JAK2 JH2 Fluorescence Polarization Assay and Crystal Structures for Complexes with Three Small Molecules.

Ana S. Newton,[†,§] Luca Deiana,[†,§] David E. Puleo,[‡] José A. Cisneros,[†] Kara J. Cutrona,[‡] Joseph Schlessinger[‡,*] and William L. Jorgensen[†,*]

[†]*Department of Chemistry, Yale University, New Haven, Connecticut 06520-8107,* [‡]*Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06520-8066*

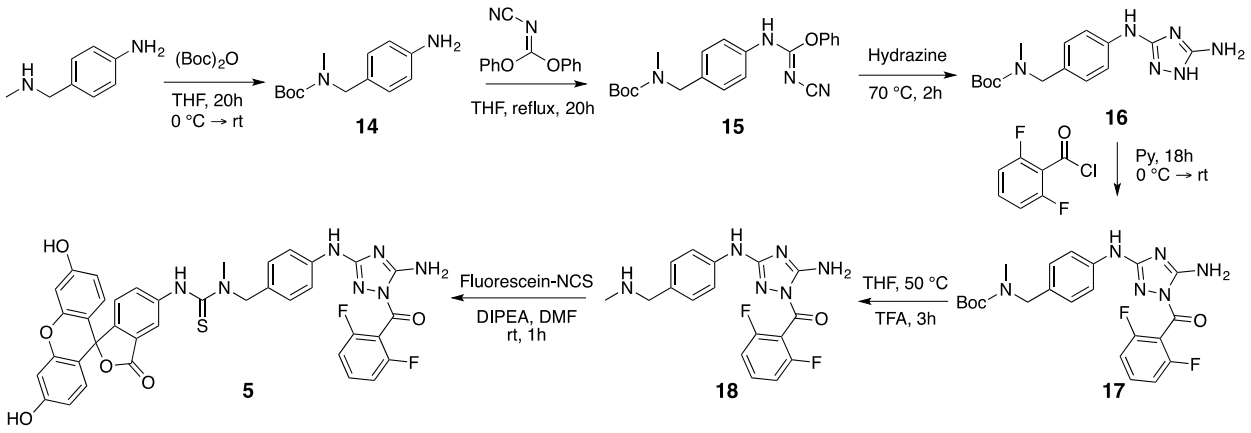
Supporting Information

Table of Contents

1. General information

NMR spectra were recorded on Agilent DD2 600 (600 MHz), and DD2 400 (400 MHz) instruments. Column chromatography was carried out using CombiFlash over redisep column cartridges employing Merck silica gel (Kieselgel 60, 63-200 μm) and Grace C18 reversed-phase (40 μm). Pre-coated silica gel plates F-254 were used for thin-layer analytical chromatography. Mass determinations were performed using electrospray ionization on water Micromass ZQ (LC-MS) and on an Agilent Technologies 6890N (GC-MS). HRMS (ESI-TOF) analyses were performed on Waters Xevo QTOF equipped with Z-spray electrospray ionization source. The purity (≥ 95%) of all final synthesized compounds was determined by reverse phase HPLC, using a Waters 2487 dual λ absorbance detector with a Waters 1525 binary pump and a Phenomenex Luna 5μ C18(2) 250 x 4.6 mm column. Samples were run at 1 mL/min using gradient mixtures of 5-100% of water with 0.1% trifluoroacetic acid (TFA) (A) and 10:1 acetonitrile:water with 0.1% TFA (B) for 22 min followed by 3 min at 100% B.

