H). HRMS (ESI+) m/z found MH$^+$ 561.2817, $C_{30}H_{37}N_6O_5$ requires 561.2820.

N-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)nicotinamide **32**: Yield 37.7%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 1H), 9.08 (dd, J = 2.4, 0.9 Hz, 1H), 8.77 (dd, J = 4.8, 1.6 Hz, 1H), 8.52 – 8.49 (m, 2H), 8.31 – 8.26 (m, 1H), 7.86 (d, J = 2.4 Hz, 1H), 7.60 – 7.55 (m 1H), 7.36 (d, J = 5.1 Hz, 1H), 6.56 (s, 1H), 4.99 (br s, 1H), 4.51 – 4.37 (m, 2H), 4.31 – 4.16 (m, 3H), 3.91 – 3.83 (m, 1H), 3.63 (s, 3H), 2.58 (d, J = 7.4 Hz, 2H), 2.43 (s, 2H), 1.22 (s, 6H). HRMS (ESI+) m/z found MH+ 539.2399, $C_{30}H_{31}N_6O_4$ requires 539.2401.

The following compounds were prepared using the procedure described for **9** and replacing acetic acid with the appropriate carboxylic acids and substituting intermediate **7** with 2-(5-amino-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-2'-yl)-6-(*tert*-butyl)-8-fluorophthalazin-1(2*H*)-one **S15**.

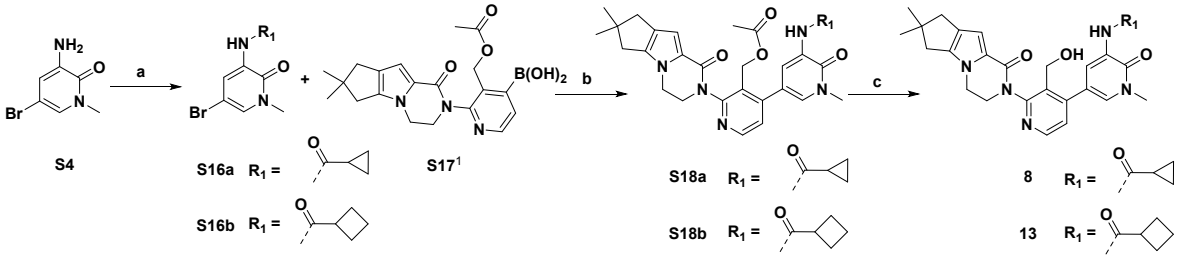
N-(2'-(6-(*tert*-butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)cyclopropanecarboxamide **23**: Yield 69.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (s, 1H), 8.55 (d, J = 5.0 Hz, 1H), 8.52 (d, J = 2.5 Hz, 1H), 8.38 (d, J = 2.4 Hz, 1H), 7.89 (d, J = 1.8 Hz, 1H), 7.80 – 7.71 (m, 2H), 7.47 (d, J = 5.0 Hz, 1H), 4.89 (t, J = 5.0 Hz, 1H), 4.43 – 4.35 (m, 2H), 3.60 (s, 3H), 2.31 – 2.20 (m, 1H), 1.39 (s, 9H), 0.85 – 0.72 (m, 4H). MS (ESI+) m/z found MH$^+$ 518.2, $C_{28}H_{29}FN_5O_4$ requires 518.2.

(1*R*,2*R*)-*N*-(2'-(6-(*tert*-butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)-2-fluorocyclopropane-1-carboxamide **24**: Yield 64.4%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 1H), 8.56 (d, J = 5.0 Hz, 1H), 8.52 (d, J = 2.6 Hz, 1H), 8.40 (d, J = 2.4 Hz, 1H), 7.89 (d, J = 1.8 Hz, 1H), 7.80 – 7.72 (m, 2H), 7.48 (d, J = 5.0 Hz, 1H), 5.01 – 4.78 (m, 2H), 4.43 – 4.36 (m, 2H), 3.60 (s, 3H), 2.49 – 2.42 (m, 1H), 1.66 – 1.53 (m, 1H), 1.39 (s, 9H), 1.20 – 1.09 (m, 1H). HRMS (ESI+) m/z found MH^+ 536.2097, $C_{28}H_{28}F_2N_5O_4$ requires 536.2104. Chiral t_R = 1.403 min (Chiralpak IB-N, isocratic 45% MeOH w/ 0.1% NH_4OH / CO_2, 4 mL/min flow, 2.5 min at 40 °C, 120 bar).

(1*R*,2*S*)-*N*-(2'-(6-(*tert*-butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)-2-fluorocyclopropane-1-carboxamide **26**: Yield 35.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.02 (s, 1H), 8.55 (d, J = 5.1 Hz, 1H), 8.52 (d, J = 2.6 Hz, 1H), 8.35 (d, J = 2.4 Hz, 1H), 7.89 (d, J = 1.7 Hz, 1H), 7.79 – 7.74 (m, 2H), 7.47 (d, J = 5.0 Hz, 1H), 4.96 – 4.73 (m, 2H), 4.44 – 4.33 (m, 2H), 3.60 (s, 3H), 2.98 – 2.85 (m, 1H), 1.56 – 1.40 (m, 1H), 1.39 (s, 9H), 1.25 – 1.14 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 169.63, 161.77, 160.05 (d), 159.16, 156.36, 155.60 (d), 153.77, 148.15, 148.01, 137.70, 132.75, 131.98, 131.11, 127.91, 125.31, 124.11, 119.71, 117.02, 116.82, 114.83, 113.85 (d), 74.97, 72.75, 56.38, 37.62, 30.49, 20.94 (d), 18.45 (d). HRMS (ESI+) m/z found MH^+ 536.2102, $C_{28}H_{28}F_2N_5O_4$ requires 536.2104. Chiral t_R = 0.586 min (Chiralpak AD, isocratic 40% IPA w/ 0.1% NH_4OH / CO_2, 4 mL/min flow, 2.5 min at 40 °C, 120 bar).

(1*S*,2*R*)-*N*-(2'-(6-(*tert*-butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)-2-fluorocyclopropane-1-carboxamide **27**: Yield 33.6%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.02 (s, 1H), 8.55 (d, J = 5.0 Hz, 1H), 8.52 (d, J = 2.5 Hz, 1H), 8.35 (d, J = 2.5 Hz, 1H), 7.89 (d, J = 1.9 Hz, 1H), 7.79 – 7.74 (m, 2H), 7.47 (d, J = 5.0 Hz, 1H), 4.94 – 4.74 (m, 2H), 4.38 (t, J = 5.2 Hz, 2H), 3.60 (s, 3H), 2.98 – 2.85 (m, 1H), 1.56 – 1.40 (m, 1H), 1.39 (s, 9H), 1.25 – 1.14 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 169.62, 161.77, 160.05 (d), 159.16, 156.36, 155.60 (d), 153.77, 148.15, 148.01, 137.70, 132.75, 131.97, 131.11, 127.89, 125.31, 124.11, 119.71, 117.02, 116.81, 114.83, 113.85 (d), 74.97, 72.75, 56.38, 37.62, 30.49, 20.94 (d), 18.45 (d). HRMS (ESI+) m/z found MH^+ 536.2103; $C_{28}H_{28}F_2N_5O_4$ requires 536.2104. Chiral t_R = 0.685 min (Chiralpak AD, isocratic 40% IPA w/ 0.1% NH_4OH / CO_2, 4 mL/min flow, 2.5 min at 40 °C, 120 bar).

Scheme S4. Preparation of **8** and **13**.[a]



[a] Reagents and conditions: (a) Cyclopropanecarboxylic acid or cyclobutanecarboxylic acid, HATU, DIPEA, DCM, 60 °C, 56 – 72% yield; (b) $Pd(dppf)Cl_2$, NaOAc, $K_3PO_4 \cdot 3H_2O$, ACN, H_2O, 100 °C, 33 – 56% yield; (c) LiOH, ^{i}PrOH/THF/H_2O, 40 °C, 47 – 65% yield.

N-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)cyclopropanecarboxamide **8**

Step 1: *N*-(5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)cyclopropanecarboxamide **S16a**

To a mixture of cyclopropanecarboxylic acid (180 mg, 2.0 mmol), HATU (570 mg, 1.5 mmol) and DIPEA (390 mg, 3.0 mmol) in DCM (8 mL) was added 3-amino-5-bromo-1-methylpyridin-2(1*H*)-one **S4** (230 mg, 1.12 mmol). The reaction mixture was stirred at 25 ℃ for 5h. The resulting mixture was evaporated under reduced pressure. The resulting residue was purified by flash column chromatography (SiO_2: MeOH / DCM) to afford 220 mg of **S16a**. Yield 72%. MS [ES-MS] (ESI+): m/z calcd for $C_{10}H_{12}BrN_2O_2$ $[M + H]^+$, 270.0; found, 270.

Step 2: [4-(5-cyclopropaneamido-1-methyl-6-oxo-1,6-dihydropyridin-3-yl)-2-{4,4-dimethyl-9-oxo-1,10-diazatricyclo[6.4.0.02,6]dodeca-2(6),7-dien-10-yl}pyridin-3-yl]methyl acetate **S18a**

A 50-mL round-bottomed flask equipped with a magnetic stirrer and a reflux condenser was charged with **S16a** (220 mg, 0.80 mmol), {3-[(acetyloxy)methyl]-2-{4,4-dimethyl-9-oxo-1,10-diazatricyclo[6.4.0.02,6]dodeca-2(6),7-dien-10-yl}pyridin-4-yl}boronic acid **S17**[1] (320 mg, 0.80 mmol), $Pd(dppf)Cl_2$ (42 mg, 0.05 mmol), NaOAc (82 mg, 1.0 mmol), $K_3PO_4 \cdot 3H_2O$ (266 mg, 1.0 mmol), water (6 drops), and ACN (6 mL). After three cycles of vacuum/argon purge, the mixture was heated at 100 ℃ for 1h. The reaction mixture was filtered, and the filtrate was evaporated under reduced pressure. The residue was purified by flash column chromatography (SiO_2: MeOH

/ DCM) to afford 150 mg of **S18a** as a solid. Yield 33%. MS [ES-MS] (ESI+): m/z calcd for $C_{30}H_{34}N_5O_5$ $[M + H]^+$ 544.3; found, 544.3.

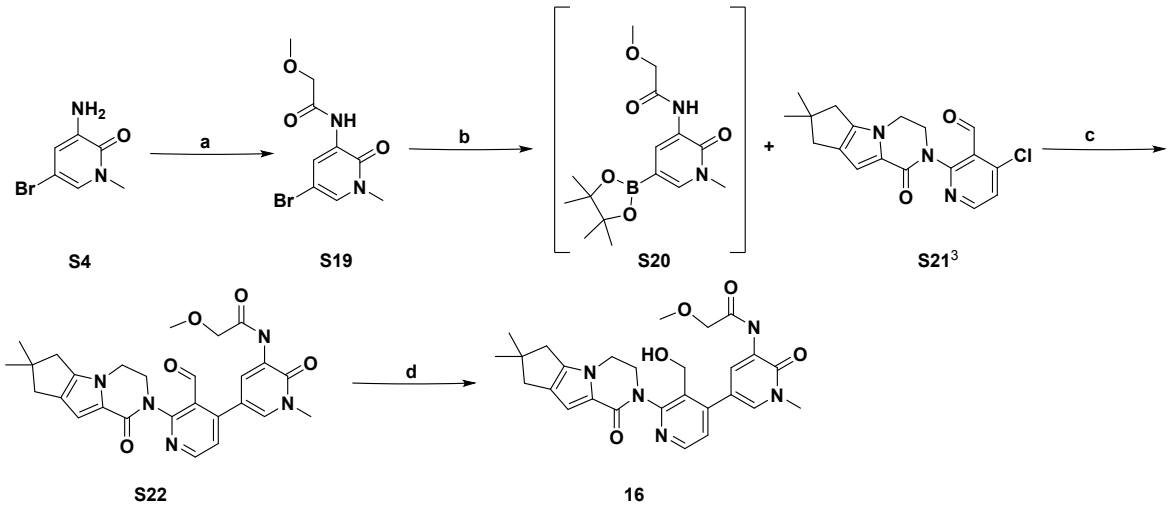
Step 3: Preparation of **8**

A mixture of **S18a** (150 mg, 0.27 mmol) and lithium hydroxide (34 mg, 1.4 mmol) in ^{i}PrOH / THF (1:1, 4 mL) and H_2O (1 mL) was stirred at 40 ℃ for 0.5h. The mixture was evaporated under reduced pressure and the residue was partitioned between ethyl acetate and water. The organic layer was concentrated under reduced pressure and the residue was purified by reverse-phase prep-HPLC to afford 65 mg of the title compound **8** as a pale yellow solid. Yield 65%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.69 (s, 1H), 8.46 (d, J = 5.0 Hz, 1H), 8.41 (d, J = 2.2 Hz, 1H), 7.73 (d, J = 2.4 Hz, 1H), 7.29 (d, J = 5.0 Hz, 1H), 6.55 (s, 1H), 4.97 – 4.89 (m, 1H), 4.48 – 4.32 (m, 2H), 4.29 – 4.13 (m, 3H), 3.89 – 3.79 (m, 1H), 3.59 (s, 3H), 2.57 (d, J = 7.4 Hz, 2H), 2.42 (s, 2H), 2.30 –2.20 (m, 1H), 1.22 (s, 6H), 0.82 – 0.72 (m, 4H). ^{13}C NMR (101 MHz, DMSO) δ 172.87, 159.20, 156.40, 154.64, 147.86, 147.71, 140.89, 132.05, 130.63, 128.13, 125.77, 125.57, 123.70, 123.44, 115.64, 108.85, 57.21, 47.79, 45.30, 41.96, 40.72, 37.60, 30.16, 30.06, 14.18, 7.67. HRMS (ESI+) m/z found MH^+ 502.2447, $C_{28}H_{32}N_5O_4$ requires 502.2449.