2. Synthesis of tracers 5 and 6



Scheme S1. Synthesis of ligand **5**

***tert*-Butyl (4-aminobenzyl)(methyl)carbamate (14).** To a solution of 4-((methylamino)methyl)aniline (500 mg, 3.67 mmol) in THF (5 mL) was added dropwise at 0 °C a solution of di-*tert*-butyl dicarbonate (880 mg, 4.04 mmol) in THF (5 mL). The reaction was warmed to room temperature and stirred for 20 hours. The solvent was removed under reduced pressure. Residue was diluted with EtOAc and the organic layer washed with water, brine and dried over anhydrous Na_2SO_4. The solvent was removed under vacuum to afford **14** (866 mg, 100%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 6.97 (d, J = 7.9 Hz, 2H), 6.63 (d, J = 7.9 Hz, 2H), 4.25 (s, 2H), 2.72 (s, 3H), 1.45 (s, 9H). MS (ESI) calcd for $[M+H]^+$ $C_{13}H_{21}N_2O_2$ 238.1, found 238.1.

***tert*-Butyl (*E*)-(4-(((cyanoimino)(phenoxy)methyl)amino)benzyl)(methyl)carbamate (15).** A solution of **14** (995 mg, 4.05 mmol) and diphenyl cyanocarbonimidate (963 mg, 4.05 mmol) in THF (15 mL) was stirred at reflux for 20 hours. The mixture was cooled to room temperature and the solvent removed under reduced pressure. The crude product was collected by filtration, washed with a mixture of hexanes/EtOAc and dried under vacuum to afford **15** (1.27 g, 83%). ^{1}H

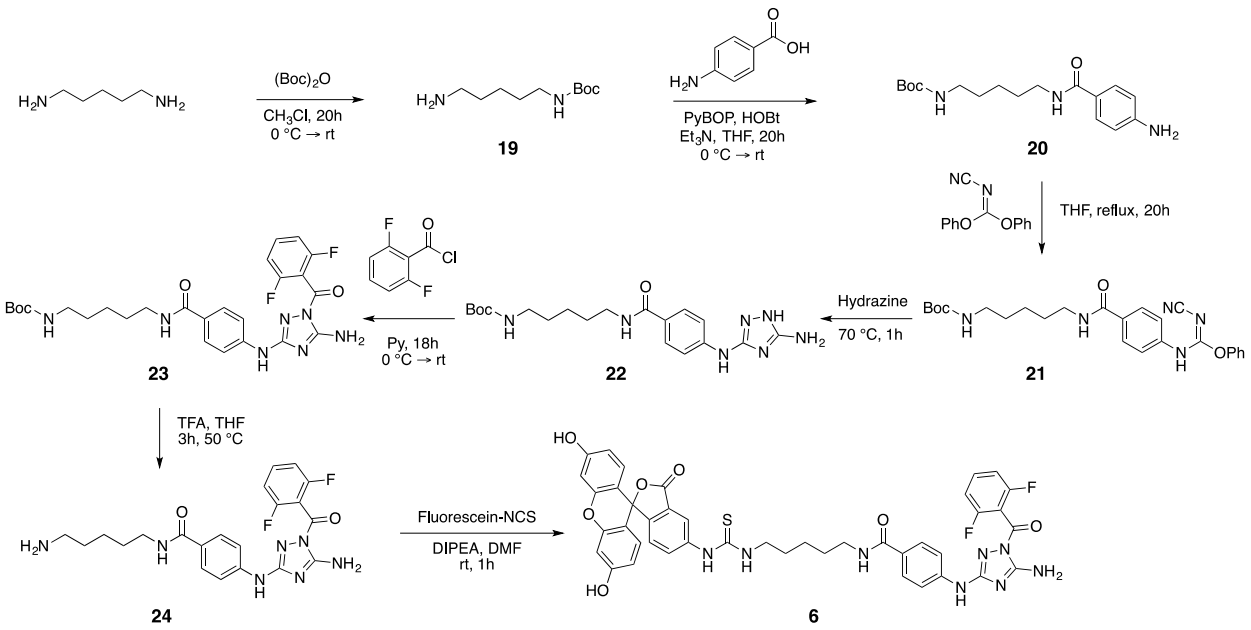
NMR (400 MHz, Acetone-d_6) δ 9.45 (bs, 1H), 7.54 (d, J = 7.9 Hz, 2H), 7.45 (d, J = 7.9 Hz, 2H), 7.33 – 7.28 (m, 3H), 7.18 (t, J = 7.8 Hz, 1H), 6.82 (d, J = 7.9 Hz, 1H), 4.44 (s, 2H), 2.78 (s, 3H), 1.45 (s, 9H). MS (ESI) calcd for $[M+H]^+$ $C_{21}H_{25}N_4O_3$ 381.2, found 381.2.

***tert*-Butyl (4-((5-amino-1-(2,6-difluorobenzoyl)-1*H*-1,2,4-triazol-3-yl)amino)benzyl) (methyl)carbamate (17).** To a solution of **15** (1.274 g, 3.35 mmol) in THF (3.6 mL), hydrazine (3.65 mL) was added at 0 °C. The reaction was stirred at 70 °C for 2 hours. After cooling to room temperature the solvent was removed under reduced pressure to yield **16**, which was used in the next step without further purification. MS (ESI) calcd for $[M+H]^+$ $C_{15}H_{23}N_6O_2$ 319.2, found 319.2. To a solution of **16** (1.276 g, 4.01 mmol) in pyridine (18 mL), 2,6-difluorobenzoyl chloride (0.92 g, 5.21 mmol) was added at 0 °C. The reaction was warmed to room temperature and stirred for 18 hours. The solvent was removed and the product purified by flash chromatography (hexanes/EtOAc) to afford **17** (1.19 g, 65%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.37 (s, 1H), 7.70 (tt, J = 8.1, 6.5 Hz, 1H), 7.47 – 7.42 (m, 2H), 7.22 (dd, J = 9.1, 7.4 Hz, 2H), 7.06 (d, J = 8.1 Hz, 2H), 4.30 (s, 2H), 2.72 (s, 3H), 1.44 (s, 9H). MS (ESI) calcd for $[M+H]^+$ $C_{22}H_{25}F_2N_6O_3$ 459.2, found 459.2.

(5-amino-3-((4-((methylamino)methyl)phenyl)amino)-1*H*-1,2,4-triazol-1-yl)(2,6-difluorophenyl)methanone (18). To a solution of **17** (103 mg, 0.23 mmol) in THF (4 mL), TFA (4 mL) was added. The reaction was stirred at 50 °C for 3 hours. After cooling to room temperature the solvent was removed to afford **18** as a salt used without further purification (70 mg, 87%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.55 (s, 1H), 8.52 (bs, 2H), 7.95 (bs, 1H), 7.67 (tt, J

= 8.1, 6.5 Hz, 1H), 7.40 – 7.26 (m, 4H), 7.22 (d, *J* = 8.3 Hz, 2H), 3.97 (t, *J* = 5.8 Hz, 2H), 2.48 (s, 3H). MS (ESI) calcd for $[M+H]^+$ $C_{17}H_{17}F_2N_6O$ 359.1, found 359.1.

1-(4-((5-amino-1-(2,6-difluorobenzoyl)-1*H*-1,2,4-triazol-3-yl)amino)benzyl)-3-(3',6'-dihydroxy-3-oxo-3*H*-spiro[isobenzofuran-1,9'-xanthen]-5-yl)-1-methylthiourea (5). DIPEA (1.5 mL) and fluorescein isothiocyanate (58 mg, 0.15 mmol) were added to a solution of **18** (70 mg, 0.15 mmol) in DMF (3 mL) and the reaction stirred at room temperature for 1 hour. The solvent was removed and the final compound purified by flash chromatography (hexanes/ EtOAc) (85 mg, 76%). ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.12 (s, 2H), 9.44 (s, 1H), 9.38 (s, 1H), 7.99 (s, 1H), 7.92 (bs, 2H), 7.81 (d, *J* = 8.2 Hz, 1H), 7.69 (p, *J* = 7.5 Hz, 1H), 7.35 - 7.31 (m, 4H), 7.19 (d, *J* = 8.2 Hz, 1H), 7.13 (d, *J* = 8.4 Hz, 2H), 6.68 (s, 2H), 6.59 (s, 4H), 5.05 (s, 2H), 3.16 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 181.2, 168.5, 159.4, 159.2, 158.9, 158.4 (d, *J* = 250.1 Hz), 158.3 (d, *J* = 252.2 Hz), 156.1, 151.8, 148.0, 142.8, 139.9, 133.4 (t, *J* = 9.8 Hz), 132.5, 128.9, 128.6, 127.9, 125.9, 123.3, 119.5, 116.8, 112.8 (t, *J* = 21.4 Hz), 112.6, 112.0 (dd, *J* = 20.4, 3.3 Hz), 109.6, 102.2, 83.0, 55.5. HRMS (ESI) calcd for $[M+H]^+$ $C_{38}H_{28}N_7O_6SF_2$ 748.1790, found 748.1796.