N-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)cyclobutanecarboxamide **13**:

Compound **13** was prepared in a similar manner to **8**, replacing cyclopropanecarboxylic acid with cyclobutanecarboxylic acid. Yield 14.7% over three steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.08 (s, 1H), 8.50 – 8.45 (m, 2H), 7.73 (d, J = 2.5 Hz, 1H), 7.31 (d, J = 5.0 Hz, 1H), 6.56 (s, 1H), 4.95 (t, J = 5.3 Hz, 1H), 4.47 – 4.34 (m, 2H), 4.31 – 4.14 (m, 3H), 3.90 – 3.81 (m, 1H), 3.57 (s, 3H), 3.57 – 3.46 (m, 1H), 2.57 (d, J = 7.4 Hz, 2H), 2.43 (s, 2H), 2.25 – 2.04 (m, 4H), 1.98 – 1.85 (m, 1H), 1.85 – 1.73 (m, 1H), 1.22 (s, 6H). ^{13}C NMR (101 MHz, DMSO) δ 173.96, 159.21, 156.41, 154.65, 147.88, 147.71, 140.90, 132.04, 130.64, 127.99, 125.77, 125.57, 123.47, 123.36, 115.71, 108.86, 57.22, 47.80, 45.31, 41.97, 40.72, 39.24, 38.97, 37.58, 30.16, 30.06, 24.61, 17.61. HRMS (ESI+) m/z found MH^+ 516.2604, $C_{29}H_{34}N_5O_4$ requires 516.2605.

Scheme S5. Preparation of **16**.[a]



[a]Reagents and conditions: (a) HATU, DIPEA, DMF, 60 °C, 78.4% yield; (b) Pin_2B_2, KOAc, XPhos, $Pd_2(dba)_3$, dioxane, microwave, 100 °C, 30 min.; (c) $K_3PO_4 \cdot 3H_2O$, 80 °C, microwave, 1 h, 62.5% yield over 2 steps; (d) $NaBH_4$, MeOH, 77.2% yield.

N-(2'-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)-2-methoxyacetamide **16**

Step 1: *N*-(5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)-2-methoxyacetamide **S19**

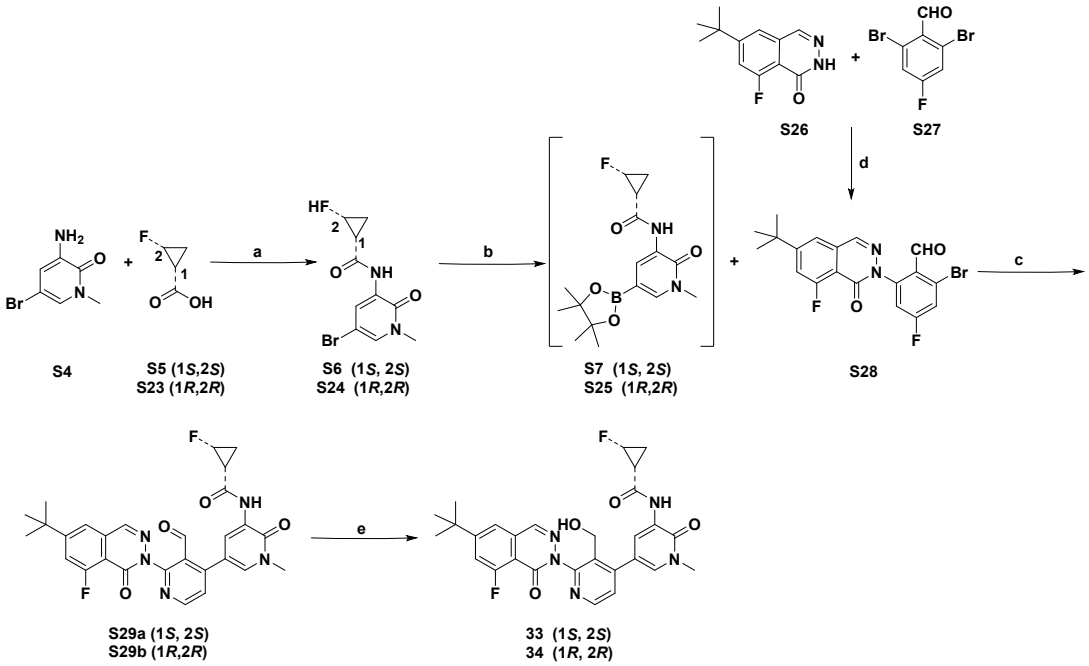
A mixture of 3-amino-5-bromo-1-methylpyridin-2(1*H*)-one **S4** (1014 mg, 4.99 mmol), 2-methoxyacetic acid (540 mg, 5.99 mmol, 1.2 equiv.), HATU (3230 mg, 8.49 mmol. 1.7 equiv.) and DIPEA (2.6 mL, 1936 mg, 15.0 mmol, 3 equiv.) in DMF (10 mL) was stirred at 60 ℃ for 16h. The resulting mixture was diluted with water and then extracted with 1:1 v/v ethyl acetate/ether (3x). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The resulting residue was purified by flash column chromatography (SiO_2: ethyl acetate / heptane) to afford 1077 mg of **S19**. Yield 78%. MS [ES-MS] (ESI+): m/z calcd for $C_9H_{12}BrN_2O_3$ $[M + H]^+$, 274.9; found, 274.9.

Step 2: Preparation of **16**

Compound **16** was prepared in a similar manner to **25**, using *N*-(5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)-2-methoxyacetamide **S19** and 4-chloro-2-(7,7-dimethyl-1-oxo-1,3,4,6,7,8-hexahydro-2*H*-cyclopenta[4,5]pyrrolo[1,2-*a*]pyrazin-2-yl)nicotinaldehyde **S21**[3] as starting materials. Yield 37.8%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.26 (s, 1H), 8.50 – 8.46 (m, 2H), 7.77

(d, J = 2.5 Hz, 1H), 7.31 (d, J = 5.1 Hz, 1H), 6.56 (s, 1H), 4.97 – 4.92 (m, 1H), 4.46 – 4.33 (m, 2H), 4.29 – 4.16 (m, 3H), 4.06 (s, 2H), 3.90 – 3.80 (m, 1H), 3.60 (s, 3H), 3.43 (s, 3H), 2.57 (d, J = 7.8 Hz, 2H), 2.42 (s, 2H), 1.22 (s, 6H). 13C NMR (101 MHz, DMSO) δ 168.34, 159.22, 156.24, 154.64, 147.94, 147.53, 140.91, 132.49, 130.71, 126.86, 125.74, 125.58, 123.51, 122.56, 115.86, 108.87, 71.50, 58.81, 57.15, 47.78, 45.30, 41.96, 40.71, 38.96, 37.56, 30.16, 30.04. HRMS (ESI+) m/z found MH^+ 506.2396, $C_{27}H_{32}N_5O_5$ requires 506.2398.

Scheme S6. Preparation of **33** and **34.** [a]



[a]Reagents and conditions: (a) HATU, DIPEA, DCM, 60 °C, 54 – 74.4% yield; (b) Pin_2B_2, KOAc, XPhos, $Pd_2(dba)_3$, dioxane, microwave, 100°C, 30 min (c) $K_3PO_4 \cdot 3H_2O$, 80 °C, microwave, 1 h, 17.6 – 25.1% yield over 2 steps; (d) XantPhos, Cs_2CO_3, $Pd_2(dba)_3$, 1,4-dioxane, 90 °C, 19h, 24.8% yield; (e) $NaBH_4$, MeOH, 63.2 – 69.8% yield.

(1*S*,2*S*)-*N*-(2'-(6-(*tert*-butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)-2-fluorocyclopropane-1-carboxamide **33**

Step 1: Preparation of 2-bromo-6-(6-(*tert*-butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-4-fluorobenzaldehyde **S28**.

In a 5-mL vial was placed 6-*tert*-butyl-8-fluoro-2*H*-phthalazin-1-one **S26** (331 mg, 1.50 mmol), 2,6-dibromo-4-fluoro-benzaldehyde **S27** (508 mg, 1.80 mmol, 1.2 equiv.), cesium carbonate (867 mg, 4.51 mmol, 3.0 equiv.), Xantphos (174 mg, 0.3006 mmol, 0.2 equiv.), $Pd_2(dba)_3$ (69 mg, 0.08

mmol, 0.05 equiv.), and dioxane (10.7 mL), and the reaction mixture was degassed for 15 min. The vial was vacuum purged / filled with N_2 (3x) and capped. The reaction mixture was stirred at 90 °C for 19h. The reaction was quenched with ethyl acetate / water and filtered. The organic phase from the filtrate was washed with water and brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (SiO_2: Ethyl acetate / heptane) to afford 157 mg of **S28** as a solid. Yield 24.8%. ^{1}H NMR (400 MHz, Chloroform-*d*) δ 10.20 (d, J = 0.9 Hz, 1H), 8.19 (d, J = 2.5 Hz, 1H), 7.52 – 7.49 (m, 2H), 7.48 (dd, J = 4.4, 2.1 Hz, 1H), 7.27 - 7.23 (m, 1H), 1.41 (s, 9H). MS (ESI+) m/z found MH^+ 421.1, $C_{19}H_{16}BrF_2N_2O_2$ requires 421.1.

Step 2: Preparation of **33**

Following the procedures described for **25**, replacing 2-(6-*tert*-butyl-8-fluoro-1-oxo-phthalazin-2-yl)-4-chloro-pyridine-3-carbaldehyde **S8** with 2-bromo-6-(6-(*tert*-butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-4-fluorobenzaldehyde **S28**, the title compound **33** was prepared. Yield 17.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (s, 1H), 8.51 (d, J = 2.5 Hz, 1H), 8.34 (d, J = 2.4 Hz, 1H), 7.88 (d, J = 1.8 Hz, 1H), 7.75 (dd, J = 13.3, 1.7 Hz, 1H), 7.65 (d, J = 2.4 Hz, 1H), 7.38 (dd, J = 9.0, 2.8 Hz, 1H), 7.28 (dd, J = 9.3, 2.8 Hz, 1H), 5.00 – 4.78 (m, 1H), 4.66 (t, J = 5.2 Hz, 1H), 4.30 (d, J = 5.3 Hz, 2H), 3.58 (s, 3H), 2.48 – 2.42 (m, 1H), 1.66 – 1.53 (m, 1H), 1.38 (s, 9H), 1.20 – 1.09 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 166.18 (d), 161.74, 159.86 (d), 159.31, 159.12, 156.23, 155.63 (d), 143.28 (d), 140.78 (d), 137.85, 132.86 (d), 131.96, 127.85, 124.28, 119.57 (d), 116.90 (d), 116.68, 116.42, 115.34, 115.12, 113.98 (d), 73.64, 71.38, 56.49, 37.49, 35.54, 30.48, 21.38 (d), 10.75 (d). HRMS (ESI+) m/z found MH^+ 553.2050, $C_{29}H_{28}F_3N_4O_4$ requires 553.2057. Chiral t_R = 0.743 min (Chiralcel OX, isocratic 50% MeOH w/ 0.1% NH_4OH / CO_2, 4 mL/min flow, 2.5 min at 40 °C, 120 bar).

(1*R*,2*R*)-*N*-(2'-(6-(*tert*-butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-5-yl)-2-fluorocyclopropane-1-carboxamide **34**

Step 1: Preparation of (1*R*,2*R*)-*N*-(5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)-2-fluorocyclopropane-1-carboxamide **S24**

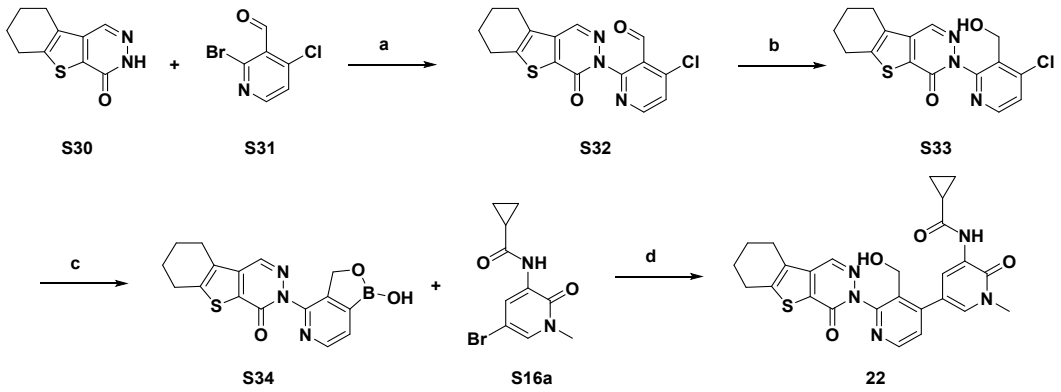
Following a similar procedure described for **S6** as previously described, replacing (1*S*,2*S*)-2-fluorocyclopropanecarboxylic acid **S5** with (1*R*,2*R*)-2-fluorocyclopropanecarboxylic acid **S23**, (1*R*,2*R*)-*N*-(5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)-2-fluorocyclopropane-1-carboxamide **S24** was prepared. Yield 74%. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.61 (br s, 1H), 8.51

(d, J = 2.5 Hz, 1H), 7.13 (d, J = 2.6 Hz, 1H), 4.89 – 4.67 (m, 1H), 3.59 (s, 3H), 1.95 – 1.83 (m, 2H), 1.27 – 1.18 (m, 1H). MS (ESI+) m/z found MH^+ 289, $C_{1o}H_{11}BrFN_2O_2$ requires 288.99.

Step 2: Preparation of **34**

Following the procedures described for **33**, replacing (1*S*,2*S*)-*N*-(5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)-2-fluorocyclopropane-1-carboxamide **S6** with (1*R*,2*R*)-*N*-(5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)-2-fluorocyclopropane-1-carboxamide **S24**, the title compound **34** was prepared. Yield 11.1%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.70 (s, 1H), 8.51 (d, J = 2.5 Hz, 1H), 8.34 (d, J = 2.4 Hz, 1H), 7.88 (d, J = 1.7 Hz, 1H), 7.75 (dd, J = 13.2, 1.7 Hz, 1H), 7.65 (d, J = 2.4 Hz, 1H), 7.38 (dd, J = 9.0, 2.8 Hz, 1H), 7.28 (dd, J = 9.3, 2.8 Hz, 1H), 5.00 – 4.78 (m, 1H), 4.66 (t, J = 5.2 Hz, 1H), 4.30 (d, J = 5.2 Hz, 2H), 3.58 (s, 3H), 2.52 – 2.42 (m, 1H), 1.66 – 1.53 (m, 1H), 1.38 (s, 9H), 1.20 – 1.09 (m, 1H). HRMS (ESI+) m/z found MH^+ 553.2051. $C_{29}H_{28}F_3N_4O_4$ requires 553.2057. Chiral t_R = 1.053 min (Chiralcel OX, isocratic 50% MeOH w/ 0.1% NH_4OH / CO_2, 4 mL/min flow, 2.5 min at 40 °C, 120 bar).

Scheme S7. Preparation of **22**.[a]



[a]Reagents: (a) CuI, sarcosine, K_2CO_3, dioxane, 80 °C, 16h, 60% yield; (b) $NaBH_4$, MeOH, DMA, dioxane, 10 °C, 1h, 59% yield; (c) $B_2(OH)_4$, XPhos-Pd-G2, XPhos, KOAc, EtOH, 80 °C, 1h, 56% yield; (d) $Pd(dppf)Cl_2$, Na_2CO_3, LiCl, dioxane / H_2O (10 mL, 3/1,v/v) 50 °C, 2 h, 10.5% yield.

N-(3'-(hydroxymethyl)-1-methyl-6-oxo-2'-(4-oxo-6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-d]pyridazin-3(4*H*)-yl)-1,6-dihydro-[3,4'-bipyridin]-5-yl)cyclopropanecarboxamide **22**

Step 1: 4-chloro-2-(4-oxo-6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyridazin-3(4*H*)-yl)nicotinaldehyde **S32**

A mixture of 6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyridazin-4(3*H*)-one (500 mg, 2.42 mmol), 2-bromo-4-chloronicotinaldehyde (1.59 g, 7.26 mmol) and K_2CO_3 (1.0 g, 7.26 mmol) in

1,4-dioxane (50 mL) was treated with CuI (462 mg, 2.42 mmol) and sarcosine (237 mg, 2.66 mmol), and the reaction mixture was stirred at 110 °C for 16 h. The reaction mixture was filtered and the filtrate was concentrated. The residue was purified by flash column chromatography (SiO_2: ethyl acetate / DCM) to afford 0.5 g of **S32** as a solid. Yield 60%.

Step 2: 3-(4-chloro-3-(hydroxymethyl)pyridin-2-yl)-6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-d]pyridazin-4(3*H*)-one **S33**

To a mixture of 4-chloro-2-(4-oxo-6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyridazin-3(4*H*)-yl)nicotinaldehyde (500 mg, 1.45 mmol) in 1,4-dioxane (10 mL) were added $NaBH_4$ (27 mg, 0.725 mmol) in DMA (252 mg, 2.9 mmol) and MeOH (139 mg, 4.35 mmol) in one portion at 10 °C. The reaction mixture was stirred at 10 °C for 1h, quenched with ice-water (10 mL), and stirred for 20 min. Volatile solvent was concentrated under reduced pressure at 50 °C, and the mixture was extracted with ethyl acetate (50 mL×3). The combined organic layers were washed with brine (20 mL×2), dried over Na_2SO_4, and concentrated under reduced pressure. The residue was purified purified by flash column chromatography (SiO_2: Ethyl acetate / petroleum ether) to afford 300 mg of **S33** as a solid. Yield 59%.

Step 3: 3-(1-hydroxy-1,3-dihydro-[1,2]oxaborolo[4,3-*c*]pyridin-4-yl)-6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyridazin-4(3*H*)-one **S34**

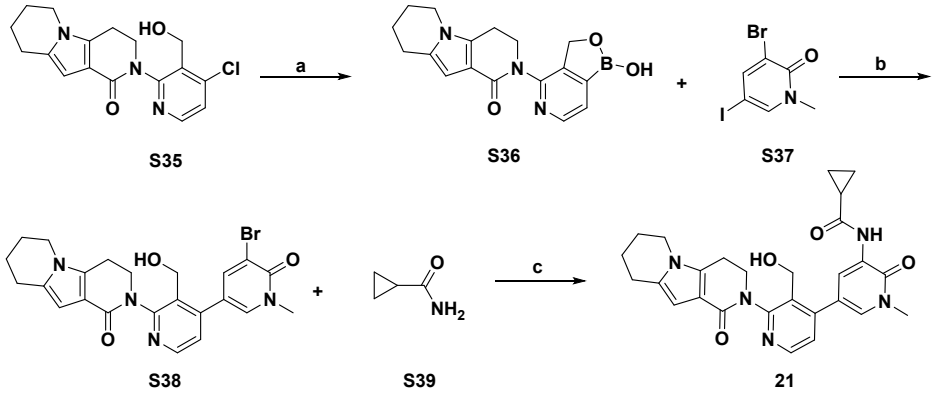
A mixture of 3-(4-chloro-3-(hydroxymethyl)pyridin-2-yl)-6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyridazin-4(3*H*)-one (360 mg, 1.04 mmol) and tetrahydroxydiborane (281 mg, 3.12 mmol) in EtOH (10 mL) was treated with Xphos-Pd-G2 (78 mg, 0.1 mol) and Xphos (95 mg, 0.2 mmol). The resulting mixture was heated to 80 °C for 1h under nitrogen. The mixture was quenched with water (20 mL) and extracted with ethyl acetate (20 mL×3). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was triturated with toluene (3 mL) to afford 200 mg of **S34** as a brown solid. Yield 56%.

Step 4: Preparation of **22**

A mixture of 3-(1-hydroxy-1,3-dihydro-[1,2]oxaborolo[4,3-*c*]pyridin-4-yl)-6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyridazin-4(3*H*)-one **S34** (136 mg, 0.5 mmol), *N*-(5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)cyclopropanecarboxamide **S16a** (170 mg 0.5 mmol), $Pd(dppf)Cl_2$ (24 mg, 0.032 mmol), Na_2CO_3 (291 mg, 2.75 mmol) and LiCl (74 mg, 1.75 mmol) in dioxane / H_2O (10 mL, 3/1,v/v) was stirred at 50 °C for 2 h. The mixture was concentrated under

reduced pressure and purified by prep-HPLC to give 26.5 mg of title compound **22** as a white solid. Yield 10.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.72 (s, 1H), 8.55 (d, J = 5.0 Hz, 1H), 8.46 (s, 1H), 8.40 (d, J = 2.5 Hz, 1H), 7.75 (d, J = 2.5 Hz, 1H), 7.49 (d, J = 5.0 Hz, 1H), 4.86 (t, J = 5.0 Hz, 1H), 4.41 – 4.28 (m, 2H), 3.59 (s, 3H), 2.98 – 2.90 (m, 2H), 2.89 – 2.81 (m, 2H), 2.31 – 2.21 (m, 1H), 1.96 – 1.79 (m, 4H), 0.81 – 0.74 (m, 4H). ^{13}C NMR (101 MHz, DMSO) δ 172.95, 156.41, 156.34, 153.69, 148.37, 148.00, 146.76, 138.70, 133.38, 132.29 (doublet), 131.77, 130.95, 128.21, 125.51, 123.57, 114.90, 56.33, 37.61, 25.20, 23.11, 22.54, 21.28, 14.18, 7.70. HRMS (ESI+) m/z found MH^+ 504.1696, $C_{26}H_{26}N_5O_4S$ requires 504.1700.

Scheme S8. Preparation of **21** *via* Buchwald amidation. [a]



[a]Reagents: (a) $B_2(OH)_4$, XPhos-Pd-G2, XPhos, KOAc, EtOH, 80 °C, 1h, 70% yield; (b) Pd(dppf)Cl_2, Na_2CO_3, LiCl, dioxane / H_2O (10 mL, 3/1,v/v) 50 °C, 2 h, 33% yield; (c) XantPhos, Cs_2CO_3, $Pd_2(dba)_3$, 1,4-dioxane, 100 °C, 1h, 24.5% yield.

N-(3'-(hydroxymethyl)-1-methyl-6-oxo-2'-(1-oxo-3,4,6,7,8,9-hexahydropyrazino[1,2-*a*]indol-2(1H)-yl)-1,6-dihydro-[3,4'-bipyridin]-5-yl)cyclopropanecarboxamide **21**

Step 1: Preparation of 2-(1-hydroxy-1,3-dihydro-[1,2]oxaborolo[4,3-*c*]pyridin-4-yl)-3,4,6,7,8,9-hexahydropyrido[3,4-*b*]indolizin-1(2*H*)-one **S36**

Following the procedure described for **22**, step 3, replacing 3-(4-chloro-3-(hydroxymethyl)pyridin-2-yl)-6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyridazin-4(3*H*)-one **S33** with 2-(4-chloro-3-(hydroxymethyl)pyridin-2-yl)-3,4,6,7,8,9-hexahydropyrido[3,4-b]indolizin-1(2*H*)-one **S35**, compound **S36** was prepared. Yield 70%.

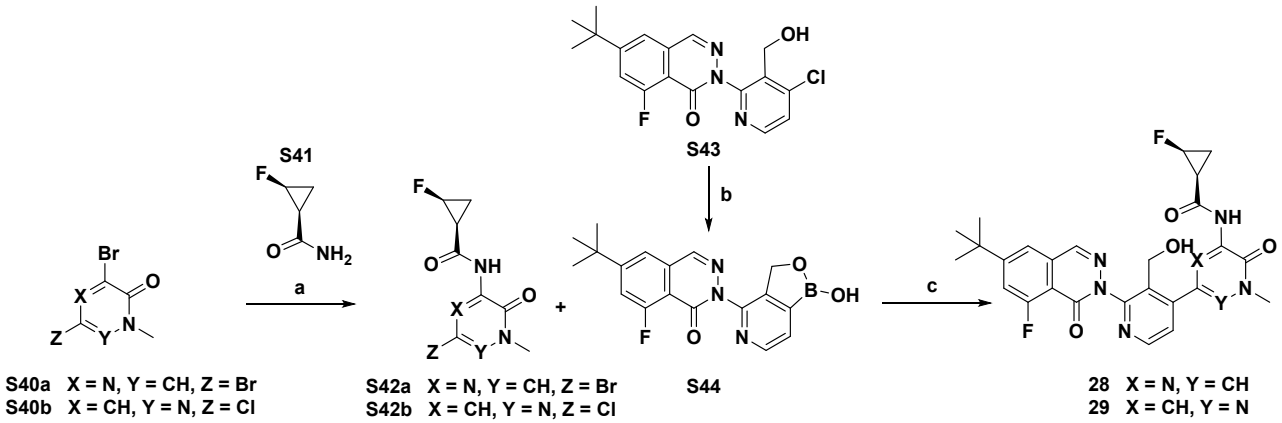
Step 2: Preparation of 2-(5-bromo-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-2'-yl)-3,4,6,7,8,9-hexahydropyrido[3,4-b]indolizin-1(2*H*)-one **S38**

Following the procedure decribed for **22**, step 4, replacing 3-(1-hydroxy-1,3-dihydro-[1,2]oxaborolo[4,3-*c*]pyridin-4-yl)-6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyridazin-4(3*H*)-one **S34** and *N*-(5-bromo-1-methyl-2-oxo-1,2-dihydropyridin-3-yl)cyclopropanecarboxamide **S16a** with 2-(1-hydroxy-1,3-dihydro-[1,2]oxaborolo[4,3-*c*]pyridin-4-yl)-3,4,6,7,8,9-hexahydropyrido[3,4-*b*]indolizin-1(2*H*)-one **S36** and 3-bromo-5-iodo-1-methylpyridin-2(1H)-one **S37**, respectively, **S38** was prepared. Yield 33%.