Scheme S2. Synthesis of ligand **6**

***tert*-Butyl (5-aminopentyl)carbamate (19).** A solution of di-*tert*-butyl dicarbonate (1.31 g, 6.0 mmol) in $CHCl_3$ (26 mL) was added dropwise to a solution of cadaverine (3.06 g, 30.0 mmol) in $CHCl_3$ (131 mL) at 0 °C. The reaction was stirred at room temperature for 20 hours. The precipitate formed was filtered off. The organic solution was washed with H_2O and brine, dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. **19** was used in the next step without further purification (1.10 g, 91%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 5.93 (s, 1H), 3.15 (t, J = 7.0 Hz, 2H), 3.08 – 3.02 (m, 2H), 1.56 (p, J = 7.3 Hz, 2H), 1.48 (p, J = 7.3 Hz, 2H), 1.43 – 1.33 (m, 11H). MS (ESI) calcd for $[M+H]^+$ $C_{10}H_{23}N_2O_2$ 203.2, found 203.2.[1]

***tert*-Butyl (5-(4-aminobenzamido)pentyl)carbamate (20).** A solution of 4-aminobenzoic acid (282 mg, 2.06 mmol), PyBOP (1.29 g, 2.47 mmol), HOBt (378 mg, 2.47 mmol), Et_3N (250 mg, 2.47 mmol) and **19** (500 mg, 2.47 mmol) was stirred at 0 °C and then warmed to room

temperature for 20 hours. The solvent was removed under reduced pressure; the mixture diluted with EtOAc and washed with H_2O and brine. The organic phase was dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash chromatography (hexanes/EtOAc) to afford **20** (622 mg, 94%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.64 (d, *J* = 8.5 Hz, 2H), 6.64 (d, *J* = 8.5 Hz, 2H), 3.36 – 3.31 (m, 2H), 3.08 – 3.03 (m, 2H), 1.58 (p, *J* = 7.3 Hz, 2H), 1.51 (p, *J* = 7.3 Hz, 2H), 1.42 – 1.35 (m, 11H). MS (ESI) calcd for $[M+H]^+$ $C_{17}H_{28}N_3O_3$ 322.2, found 322.2.[2]

***tert*-Butyl (*E*)-(5-(4-(((cyanoimino)(phenoxy)methyl)amino)benzamido)pentyl)carbamate (21).** A solution of **20** (622 mg, 1.94 mmol) and diphenyl cyanocarbonimidate (461 mg, 1.94 mmol) in THF (7 mL) was stirred at 65 °C for 20 hours. Then, the reaction was cooled to room temperature and the solvent removed under reduced pressure. The product was collected by filtration, washed with a mixture of hexanes/EtOAc and dried under vacuum. **21** was used in the next step without further purification (811 mg, 90%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.93 (d, *J* = 8.7 Hz, 2H), 7.63 (d, *J* = 8.5 Hz, 2H), 7.53 – 7.45 (m, 3H), 7.34 – 7.31 (m, 2H), 3.39 (q, *J* = 6.7 Hz, 2H), 3.06 (q, *J* = 6.6 Hz, 2H), 1.62 (p, *J* = 7.4 Hz, 2H), 1.52 (p, *J* = 7.1 Hz, 2H), 1.43 – 1.35 (m, 11H). MS (ESI) calcd for $[M+H]^+$ $C_{25}H_{32}N_5O_4$ 466.2, found 466.2.