Step 3: Preparation of **21**

To a mixture of 2-(5-bromo-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-2'-yl)-3,4,6,7,8,9-hexahydropyrido[3,4-b]indolizin-1(2*H*)-one (100 mg, 0.21 mmol) and cyclopropanecarboxamide (53.5 mg, 0.63 mmol) in 1,4-dioxane (4 mL) were added Xantphos (12.2 mg, 0.02 mmol), Cs_2CO_3 (205 mg, 0.63 mmol), followed by $Pd_2(dba)_3$ (10 mg, 0.0105 mmol). The resulting mixture was stirred at 100 °C for 1h. The reaction mixture was diluted in water and filtered. The filtrate was extracted with ethyl acetate (20 mL x 3). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash column chromatography (SiO_2: methanol / ethyl acetate) to give 25 mg of the title compound as a white solid. Yield 24.5%. ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.68 (s, 1H), 8.47 – 8.38 (m, 2H), 7.75 (d, J = 2.4 Hz, 1H), 7.25 (d, J = 5.0 Hz, 1H), 6.03 (s, 1H), 4.84 (s, 1H), 4.43 – 4.26 (m, 2H), 4.19 – 4.08 (m, 1H), 3.99 – 3.89 (m, 1H), 3.83 – 3.74 (m, 2H), 3.59 (s, 3H), 3.06 – 2.88 (m, 2H), 2.71 (t, J = 6.4 Hz, 2H), 2.31 – 2.20 (m, 1H), 1.96 –1.86 (m, 2H), 1.83 – 1.67 (m, 2H), 0.81 – 0.73 (m 4H). ^{13}C NMR (101 MHz, DMSO) δ 172.84, 166.4, 164.90, 156.40, 155.72, 147.80, 147.58, 135.79, 132.01, 130.57, 129.91, 128.12, 123.75, 123.09, 115.83, 112. 31, 102.03, 57.32, 48.78, 42.51, 37.60, 23.07, 22.66, 20.69, 20.40, 14.18, 7.66. HRMS (ESI+) m/z found MH^+ 488.2287, $C_{27}H_{30}N_5O_4$ requires 488.2292.

Scheme S9. Preparation of **28** and **29**.[a]



[a]Reagents: (a) $Pd_2(dba)_3$, Xantphos, Cs_2CO_3, 1,4-dioxane, 110 °C, 1h, 30–76% yield; (b) $B_2(OH)_4$, XPhos-Pd-G2, XPhos, KOAc, EtOH, 80 °C, 1h, 92% yield; (c) $Pd(dppf)Cl_2$, Na_2CO_3, LiCl, 1,4-dioxane, H_2O, 80 °C, microwave, 1h, 13–20% yield.

(1*S*,2*S*)-*N*-(6-(2-(6-(*tert*-butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-3-(hydroxymethyl)pyridin-4-yl)-4-methyl-3-oxo-3,4-dihydropyrazin-2-yl)-2-fluorocyclopropanecarboxamide **28**

Step 1: (1*S*,2*S*)-*N*-(6-bromo-4-methyl-3-oxo-3,4-dihydropyrazin-2-yl)-2-fluorocyclopropanecarboxamide **S42a**

To a mixture of 3,5-dibromo-1-methylpyrazin-2(1*H*)-one **S40a** (200 mg, 0.7 mmol) and (1*S*,2*S*)-2-fluorocyclopropanecarboxamide **S41** (72 mg, 0.7 mmol) in 1,4-dioxane (20 ml) were added Xantphos (41 mg, 0.07 mmol), cesium carbonate (456 mg, 1.4 mmol), followed by tris(dibenzylideneacetone)dipalladium(0) (32 mg, 0.035 mmol). The resulting mixture was stirred at 110 °C for 1h under nitrogen and then diluted with water and filtered. The filtrate was extracted with ethyl acetate (20 mL×3). The combined organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The residue was purified by flash column chromatography (SiO_2: MeOH / DCM) to afford 80 mg of **S42a** as a yellow solid. Yield 30%. MS (ESI+) m/z found MH^+ 290, $C_9H_{10}BrFN_3O_2$ requires 289.99.

Step 2: Preparation of 6-(tert-butyl)-8-fluoro-2-(1-hydroxy-1,3-dihydro-[1,2]oxaborolo[4,3-*c*]pyridin-4-yl)phthalazin-1(2*H*)-one **S44**

Following the procedure described for **22**, step 3, replacing 3-(4-chloro-3-(hydroxymethyl)pyridin-2-yl)-6,7,8,9-tetrahydrobenzo[4,5]thieno[2,3-*d*]pyridazin-4(3*H*)-one

S33 with 6-(*tert*-butyl)-2-(4-chloro-3-(hydroxymethyl)pyridin-2-yl)-8-fluorophthalazin-1(2*H*)-one **S43**, compound **S44** was prepared. Yield 92%.

Step 3: Preparation of title compound **28**

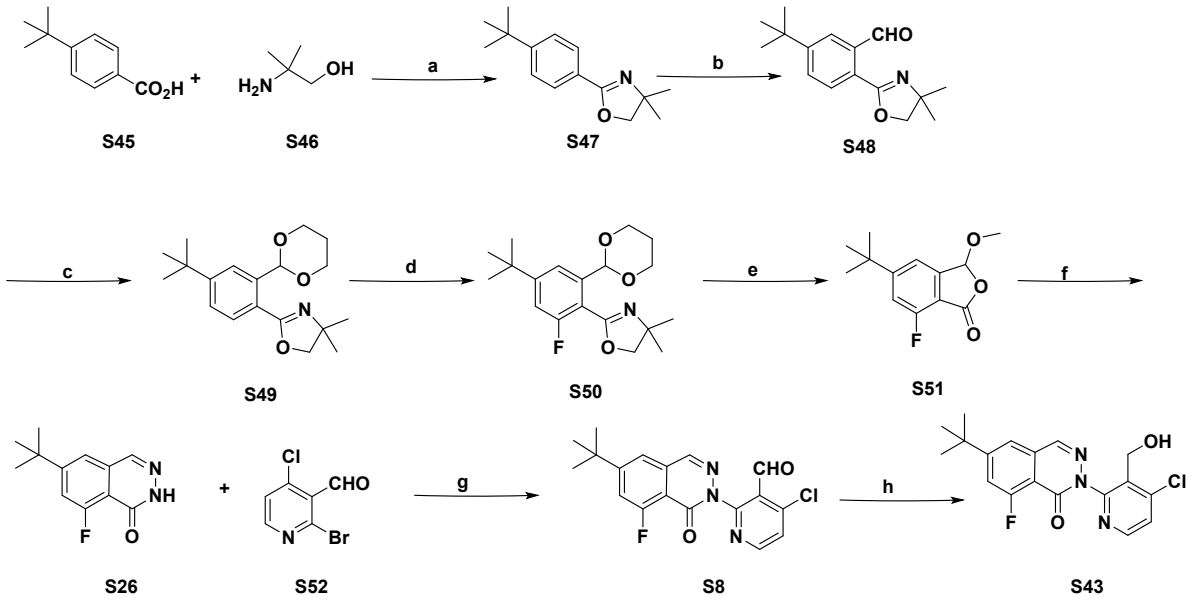
To a mixture of (1*S*,2*S*)-*N*-(6-bromo-4-methyl-3-oxo-3,4-dihydropyrazin-2-yl)-2-fluorocyclopropanecarboxamide **S42a** (70 mg, 0.24 mmol) and 6-(*tert*-butyl)-8-fluoro-2-(1-hydroxy-1,3-dihydro-[1,2]oxaborolo[4,3-*c*]pyridin-4-yl)phthalazin-1(2*H*)-one **S44** (85 mg, 0.24 mmol) in 1,4-dioxane (10 mL) and water (2 mL) were added sodium carbonate (127 mg, 1.2mmol), lithium chloride (30 mg, 0.72 mmol) and 1,1'-bis(diphenylphosphino)ferrocene palladium dichloride dichloromethane (9.8 mg, 0.012 mmol). The mixture was stirred at 80 °C for 1h under nitrogen. The resulting mixture was diluted with water, and extracted with ethyl acetate (20 mL×3). The organic layers were dried over anhydrous sodium sulfate and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (SiO_2: MeOH / DCM) to afford 17 mg of the title compound **28** as a white solid. 13% Yield. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.59 (d, J = 6.7 Hz, 1H), 8.57 (d, J = 5.0 Hz, 1H), 8.54 (d, J = 2.6 Hz, 1H), 8.07 (s, 1H), 7.91 (d, J = 1.8 Hz, 1H), 7.78 (dd, J = 13.2, 1.7 Hz, 1H), 7.65 (d, J = 5.0 Hz, 1H), 5.30 – 5.19 (m, 1H), 5.04 – 4.80 (m, 1H), 4.42 – 4.30 (m, 1H), 4.28 – 4.18 (m, 1H), 3.60 (s, 3H), 2.63 – 2.53 (m, 1H), 1.65 – 1.52 (m, 1H), 1.39 (s, 9H), 1.24 – 1.12 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 165.78 (d), 161.70, 160.16 (d), 159.09, 155.61 (d), 154.05, 150.35, 148.17, 147.15, 144.09, 137.70, 131.91, 131.37, 127.63, 126.86, 124.17, 119.78 (d), 117.13, 116.92, 73.89, 71.63, 56.31, 37.23, 35.60, 30.48, 21.77 (d), 11.14 (d). HRMS (ESI+) m/z found MH^+ 537.2050, $C_{27}H_{27}F_2N_6O_4$ requires 537.2056.

(1*S*,2*S*)-*N*-(6-(2-(6-(*tert*-butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-3-(hydroxymethyl)pyridin-4-yl)-2-methyl-3-oxo-2,3-dihydropyridazin-4-yl)-2-fluorocyclopropane-1-carboxamide **29**

Compound **29** was prepare in a similar manner to **28**, replacing 3,5-dibromo-1-methylpyrazin-2(1*H*)-one **S42a** with 4-bromo-6-chloro-2-methylpyridazin-3(2*H*)-one **S42b**. Yield 15.2% over two-steps. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.42 (s, 1H), 8.63 (d, J = 5.0 Hz, 1H), 8.54 (d, J = 2.5 Hz, 1H), 8.31 (s, 1H), 7.90 (d, J = 1.8 Hz, 1H), 7.77 (dd, J = 13.1, 1.8 Hz, 1H), 7.60 (d, J = 5.0 Hz, 1H), 5.07 – 4.83 (m, 2H), 4.46 – 4.34 (m, 2H), 3.80 (s, 3H), 2.69 – 2.58 (m, 1H), 1.72 – 1.57 (m, 1H), 1.39 (s, 9H), 1.28 –1.16 (m, 1H). ^{13}C NMR (101 MHz, DMSO) δ 167.80 (d), 161.77, 160.18 (d), 155.57 (d), 154.61, 153.03, 148.25, 146.46, 144.43, 137.95, 135.75, 135.70, 131.96

(d), 124.92, 119.78, 117.14, 116.93, 113.71 (d), 113.06, 73.97, 71.70, 56.20, 40.37, 35.60, 30.47, 21.52 (d), 11.33 (d). HRMS (ESI+) m/z found MH^+ 537.2049, $C_{27}H_{27}F_2N_6O_4$ requires 537.2056.

Scheme S10. Preparation of **S8** and **S43**.[a]



[a]Reagents and conditions: (a) (i) SO_2Cl_2, reflux, 3h; (ii) DCM, 0 °C to RT, overnight; (iii) $SOCl_2$, reflux, 1h, 41% yield; (b) (i) *n*BuLi (2.4M in hexane), THF, 78 °C to -20 °C 4h; (ii) DMF, -78 °C to RT, 2h 15 min, 62% yield; (c) 1,3-propanediol, PPTS, toluene, reflux, overnight, 22% yield; (d) 50% aq. H_2SO_4, MeOH, reflux, overnight; (e) (i) *n*BuLi (2.4 M in hexane), THF, -78 °C to -17 °C, 3h; (ii) NFSI, THF, -78 °C to -20 °C to RT, 1h (f) $NH_2NH_2 \cdot H_2O$, glacial AcOH, 0 to 50 °C, 1.5h, 38% yield over 2 steps; (g) CuI, 4,7-dimethoxy-1,10-phenanthroline, KOAc, dioxane, 90 °C, 10h, 25% yield; (h) $NaBH_4$, MeOH, RT, 1h, 90% yield.

2-(6-(*tert*-Butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-4-chloronicotinaldehyde **S8**

Step 1: 2-(4-(*tert*-butyl)phenyl)-4,4-dimethyl-4,5-dihydrooxazole **S47**

A mixture of 4-(*tert*-butyl)benzoic acid (1000 g, 5.6 mol) in SO_2Cl_2 (1.5 L) was refluxed for 3 hours. The solvent was then concentrated under reduced pressure to give crude 4-(tert-butyl)benzoyl chloride and DCM (200 mL) was added. To this reaction mixture at 0 °C was added dropwise a solution of 2-amino-2-methylpropan-1-ol (1000 g, 10.5 mol) in DCM (2000 mL), while keeping the internal temperature between 0 to 10 °C. A white precipitate formed 5 minutes after

the initial addition. The slurry was stirred at room temperature overnight, and the resulting white precipitate was filtered and rinsed with DCM (1000 mL). The filtrate was concentrated under reduced pressure to give 4-(*tert*-butyl)-*N*-(1-hydroxy-2-methylpropan-2-yl)benzamide as a light yellow solid which was used in the next step without further purification. A stirred mixture of crude 4-(*tert*-butyl)-*N*-(1-hydroxy-2-methylpropan-2-yl)benzamide (1000 g, 4 mmol) in $SOCl_2$ (1.5 L) was refluxed for 1 hour. The reaction mixture was cooled to room temperature and slowly added to stirred ether (500 mL), during which time a white precipitate formed. The precipitate was collected by filtration and washed with additional ether. The solid was then dissolved in water (500 mL) and neutralized with 25% aq. NaOH solution. The aqueous solution was extracted with EtOAC (3 x 500 mL), and the combined organic layers were washed with brine (500 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford 530 g of **S47** as a white solid. Yield: 41%. ^{1}HNMR (300 MHz, Chloroform-*d*) δ 7.87 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 8.4 Hz, 2H), 4.08 (s, 2H), 1.37 (s, 6H), 1.33 (s, 9H).

Step 2: 5-(*t*ert-Butyl)-2-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)benzaldehyde **S48**

To a solution of 2-(4-(*tert*-butyl)phenyl)-4,4-dimethyl-4,5-dihydrooxazole **S47** (50 g, 0.22 mol) in anhydrous THF (750 mL) under N_2 was added *n*-butyllithium (230 mL, 0.55 mol, 2.5 equiv., 2.4 M in hexane) at -78 °C. The amber solution was warmed to -20 °C and stirred for 4 h. The reaction mixture became cloudy and has a dark red amber color. The mixture was re-cooled to -78 °C and stirred rapidly before DMF (72 mL, 0.95 mol, 4.3 equiv.) was added dropwise at such a rate that the temperature was controlled below -60 °C. After the addition, the reaction mixture was stirred at -78 °C for 15 min, at -20 °C for 1 h, and at RT for 1 h more. The reaction mixture was quenched with 0.5 M $KHSO_4$ (200 mL). Additionl 0.5 M $KHSO_4$ was added to the reaction mixture until the pH was adjusted to 4-5. The aqueous phase was extracted with EtOAc (3 x 500 mL), and the combined organic layers were washed with brine (400 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to give 35 g of **S48** as a yellow solid. Yield: 62%. MS [ES-MS] (ESI+) m/z found MH^+ 260, $C_{16}H_{22}NO_2$ requires 260.

Step 3: 2-(4-(*tert*-Butyl)-2-(1,3-dioxan-2-yl)phenyl)-4,4-dimethyl-4,5-dihydrooxazole **S49**

A mixture of 5-(*t*ert-butyl)-2-(4,4-dimethyl-4,5-dihydrooxazol-2-yl)benzaldehyde **S48** (60 g, 0.23 mol), PPTS (4 g, 0.02 mol) and 1,3-propanediol (60 mL, 0.83 mol, 3.6 equiv.) in toluene (500 mL) was heated to reflux overnight and then cooled to room temperature. The reaction mixture

was quenched with 50% aq. $NaHCO_3$ solution (200 mL), and the organic layer was washed with water (200 mL), brine (200 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (SiO_2: ethyl acetate / petroleum ether) to afford 16 g of **S49** as a yellow gum. Yield: 22%. ^{1}HNMR (300 MHz, Chloroform-*d*) δ 7.79 (s, 1 H), 7.67 (d, J = 8.0 Hz, 1H), 7.36 (dd, J = 8.4, 1.6 Hz, 1H), 6.32 (s, 1H), 4.23 (dd, J = 5.2, 11.2 Hz, 2H), 4.06 (s, 2H), 4.04 – 3.98 (m, 2H), 2.27 – 2.21 (m, 1H), 1.48 – 1.38 (m, 1H), 1.38 (s, 6H), 1.32 (s, 9H). MS [ES-MS] (ESI+) m/z found MH^+ 318, $C_{19}H_{28}NO_3$ requires 318.

Step 4: 2-(4-(*tert*-Butyl)-2-(1,3-dioxan-2-yl)-6-fluorophenyl)-4,4-dimethyl-4,5-dihydrooxazole **S50**

To a solution of 2-(4-(*tert*-butyl)-2-(1,3-dioxan-2-yl)phenyl)-4,4-dimethyl-4,5-dihydrooxazole **S49** (20 g, 63 mmol) in anhydrous THF (400 mL) was added *n*-BuLi (65 mL, 157 mmol, 2.5 equiv., 2.4 M in hexane) at -78 °C under N_2. The clear yellow solution was stirred at -17 °C for 3 h. The deep red-orange color reaction solution was re-cooled to -78 °C, and a solution of NFSI (29 g, 92 mmol) in anhydrous THF (100 mL) was added dropwise over 10 min. The reaction mixture was stirred at -78°C for 5 min, -20 °C for 30 min, then at room temperature for 1h. The reaction mixture was quenched with 50% aq. NH_4CI solution (150 mL) and extracted EtOAc (300 mL). The organic layer was washed with water (150 mL) and brine (150 mL), dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (SiO_2: ethyl acetate / DCM) to afford 7 g of **S50** as yellow solid. Yield: 33%. ^{1}HNMR (300 MHz, Chloroform-*d*) δ 7.53 (d, J = 1.6 Hz, 1H), 7.08 (dd, J = 12.0, 2.0 Hz, 1 H), 5.90 (s, 1H), 4.24 (dd, J = 10.8. 5.2 Hz, 2H), 4.06 (s, 2H), 3.98 – 3.91 (m, 2H), 2.26 2.19 (m, 1H), 1.45 – 1.42 (m, 1H), 1.42 (s, 6H), 1.30 (s, 9H). MS [ES-MS] (ESI+) m/z found MH^+ 336, $C_{19}H_{27}FNO_3$ requires 336.

Step 5: 5-(*tert*-Butyl)-7-fluoro-3-methoxyisobenzofuran-1(3*H*)-one **S51**

A stirred mixture of 2-(4-(*tert*-butyl)-2-(1,3-dioxan-2-yl)-6-fluorophenyl)-4,4-dimethyl-4,5-dihydrooxazole **S50** (68.8 g, 205.4 mmol) in MeOH (1.34 L) and 50% aq. H_2SO_4 (881 mL) was stirred at reflux overnight. The reaction mixture was diluted with water (400 mL) and extracted with DCM (3 x 1L). The combined organic layers were washed with brine (400 mL), dried over

Na_2SO_4, filtered, and concentrated under reduced pressure to provide 43 g of **S51** as an off-white solid which was used in the next step without further purification.

Step 6: 6-*tert*-Butyl-8-fluorophthalazin-1(2*H*)-one **S26**

To a stirred solution of 5-(*tert*-butyl)-7-fluoro-3-methoxyisobenzofuran-1(3*H*)-one **S51** (40 g, 168 mmol) in glacial acetic acid (360 mL) was added hydrazine monohydrate (240 mL) at 0 °C under N_2. The resulting slurry was stirred under at 50 °C for 1.5 h. The reaction mixture was diluted with water (300 mL), and the aqueous layer was extracted with DCM (2 x 500 mL). The combined organic layers was dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was recrystallized from DMC and Et_2O to provide 17g of **S26** as an off-white solid. Yield: 38% over 2 steps. ^{1}HNMR (300 MHz, Chloroform-*d*) δ 8.11 (d, J = 2.7 Hz, 1H), 7.52 – 7.41 (m, 2H), 1.39 (s, 9 H), NH not seen. MS [ES-MS] (ESI+) m/z found MH^+ 221, $C_{12}H_{14}FN_2O$ requires 221.

Step 7: 2-(6-(*tert*-Butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-4-chloronicotinaldehyde **S8**

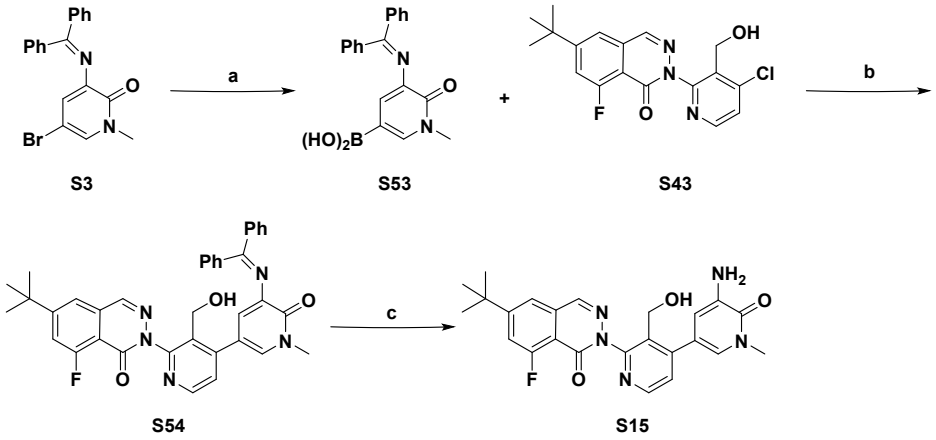
To a stirred mixture of 6-*tert*-butyl-8-fluorophthalazin-1(2*H*)-one **S26** (300 mg, 1.36 mmol) and 2-bromo-4-chloronicotinaldehyde **S52** (446 mg, 2.05 mmol, 1.5 equiv.) in dioxane (50 mL) was added KOAc (267 mg, 2.72 mmol, 2.0 equiv.), Cul (259 mg, 1.36 mmol, 1.0 equiv.), and 4,7-dimethoxy-l,10-phenanthroline (327 mg, 1.36 mmol, 1.0 equiv.). Nitrogen was bubbled through the reaction mixture for 30 min, and the mixture was stirred at 90 °C for 10 h. Water (100 mL) was added to the cooled reaction, and the aqueous layer was extracted with EtOAc (2 x 200 mL). The combined organic layers were washed with brine (100 mL) and dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (SiO_2: ethyl acetate / petroleum ether) to afford 120 mg of **S8**. Yield: 25%. ^{1}HNMR (500 MHz, Chloroform-*d*) δ 10.36 (s, 1H), 8.69 (d, J = 5.5 Hz, 1H), 8.28 (d, J = 2.0 Hz, 1H), 7.28 – 7.56 (m, 3H), 1.49 (s, 9H). MS [ES-MS] (ESI+) m/z found MH^+ 360, $C_{18}H_{16}ClFN_3O_2$ requires 360.

6-(*tert*-Butyl)-2-(4-chloro-3-(hydroxymethyl)pyridin-2-yl)-8-fluorophthalazin-1(2*H*)-one **S43**

To a solution of 2-(6-(*tert*-butyl)-8-fluoro-1-oxophthalazin-2(1*H*)-yl)-4-chloronicotinaldehyde **S8** (2.0 g, 5.5 mmol) in MeOH (30 mL) was added $NaBH_4$ (700 mg, 16.5 mmol, 3.0 equiv.) at room temperature. The reaction mixture was stirred for 1 h and quenched with water (30 mL). Volatile solvent was removed under reduced pressure, and the aqueous phase was extracted with DCM (3 x 30mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and

evaporated under reduced pressure to afford 1.8 g of **S43** as a white solid. Yield: 90%. MS [ES-MS] (ESI+) m/z found MH^+ 362, $C_{18}H_{18}ClFN_3O_2$ requires 362.

Scheme S11. Preparation of **S15**.[a]



[a]Reagents and conditions: (a) Pin_2B_2, KOAc, XPhos, $Pd_2(dba)_3$, dioxane, 60°C, 3 h, 93% yield; (b) $Pd(dppf)Cl_2$, K_3PO_4, NaOAc, ACN, H_2O, 100 °C, 2 h, 40% yield; (c) 4M HCl in dioxane, 25 °C, 1 h, 50% yield.