***tert*-Butyl (5-(4-((5-amino-1*H*-1,2,4-triazol-3-yl)amino)benzamido)pentyl)carbamate (22).** To a solution of **21** (0.811 g, 1.74 mmol) in THF (1.9 mL), hydrazine (1.9 mL) was added at 0 °C. The reaction was allowed to warm to room temperature and then stirred at 70 °C for 2 hours.

The reaction was cooled to room temperature. Hexanes was added to the solution and the white precipitate was collected by filtration, washed with hexanes and dried under vacuum. **22** was used directly in the next step without further purification (0.65 g, 93%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.25 (bs, 1H), 8.99 (s, 1H), 8.11 (t, *J* = 5.7 Hz, 1H), 7.67 (d, *J* = 8.5 Hz, 2H), 7.50 (d, *J* = 8.6 Hz, 2H), 6.79 – 6.76 (m, 1H), 5.91 (bs, 2H), 3.19 (q, *J* = 6.6 Hz, 2H), 2.90 (q, *J* = 6.6 Hz, 2H), 1.48 (p, *J* = 7.4 Hz, 2H), 1.42 – 1.34 (m, 11H), 1.25 (p, *J* = 7.2 Hz, 2H). MS (ESI) calcd for $[M+H]^+$ $C_{19}H_{30}N_7O_3$ 404.2, found 404.2.

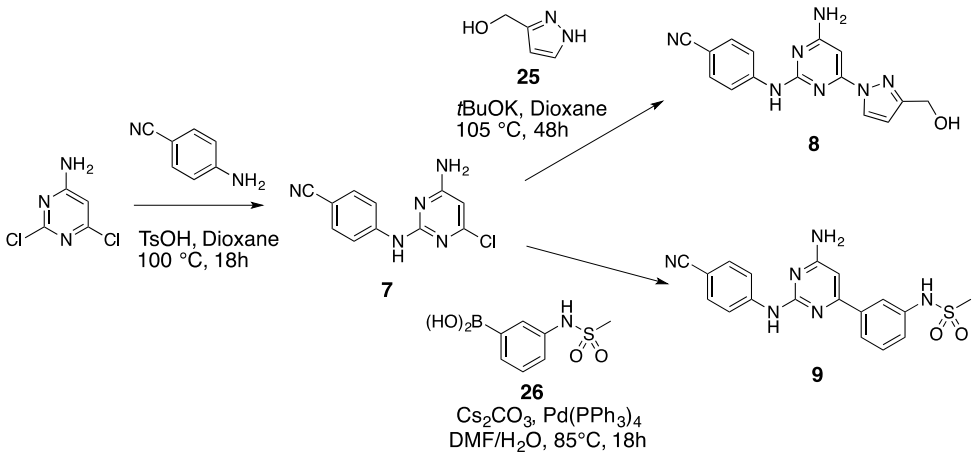
***tert*-Butyl (5-(4-((5-amino-1-(2,6-difluorobenzoyl)-1*H*-1,2,4-triazol-3-yl)amino)benzamido) pentyl)carbamate (23).** To a solution of **22** (0.655 g, 1.62 mmol) in pyridine (7.5 mL), 2,6-difluorobenzoyl chloride (0.372 g, 2.11 mmol) was added at 0 °C. The reaction was warmed to room temperature and stirred for 18 hours. The solvent was removed and the product purified by flash chromatography (hexanes/EtOAc) (0.37 g, 42%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 9.66 (s, 1H), 8.17 (t, *J* = 5.0 Hz, 1H), 7.97 (bs, 2H), 7.76 – 7.68 (m, 1H), 7.63 (d, *J* = 8.7 Hz, 2H), 7.37 – 7.32 (m, 4H), 6.76 (t, *J* = 5.0 Hz, 1H), 3.18 (q, *J* = 6.0 Hz, 2H), 2.89 (q, *J* = 6.0 Hz, 2H), 1.47 (p, *J* = 7.1 Hz, 2H), 1.41 – 1.33 (m, 11H), 1.24 (p, *J* = 7.0 Hz, 2H). MS (ESI) calcd for $[M+H]^+$ $C_{26}H_{32}F_2N_7O_4$ 544.2, found 544.2.

4-((5-amino-1-(2,6-difluorobenzoyl)-1*H*-1,2,4-triazol-3-yl)amino)-*N*-(5-aminopentyl) benzamide (24). To a solution of **23** (100 mg, 0.184 mmol) in THF (4 mL), TFA (4 mL) was added. The reaction was stirred at 50 °C for 3 hours. The reaction was cooled to room temperature and the solvent removed under reduced pressure. The crude mixture was diluted

with EtOAc, and neutralized with a saturated aqueous solution of $NaHCO_3$. The aqueous layer was extracted with EtOAc, the organic phase dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure to give **24** used without further purification (58 mg, 71%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.92 (bs, 1H), 9.67 (bs, 1H), 8.19 (t, *J* = 5.0 Hz, 1H), 7.98 (bs, 2H), 7.76 – 7.68 (m, 1H), 7.63 (d, *J* = 8.4 Hz, 2H), 7.38 – 7.32 (m, 4H), 7.22 (t, *J* = 8.3 Hz, 1H) 3.21 (q, *J* = 6.1 Hz, 2H), 2.77 (q, *J* = 6.8 Hz, 2H), 1.58 – 1.46 (m, 4H), 1.38 – 1.27 (m, 2H). MS (ESI) calcd for $[M+H]^+$ $C_{21}H_{24}F_2N_7O_2$ 444.2, found 444.2.

4-((5-amino-1-(2,6-difluorobenzoyl)-1*H*-1,2,4-triazol-3-yl)amino)-*N*-(5-(3-(3',6'-dihydroxy-3-oxo-3*H*-spiro[isobenzofuran-1,9'-xanthen]-5-yl)thioureido)pentyl)benzamide (6). To a solution of **24** (58 mg, 0.13 mmol) in DMF (3 mL) was added DIPEA (34 µL), fluorescein isothiocyanate (51 mg, 0.13 mmol) and the reaction was stirred at room temperature for 1 hour. The solvent was removed and the final product purified by reverse phase chromatography (75 mg, 69%). ^{1}H NMR (600 MHz, Methanol-d_4) δ 8.26 (t, *J* = 5.9 Hz, 1H), 8.10 (s, 1H), 7.72 (d, *J* = 8.1 Hz, 1H), 7.65 – 7.59 (m, 3H), 7.39 (d, *J* = 8.4 Hz, 2H), 7.17 – 7.11 (m, 3H), 6.70 – 6.65 (m, 3H), 6.54 (d, *J* = 8.4 Hz, 2H), 3.63 (bs, 2H), 3.40 – 3.34 (m, 2H), 1.71 (p, *J* = 6.9 Hz, 2H), 1.66 (p, *J* = 7.0 Hz, 2H), 1.47 (p, *J* = 6.9 Hz, 2H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 182.8, 171.2, 169.9, 169.9, 161.7, 160.7 (d, *J* = 251.8 Hz), 160.7 (d, *J* = 251.7 Hz),159.8, 158.0, 154.3, 145.0, 142.4, 140.2, 134.3 (t, *J* = 9.9 Hz), 131.7, 130.4, 129.1, 127.3, 127.3, 125.8, 120.0, 117.4, 114.2 (t, *J* = 21.0 Hz), 113.8, 112.7 (dd, *J* = 20.8, 3.9 Hz), 111.6, 103.5, 45.6, 40.7, 30.3, 29.6, 25.4. HRMS (ESI) calcd for $[M+H]^+$ $C_{42}H_{35}F_2N_8O_7S$ 833.2317, found 833.2317.