2-(5-Amino-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-2'-yl)-6-(*tert*-butyl)-8-fluorophthalazin-1(2*H*)-one **S15**

Step 1: (5-((Diphenylmethylene)amino)-1-methyl-6-oxo-1,6-dihydropyridin-3-yl)boronic acid **S53**

A 100-mL round-bottomed flask equipped with a reflux condenser was charged with 5-bromo-3-((diphenylmethylene)amino)-1-methylpyridin-2(1*H*)-one (3.0 g, 8.0 mmol), Pin_2B_2 (6.1 g, 24.0 mmol, 3.0 equiv.), $Pd_2(dba)_3$ (290 mg, 0.40 mmol, 0.05 equiv.), XPhos (385 mg, 0.80 mmol, 0.1 equiv.), KOAc (1.6 g, 16.0 mmol, 2.0 equiv.), and 1,4-dioxane (30 mL). After three cycles of vacuum/argon flush, the mixture was stirred at 60°C for 3h. The reaction was cooled and filtered, and the filtrate was evaporated under reduced pressure. The crude residue was washed with PE to afford 2.5 g of S53 as brown oil, which was used directly without further purification. Yield: 93%. MS [ES-MS] (ESI+) m/z found MH^+ 333, $C_{19}H_{18}BN_2O_2$ requires 333.

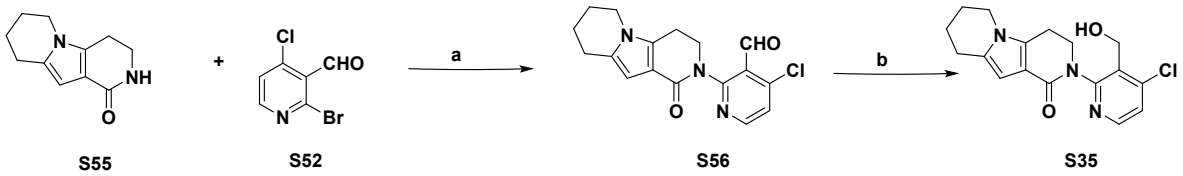
Step 2: 6-(*tert*-Butyl)-2-(5-((diphenylmethylene)amino)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-2'-yl)-8-fluorophthalazin-1(2*H*)-one **S54**

A 50-mL round-bottomed flask equipped with a reflux condenser was charged with (5-((diphenylmethylene)amino)-1-methyl-6-oxo-1,6-dihydropyridin-3-yl)boronic acid **S53** (2.0 g, 6.0 mmol), 6-(*tert*-butyl)-2-(4-chloro-3-(hydroxymethyl)pyridin-2-yl)-8-fluorophthalazin-1(2*H*)-one **S43** (2.17 g, 6.0 mmol, 1.0 equiv.), K_3PO_4(2.54 g, 12.0 mmol, 2.0 equiv,), NaOAc (1.0 g, 12.0 mmol, 2.0 equiv.), Pd(dppf)$C1_2$ (245 mg, 0.3 mmol, 0.05 equiv.), ACN (15 mL), and H_2O (12 mL). After three cycles of vacuum/argon flush, the reaction mixture was stirred at 100°C under N_2 for 2 h.. The reaction was cooled to room temperature and filtered. The filtrate was concentrated under reduced pressure. The residue was diluted with DCM (20 mL) and water (10 mL). The aqueous layer was extracted with DCM (2x10 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude residue was purified by flash column chromatography (SiO_2: MeOH / DCM) to afford 1.6 g of **S54** as yellow solid. Yield: 40%. MS [ES-MS] (ESI+) m/z found MH^+ 614, $C_{37}H_{33}FN_5O_3$ requires 614.

Step 3: 2-(5-Amino-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-2'-yl)-6-(*tert*-butyl)-8-fluorophthalazin-1(2*H*)-one **S15**

A mixture of 6-(*tert*-butyl)-2-(5-((diphenylmethylene)amino)-3'-(hydroxymethyl)-1-methyl-6-oxo-1,6-dihydro-[3,4'-bipyridin]-2'-yl)-8-fluorophthalazin-1(2*H*)-one **S54** (1.6 g, 2.6 mmol) in 4M HCl in dioxane (10 mL) was stirred at 25 °C for 1 h. The mixture was evaporated under reduced pressure, and the crude residue was purified by reverse-phase prep-HPLC to afford 580 mg of **S15** as a pale yellow solid. Yield: 50%. ^{1}H NMR (500 MHz, DMSO-d_6) δ 8.53 – 8.52 (m, 2H), 7.90 (d, J = 1.0 Hz, 1H), 7.79 – 7.76 (m, 1H), 7.45 (d, J = 5.0 Hz, 1H), 7.24 (d, J = 2.5 Hz, 1H), 6.65 (d, J = 2.0 Hz, 1H), 5.33 (s, 2H), 4.95 – 4.93 (m, 1H), 4.39 (s, 2H), 3.52 (s, 3H), 1.38 (s, 9H). MS [ES-MS] (ESI+) m/z found MH^+ 450, $C_{24}H_{25}FN_5O_3$ requires 450.

Scheme S12. Preparation of **S35**.[a]



S55 S52 S56 S35

[a]Reagents and conditions: (a) $Pd_2(dba)_3$, XantPhos, K_2CO_3, dioxane, 80 °C, overnight, 50% yield; (b) $NaBH_4$, MeOH, 30 °C, 1 h, 92% yield.

2-(4-Chloro-3-(hydroxymethyl)pyridin-2-yl)-3,4,6,7,8,9-hexahydropyrido[3,4-*b*]indolizin-1(2*H*)-one **S35**

Step 1: 4-Chloro-2-(1-oxo-3,4,6,7,8,9-hexahydropyrido[3,4-*b*]indolizin-2(1*H*)-yl)nicotinaldehyde **S56**

A 250-mL single-neck round-bottomed flask equipped with a magnetic stirrer and reflux condenser was charged with 1,4-dioxane (50 mL), 3,4,6,7,8,9-hexahydropyrido[3,4-*b*]indolizin-1(2*H*)-one **S55** (0.6 g, 3.2 mmol), 2-bromo-4-chloronicotin-aldehyde **S52** (1.4 g, 6.4 mmol, 2.0 equiv.), $Pd_2(dba)_3$ (293 mg, 0.32 mmol, 0.1 equiv.), XantPhos (370 mg, 0.64 mmol, 0.2 equiv.), and potassium carbonate (627 mg, 6.4 mmol, 2.0 equiv.). After three cycles of vacuum/argon flush, the mixture was stirred at 80 °C overnight. The reaction was cooled to room temperature and filtered, and the filtrate was evaporated under reduced pressure. The crude residue was purified by flash column chromatography (SiO_2: MeOH / DCM) to afford 528 mg of **S56** as a yellow solid. Yield: 50%. ^{1}H NMR (500 MHz, Chloroform-*d*) δ 10.09 (s, 1H), 8.37 (d, J = 5.5 Hz, 1H), 7.16 (d, J = 5.5 Hz, 1H), 6.25 (s, 1H), 4.29 – 4.32 (m, 2H), 3.83 – 3.86 (m, 2H), 2.96 – 2.99 (m, 2H), 2.75 – 2.78 (m, 2H), 2.00 – 2.07 (m, 2H), 1.82 – 1.85 (m, 2H). MS [ES-MS] (ESI+) m/z found MH^+ 330, $C_{17}H_{17}ClN_3O_2$ requires 330.

Step 2: 2-(4-Chloro-3-(hydroxymethyl)pyridin-2-yl)-3,4,6,7,8,9-hexahydropyrido[3,4-*b*]indolizin-1(2*H*)-one **S35**

To a solution of 4-chloro-2-(1-oxo-3,4,6,7,8,9-hexahydropyrido[3,4-b]indolizin-2(1*H*)-yl)nicotinaldehyde **S56** (1.0 g, 3.0 mmol) in MeOH (30 mL) was added $NaBH_4$ (380 mg, 9.0 mmol, 3.0 equiv.). The reaction mixture was stirred at 30 °C for 1 h and then quenched with water (10 mL). Volatile solvent was concentrated under reduced pressure, and the crude was extracted with DCM (3 x 20 mL). The combined organic layers were dried over anhydrous Na_2S0_4, filtered, and evaporated under reduced pressure to afford 920 mg of a yellow solid, which was used as is in the next reaction step. Yield: 92%. MS [ES-MS] (ESI+) m/z found MH^+ 332, $C_{17}H_{19}ClN_3O_2$ requires 332.

IN VITRO PHARMACOLOGY

Btk assays.

Btk kinase activity and inhibition was assessed using a peptide phosphorylation assay as previously described (1). The inhibitor concentration at 50% inhibition (IC_{50}) values were calculated from inhibitor titration data by plotting the % of Control Btk activity against test article concentration and fitting the data to a standard 4-parameter sigmoidal inhibition equation using Prism v5 software (GraphPad Software LLC; San Diego, CA, USA).

In vitro whole blood pBTK assay.

Heparinized human whole blood was treated with test article for 1 h at 37 °C (0.4% DMSO final concentration). Blood was lysed with 2X lysis buffer (Cell Signaling Technology) containing protease (Roche Applied Science) and phosphatase inhibitors (cocktail II and III) (Sigma). Phosphorylation of Btk at the Y223 residue was evaluated using Meso Scale Discovery (MSD) technology. Mouse anti-total Btk Ab (BD Bioscience) and rabbit anti-pBtk-Y223 mAbs (Epitomics) followed by a SULFO-TAG anti-rabbit Ab (MSD) were used for detection.

Human whole blood CD69 assay.

Heparinized human whole blood was incubated with test article for 1 h at 37 °C (11-point titration, with 3-fold dilution with a top concentration of 4.76 μM) or 0.4% DMSO vehicle control. Blood was then stimulated with 50 μg/mL of goat anti-IgM F(ab')2 (Southern Biotech) for 18 h. After incubation, B cells were stained with anti-mouse CD19 PerCP (BD Biosciences, clone SJ25C1), anti-mouse CD27 FITC (BD Biosciences, clone L128) and anti-mouse CD69 PE (BD Biosciences, clone FN50) or Isotype IgG1 PE antibodies for 30 min at room temperature followed by RBC lysis using 1X BD lysis buffer. Cells were washed with FACS buffer and fixed with 2% paraformaldehyde. Samples were acquired on BD LSR II and analyzed using BD FACSDiva software. B cells were gated as CD19+CD27- and B cell activation was assessed based on CD69 PE mean fluorescence intensity (MFI).

Measure of error / number of replicates for CD69 and pBtk HWB assays

Mean IC_{50} +/- SD (n=determinations)

Cmpd **6**: CD69 = 8.5 +/- 5.6 nM (n=6); pBTK = 11 +/- 4.2 nM (n=6)

Cmpd **25**: CD69 = 78 +/- 57 nM (n=3); pBTK = 79 +/- 13 nM (n=7)

Liver Microsome Metabolic Stability Assays

Metabolic stability of test compounds was evaluated in pooled human, mouse, and rat liver microsomes (BD Biosciences, San Jose, CA). The final incubations contained: 1 μM of the tested compound, 1 mM NADPH, 0.5 mg/mL microsomal protein in 0.1 M potassium phosphate buffer (pH 7.4). Following a 5-minute pre-incubation period, the enzymatic reactions were initiated by the addition of NADPH and test compound to the microsomes diluted in phosphate buffered saline. The mixtures were incubated at 37 °C for 0, 20, 40, and 60 min. Compound concentrations were assessed by LCMS/MS. Intrinsic clearance based upon microsomal stability data was determined using a substrate depletion method and scaled to hepatic clearance using the well-stirred model.[2]

Hepatocyte Metabolic Stability Assays

Metabolic stability assays of test compounds were carried out using cryopreserved pooled donor mouse, rat, dog, cynomolgus monkey, and human hepatocytes (CellzDirect; Durham, NC). Membrane integrity of the cells was assessed by trypan blue exclusion. Test compounds (1 μM with 0.1% DMSO) were incubated with cells (0.5 million cells/mL) at 37 °C in a 95% air/5% CO_2 atmosphere for 0, 20, 40, or 60 min. Concentrations of test compounds in hepatocyte incubations were determined by LC-MS/MS. Intrinsic clearance was determined using a substrate depletion method and scaled to hepatic clearance using the well-stirred model (*vide supra*).

In Vitro Permeability Assay in MDCK (Madin-Darby Canine Kidney) Cells

The permeability of test compounds was determined in MDCK cells (American Type Culture Collection; Manassas, VA). Four days prior to use, MDCK cells were seeded at a density of 2.5×105 cells/mL in 24 well plates. Compounds were dissolved in transport buffer consisting of Hank's Balanced Salt Solution with 10 mM HEPES (Invitrogen Corporation, Grand Island, NY) at a concentration of 10 μM, and permeability was assessed in the apical to basolateral (A-B) and basolateral to apical (B-A) directions following a 3 hour incubation. Lucifer Yellow (Sigma Aldrich, St. Louis, MO) was used as the cell monolayer integrity marker. Test compound concentrations in the donor and receiving compartments were determined by LC-MS/MS. The apparent permeability (Papp) of test compounds was determined as follows:

$P_{app} = (dQ/dt)*(1/AC_0)$

Where dQ/dt is the rate of compound appearance in the receiver compartment, Q is the quantity of compound), C0 is the concentration in the donor compartment and A is the surface area of the insert. Efflux ratio was calculated as $P_{app, B-A}/ P_{app, A-B}$.

In Vitro Plasma Protein Binding

In vitro plasma protein binding (n = 2) was determined in pooled mouse, rat, and human plasma (Bioreclamation, Inc., Hicksville, NY) by equilibrium dialysis using a Rapid Equilibrium Dialysis (RED) device (Pierce Biotechnology / Thermo Fisher Scientific; Rockford, IL) with a molecular weight cut-off of 8000 Daltons. Test compounds were added to plasma. Plasma samples were equilibrated with phosphate-buffered saline at 37 ºC for 4 hours. Compound concentrations in post-dialysis plasma and buffer samples were measured by LC-MS/MS. The percent unbound fraction in plasma for each compound was calculated by dividing the compound concentration in the post-dialysis buffer by that measured in the post-dialysis plasma and multiplying by 100%.

Reversible CYP and Inhibition Studies in Human Liver Microsomes[3]

Compound **25** was incubated with human liver microsomes (150 donor pool, BD Biosciences, San Jose, CA) fortified with NADPH and isoform-specific probe substrates. Reactions were stopped by the addition of cold acetonitrile/formic acid (94:6 v/v) containing internal standard. Five concentrations of each compound were tested (10, 5, 1, and 0.1 μM as well as a solvent control) S30 to generate IC_{50} values.

Aqueous Solubility Determinations

Compounds were dissolved in DMSO to a concentration of 10 mM. These solutions were diluted into PBS buffer (pH 7.2, composed with NaCl, KCl, Na_2HPO_4, and KH_2PO_4) to a final compound concentration of 100 μM, DMSO concentration of 2%, at pH 7.4. The samples were shaken for 24 hours at room temperature followed by filtration. LC/CLND was used to determine compound concentration in the filtrate, with the concentration calculated by a caffeine calibration curve and the sample's nitrogen content. An internal standard compound was spiked into each sample for accurate quantification.

hERG Automatic Patch Clamp Assay

The in vitro effects of test articles on hERG potassium channel current expressed in mammalian cells were evaluated at room temperature by using automated patch-clamp technique, the QPatch HT (Sophion Bioscience A/S, Denmark) or QPatch HTX (Sophion Bioscience) at Chantest (Cleveland, OH) or Wuxi AppTec (Shanghai, China), respectively. IC_{50s} of **24**, **25**, **33**, and **34** were determined based on the data from five concentrations tested (0.1, 0.3, 1, 3, and 10 μM).

Induced Pluripotent Stem Cell (iPSC)-derived Cardiomyocyte Multi-electrode Array Assay

iPSC-derived cardiomyocytes (icell^4 cardiomyocytes, Fujifilm Cellular Dynamics, Madison, WI) were thawed and plated on 96-well multi-electrode array (MEA) plates (Axion Biosystems, Atlanta, GA) according to manufacturer directions. Briefly, cells were spotted in 5μL droplets over electrodes at a density of 60k/well on fibronectin (Roche, Pleasanton, CA) and maintained for 7 days. A 100% media change was performed one day prior to compound treatment. Compounds were initially diluted in DMSO, and added to wells at 10x target concentration in media for a final DMSO concentration of 0.1%. A single dose per well scheme was utilized with 4 replicates per condition, and time matched controls included untreated (media only), vehicle, cisapride (hERG channel blocker, Sigma Aldrich, St Louis, MO), and nifedipine (calcium channel blocker, Sigma Aldrich). On the day of the experiment, plates were moved from the incubator to the MEA device (Maestro, Axion Biosystems) with environmental controls (37°C and 5% CO_2) and allowed to equilibrate for 5 minutes prior to recording a baseline measurement. Dosing was performed manually in a biosafety cabinet in under 10 minutes, after which plates were returned to the MEA platform for post-dose recordings. All recordings were at least 5 minutes in length and analyzed using instrument specific software with semi-automated analysis of field potential signals. Endpoints included sodium spike amplitude and field potential duration, which was corrected for beat rate using the Fridericia correction (2). Values were normalized first to baseline, then vehicle. Additionally, beat waveforms were inspected for arrhythmic events and drug-induced beat cessation (quiescence).

Pharmacokinetic (PK) study protocols.

Rat pharmacokinetic study.

Nine male Sprague Dawley rats (275-300g) obtained from Charles River Laboratories (Hollister, CA) were divided evenly into three dose groups (n=3 rats/group). Animals in Group 1 were given a single IV dose of 1 mg/kg compound **25** in 60% polyethylene glycol 400 (PEG400)/15% EtOH/50mM NaCitrate, pH 4.2 at 1 mL/kg. Animals in Group 2 were given 5 mg/kg PO dose of **25** in 0.5% hydroxypropyl methyl cellulose (HPMC) /50mM sodium citrate (NaCitrate), pH 3.0, as an amorphous solid dispersion (ASD) suspension at 2 mL/kg, and animals in Group 3 were given 5 mg/kg PO dose of **25** in 1% HPMC/0.2% polysorbate 80 (Tween80) /50 mM NaCitrate, pH 3.0, as crystalline suspension at 2 mL/kg. The blood samples were collected at 0.033, 0.167, 0.5, 1, 2, 4, 6 and 8 hours after IV administration. For PO administration, the blood samples were collected at 0.083, 0.25, 0.5, 1, 2, 4, 6, and 8 hours post dose. Blood was centrifuged to harvest plasma. Concentration of **25** in each plasma sample was determined by a non-validated liquid chromatography tandem mass spectrometry assay at Genentech, Inc. Pharmacokinetic (PK) analysis was performed using non-compartmental methods and the IV bolus input model (Model 201) or extravascular (Model 200), WinNonlin-Enterprise®, version 5.2.1. Following PO administration (Groups 2 and 3), percent bioavailability (% F) was determined for each animal by dividing the dose-normalized area under the plasma concentration-time curve extrapolated to infinity (AUCinf) obtained following each PO dose by the mean dose-normalized AUCinf of the animals dosed by IV injection (Group 1).

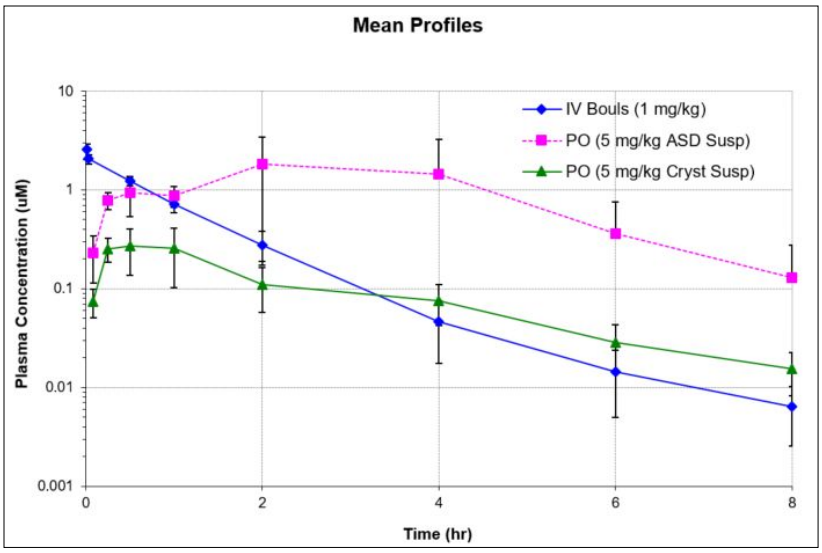


Figure S1. Mean total plasma concentration vs. time profiles of compound **25** after an IV bolus dose of 1 mg/kg and a PO dose of 5 mg/kg (ASD suspension) and a PO dose of mg/kg (Crystalline suspension) in male Sprague-Dawley rats, n=3, mean ± SD.

Dog pharmacokinetic study.

Six male beagle dogs (7-12 kg) obtained from Marshall Bioresources (Beijing, China) were divided evenly into two dose groups (n=3 dogs/group). Animals in Group 1 were given a single IV dose of 1 mg/kg compound **25** in 60% PEG400 /15% EtOH/50mM NaCitrate, pH 4.2 at 1 mL/kg. Animals in Group 2 were given 5 mg/kg PO dose of **25** in 0.5%HPMC/50mM NaCitrate, pH 3.0, as a suspension at 2 mL/kg. The blood samples were collected at 0.033, 0.083, 0.25, 0.5, 1, 3, 6, 9 and 24 after IV and PO administration. Blood was centrifuged to harvest plasma. Concentration of **25** in each plasma sample was determined by a non-validated liquid chromatography tandem mass spectrometry assay at WuXi AppTec. Pharmacokinetic (PK) analysis was performed at Genentech, Inc., using non-compartmental methods and the IV bolus input model (Model 201) or extravascular (Model 200), WinNonlin-Enterprise®, version 5.2.1. Following PO administration (Group 2), percent bioavailability (% F) was determined for each animal by dividing the dose-normalized area under the plasma concentration-time curve extrapolated to infinity (AUCinf) obtained following each PO dose by the mean dose-normalized AUCinf of the animals dosed by IV injection (Group 1).

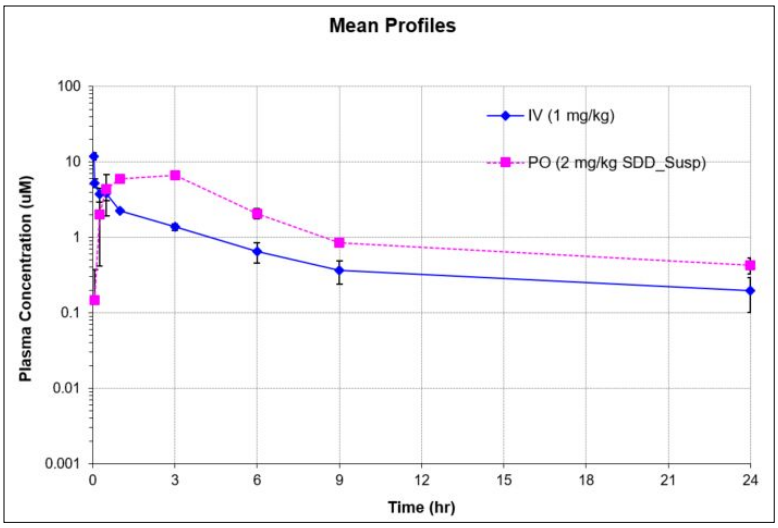


Figure S2. Mean total plasma concentration vs. time profiles of compound **25** after an IV dose of 1 mg/kg and a PO dose of 5 mg/kg (selective decontamination of the digestive tract [SSD] suspension) in male Beagle dogs, n=3, mean ± SD.

Animal Care:

Animals were pair-housed in cages that are in accordance with applicable animal welfare laws and regulations during acclimation period.

The room was controlled and monitored for humidity (targeted mean range 40% to 70%, and any excursion from this range for more than 3 hours will be documented as a deviation) and temperature (targeted mean range 18° to 26°C, and any excursion from this range will be documented as a deviation) with 10 to 20 air changes/hour. The room was on a 12-hour light/dark cycle except when interruptions are necessitated by study activities.

The dogs were individually housed in cages during experiment. The animals were fed twice daily. Stock dogs were fed approximately 220 grams of Certified Dog Diet daily (Beijing Vital Keao Feed Co., Ltd. Beijing, P. R. China). These amounts were adjusted as necessary based on food consumption of the group or an individual, body weight changes of the group or an individual and/or changes in the certified diet.

For fasted group (PO dose group), animals were fed the afternoon (at 3:30 to 4:00 pm) prior to the day of oral dosing and the remaining food were removed at about 8:00 pm. Food were withheld until 4-hour post-dose unless specified in this protocol. Fasted animals were fed once on the day of dosing, with the amount of approximately 220 g.

Fresh drinking water were available to all animals, ad libitum. Feed and water analyses were maintained.

KINASE SELECTIVITY

Table S1. Selectivity of **6** and **25** tested in duplicate at 1 μM in the SelectScreen kinase assay panel (ThermoFisher Scientific; Madison, WI); the mean percent inhibition is shown. The [ATP] column indicates the concentration of ATP used in each assay: Km app means the ATP concentration was within 2-fold of the experimentally determined apparent Km in the assay; NA (Not Applicable) denotes these were binding assays and no ATP was present; and 10 or 100 are micromolar concentrations of ATP. Gray cells indicate the kinase was not tested.

Compound		6	25
Kinase Count		295	220
Kinase	[ATP]	%Inh@1uM	%Inh@1uM
BTK	Km app	99	96
Src	Km app	70	56
Fgr	Km app	69	40
Bmx	Km app	56	14
Yes	Km app	46	14
Lck	Km app	45	29
Fyn	Km app	32	
TEC	NA	23	23
Flt3(D835Y)	Km app	23	
LIMK1	NA	22	13
Lyn	Km app	22	15
LynB	Km app	21	
CamKII_delta	Km app	21	
Flt4	Km app	20	17
LRRK2	Km app	19	-2
CamKIV	Km app	19	-11
FGFR3	Km app	18	2
GSK3_beta	Km app	18	20
PKG2	Km app	18	
ROCK2	Km app	17	-2
MEK1	100	16	
RSK2	Km app	16	7
MKNK2	NA	16	-1
Flt3	Km app	16	11
EphA1	Km app	16	8
CDK7/cyclinH	Km app	15	-12
CSF1R	Km app	15	5
MAPKAPK3	Km app	15	-1
MYLK3(caMLCK)	NA	15	4
CamKII_alpha	Km app	15	-5
MuSK	Km app	15	-6
Aurora_A	Km app	15	4
Srm	Km app	15	10
SPHK1	Km app	14	7
MARK4	Km app	14	
PRKAA2	Km app	13	
MEKK3	NA	13	
CAMKK1	NA	13	-4
ARK5	Km app	13	-14
SGK3	Km app	13	-1
FGFR1	Km app	12	17
PI4Kb	Km app	12	
CDK9/cyclinK	NA	12	
KDR	Km app	12	10
JNK1_alpha1	NA	12	1
PKC_gamma	Km app	12	
TTK	NA	12	15
Mink1	Km app	11	17
Hck	Km app	11	
RSK3	Km app	11	2
TrkA	Km app	11	-11
PKC_eta	Km app	10	10
WEE1	NA	10	2
CAMKK2	NA	10	-2
Axl	Km app	10	-4
PKC_alpha	Km app	9	7
TSSK1	Km app	9	0
p38_alpha	100	9	
GCK	Km app	9	
Tpl2	100	9	14
DAPK1	Km app	9	-1
MKK6	NA	9	2
PDGFR_alpha(V561D)	Km app	9	
PDGFR_beta	Km app	9	
EGFR(T790M)	Km app	9	
IRAK1	Km app	9	7
PKC_beta2	Km app	9	
FGFR4	Km app	8	10
IKK_epsilon	Km app	8	-9
JNK1_alpha1	100	8	
PDK1(direct)	Km app	8	3
TSSK2	Km app	8	
TrkC	Km app	8	
DNA-PK	Km app	8	-3
FGFR3(K650E)	Km app	8	
DRAK1	NA	8	3
PRK1	Km app	8	-2
WNK2	NA	8	3
FGFR2	Km app	8	
MAP4K4	Km app	8	11
Fes	Km app	7	-1
JNK3	NA	7	3
Arg	Km app	7	
Met	Km app	7	15
p38_gamma	Km app	7	11
MARK2	Km app	7	
eEF-2K	Km app	7	4
Ret	Km app	7	10
MEK1	NA	7	4
TAK1-TAB1	NA	7	-8
TGFBR1	NA	7	7
PKA	Km app	6	-7
PKD1	Km app	6	13
CK2_alpha2	Km app	6	
TNK2	NA	6	2
EphA3	NA	6	7
Hyl	Km app	6	2
MEKK2	NA	6	1
FAK	Km app	6	8
Blk	Km app	6	15
ZAK	NA	6	0

Compound		6	25
Kinase Count		295	220
Kinase	[ATP]	%Inh@1uM	%Inh@1uM
CaMKI	100	6	1
PDGFR_alpha	Km app	6	0
Abl	Km app	6	7
CSK	Km app	6	16
ERK2	Km app	6	17
GSK3_alpha	Km app	6	3
EphB2	Km app	5	
MELK	Km app	5	5
PAK4	Km app	5	10
PKC_iota	Km app	5	
p38_alpha(direct)	Km app	5	19
CLK1	Km app	5	0
ERK1	Km app	5	
Flt1	Km app	5	8
TBK1	Km app	5	3
SLK	NA	5	0
TYK2	Km app	5	5
EphA4	Km app	5	
JAK3	Km app	5	1
LRRK2(G2019S)	Km app	5	
MLK2	NA	5	-1
PRAK	Km app	5	2
Kit(T670I)	Km app	5	
DYRK1A	Km app	5	6
CLK2	Km app	5	-4
EphA8	Km app	5	0
NEK6	Km app	5	3
CK1_gamma2	Km app	5	6
DDR1	NA	5	0
EGFR(T790M,L858R)	Km app	5	-1
JNK2	NA	5	1
Syk	Km app	5	11
ZAP-70	Km app	5	0
CK1_delta	Km app	4	1
Kit(V654A)	NA	4	
MLK3	NA	4	
RIPK2	NA	4	5
GRK2	Km app	4	1
PI3KC2a	Km app	4	
CDK8/cyclinC	NA	4	6
MRCK_alpha	Km app	4	11
CDK5/p25	Km app	4	3
FAK2	Km app	4	
TAO2	Km app	4	0
MKK6	100	4	
Ros	Km app	4	3
Aurora_B	Km app	4	12
DYRK1B	Km app	4	
NEK7	Km app	4	
PI3KC2b	10	4	
IRAK4	Km app	4	12
RSK1	Km app	4	8
ZIPK	Km app	4	9
MRCK_beta	Km app	4	
STK16	NA	4	1
TXK	Km app	4	6
ACVR2B	NA	4	5
CDK9/cyclinT1	Km app	4	9
MKNK1	Km app	4	2
MSK2	Km app	4	
PKD2	Km app	4	
mTOR	Km app	4	0
CDK1/cyclinB	Km app	3	9
IKK_alpha	Km app	3	9
InsR	Km app	3	4
Mer	Km app	3	4
CK1_gamma3	Km app	3	
PrKX	Km app	3	1
CLK4	NA	3	5
PKC_beta1	Km app	3	5
KHS1	Km app	3	14
Ron	Km app	3	-2
EGFR(L858R)	Km app	3	
EphB3	Km app	3	4
Frk	Km app	3	13
MST3	Km app	3	13
TrkB	Km app	3	0
BMPR1A	NA	3	3
CK1_alpha1	Km app	3	7
AKT1	Km app	2	3
DMPK	NA	2	1
MSK1	Km app	2	-1
PKC_theta	Km app	2	15
PKD3	Km app	2	
DCAMKL2	Km app	2	0
IGF1R	Km app	2	6
PRKAA1	Km app	2	4
PAK1	Km app	2	8
SRPK2	Km app	2	
GRK3	Km app	2	2
PLK1	Km app	2	3
CaMKII_beta	Km app	2	-5
HIPK4	Km app	2	5
MYLK(smMLCK)	NA	2	4
PDK1	100	2	
DYRK3	Km app	2	1
CDK2/cyclinA	Km app	2	7
PASK	Km app	2	9
SRPK1	Km app	2	1
PKC_zeta	Km app	2	14
EphA2	Km app	2	

Compound		6	25
Kinase Count		295	220
Kinase	[ATP]	%Inh@1uM	%Inh@1uM
STK33	NA	2	4
CK1_epsilon1	Km app	1	8
CaMKI_delta	Km app	1	-4
Met(1250T)	Km app	1	
Brk	Km app	1	10
PIM2	Km app	1	
HIPK1	Km app	1	1
PKC_epsilon	Km app	1	4
LTK	Km app	1	3
AKT3	Km app	1	
CHK1	Km app	1	8
MARK1	Km app	1	6
NEK4	Km app	1	2
MST1	Km app	1	5
Rse	Km app	1	5
p70S6K	Km app	1	3
ErbB4	Km app	1	1
p38_delta	Km app	1	5
CK1_gamma1	Km app	0	7
EphA5	Km app	0	
PIM1	Km app	0	2
CHK2	Km app	0	4
GRK7	Km app	0	
PKG1_alpha	Km app	0	-2
AKT2	Km app	0	2
MAPKAPK2	Km app	0	7
JNK2	100	0	
SIK2	Km app	0	11
MSSK1	Km app	-1	10
BrSK1	Km app	-1	1
ITK	Km app	-1	7
MKK6(S207E,T211E)	NA	-1	
MARK3	Km app	-1	1
ErbB2	Km app	-1	4
RAF1(Y340D,Y341D)	NA	-1	-4
RSK4	Km app	-1	
NLK	NA	-1	2
RAF1(Y340D,Y341D)	100	-1	
Fer	Km app	-1	
PAK7	Km app	-1	
SGK1	Km app	-1	3
PAK6	Km app	-2	0
PKC_delta	Km app	-2	2
Tie2	Km app	-2	-1
CDK5/p35	Km app	-2	
SPHK2	10	-2	
DDR2	NA	-2	
JNK3	100	-2	
BRAF	NA	-2	2
PhK_gamma1	Km app	-2	-16
SGK2	Km app	-2	2
MST4	Km app	-2	16
CLK3	Km app	-2	6
ROCK1	Km app	-2	4
MST2	Km app	-3	5
PLK2	Km app	-3	4
NEK1	Km app	-3	12
NEK2	Km app	-3	
IKK_beta	Km app	-3	11
NEK9	Km app	-3	3
PDGFR_alpha(T674I)	Km app	-3	
HIPK2	Km app	-3	0
GRK5	Km app	-3	-1
JAK1	Km app	-3	4
LIMK2	NA	-3	
PI3K-G	Km app	-3	8
EphB4	Km app	-4	
PhK_gamma2	Km app	-4	3
Aurora_C	Km app	-4	
MEK2	100	-4	
PI3KC3_hVPS34	Km app	-4	
p38_beta	Km app	-4	10
BRAF	100	-5	
JAK2	Km app	-5	2
EphB1	Km app	-5	-7
DYRK4	Km app	-5	1
GRK6	Km app	-5	3
PDGFR_alpha(D842V)	Km app	-5	
TAO3	NA	-5	
GRK4	Km app	-5	
Haspin	Km app	-5	
MLK1	Km app	-6	6
NIK	NA	-6	
YSK1	Km app	-6	15
EGFR	Km app	-7	11
CK2_alpha1	Km app	-7	8
PLK3	Km app	-8	-10
PAK3	Km app	-8	8
ASK1	NA	-8	-3
ALK2	NA	-9	5
EphA7	NA	-9	7
Kit	Km app	-9	13
PI3K-A	Km app	-10	-8
PI4Ka	10	-10	
IRR	Km app	-11	-1
MYLK2(skMLCK)	Km app	-11	
MEK3	NA	-13	6
ACVR1B	Km app	-13	6
PI3K-D	Km app	-13	
PAK2	Km app	-19	

Table S2. Comparison of kinome selectivity of **25** vs. **6** against the top hits in the SelectScreen kinase assay panel (ThermoFisher Scientific; Madison, WI).

	25	**6**
Kinases	**%Inh @ 1μM (IC_{50})**	**%Inh @ 1μM (IC_{50})**
Btk	96 (2.4 nM)	99 (2.3 nM)
Src	56 (1330 nM)	70 (302 nM)

Fgr	40	69 (387 nM)
Bmx	14	56 (351 nM)

CRYSTALLOGRAPHY

Btk kinase domain protein was expressed in insect cells, purified, and crystallized as previously described[3]. Diffraction data for the complex of Btk and **25** were collected at beamline 21-IDF of the Advanced Photon Source, and the data were integrated and scaled in HKL2000[5]. The structure was determined by molecular replacement in space group $P6_1$ with PHASER[6], and with unit cell dimensions a=b=108.3Å, c=42.2Å, α=β=90°, and γ=120°, with one protein molecule in the asymmetric unit. Clear electron density of the inhibitor (Figure S3) was observed in all protein molecules. The structure was refined in PHENIX[7] (Table S3).

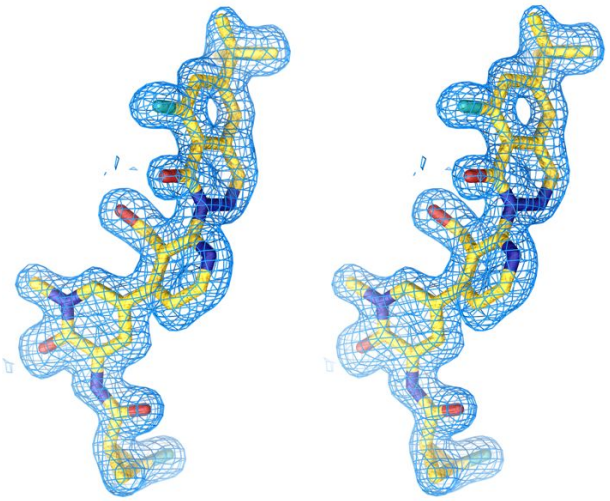


Figure S3: Electron density map of inhibitor 25 in complex with Btk. Divergent eye stereo diagram of the simulated annealing composite omit difference electron density map, $(2m|F_o| - D|F_c|) \exp(i\alpha_c)$, of the Btk inhibitor **25**, contoured at 1σ.

Table S3: Btk crystallographic data collection and refinement statistics.

PDB code	**6XE4**
Compound #	**25**
Beamline	APS 21-IDF
Wavelength (Å)	0.97872
Space group	$P6_1$
Kinase molecules in ASU	1
Resolution range (Å)	35.0-1.60
Highest resolution bin (Å)	1.63 – 1.60
Redundancy	7.6 (6.0)
Completeness (%)	98.8 (93.9)
Mean I/σ_I	13.8 (2.4)
R_{merge} (%)	8.5 (80.2)
CC ½ highest bin	0.54
Refinement	
Resolution range (Å)	33.3 – 1.60
No. reflections (R_{free} set)	37,117 (1,912)
R_{work}, R_{free} (%)	18.0, 15.6
No. non-hydrogen atoms in ASU	2,498
No. water molecules in ASU	276
Rmsd bond lengths (Å)	0.005
Rmsd bond angles (°)	0.792
Molprobity clash score	0.69
Ramachandran most favored region	98.1%
Ramachandran outlier region	0%

Values in parentheses represent data from the highest resolution shell.

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