3. Synthesis of Compounds 7-13

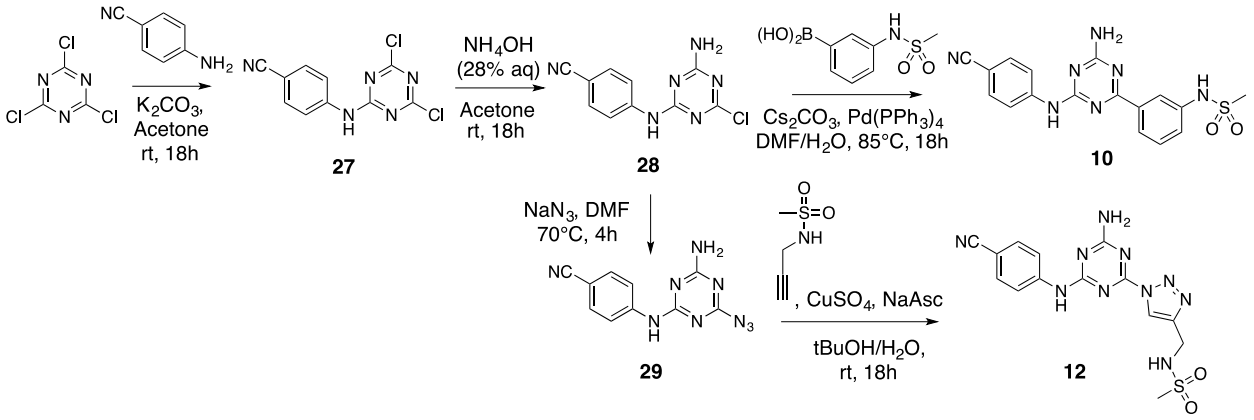


Scheme S3. Synthesis of **7, 8** and **9**.

4-((4-amino-6-chloropyrimidin-2-yl)amino)benzonitrile (7). To a solution of 2,6-dichloropyrimidin-4-amine (200 mg, 1.23 mmol) in dioxane (15 mL), *p*-toluenesulfonic acid (211 mg, 1.23 mmol) was added and the solution stirred for 30 minutes at room temperature. Then, 4-aminobenzonitrile (121 mg, 1.03 mmol) was added and the reaction stirred overnight at 100 °C. The mixture was cooled to room temperature and the solvent removed under reduced pressure. The organic phase was extracted with EtOAc, washed with a saturated aqueous solution of $NaHCO_3$, and dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to yield the desired intermediate as a pure white solid (268 mg, 89%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.83 (s, 1H), 8.06 (d, J = 8.8 Hz, 2H), 7.64 (d, J = 8.7 Hz, 2H), 6.56 (bs, 2H), 6.16 (s, 1H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 165.5, 159.1, 158.6, 145.0, 132.5, 119.1, 118.3, 103.0, 95.2. HRMS (ESI) calcd for $[M+H]^+$ $C_{11}H_9ClN_5$ 246.0541, found 246.0546.

4-((4-amino-6-(3-(hydroxymethyl)-1*H*-pyrazol-1-yl)pyrimidin-2-yl)amino)benzonitrile (8). A solution of **7** (50 mg, 0.20 mmol), (1*H*-pyrazol-3-yl)methanol (29 mg, 0.30 mmol) and potassium *tert*-butoxide (34 mg, 0.30 mmol) in dioxane (2 mL) were stirred for 48 hours at 105 °C. The reaction mixture was cooled to room temperature, solvent evaporated, and the intermediate purified by flash chromatography (hexanes/EtOAc) (15 mg, 24%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 8.81 (s, 1H), 8.43 (d, *J* = 2.3 Hz, 1H), 8.10 (d, *J* = 8.7 Hz, 2H), 7.67 (d, *J* = 8.7 Hz, 2H), 6.64 (s, 1H), 6.51 (d, *J* = 2.4 Hz, 1H), 6.46 (bs, 2H), 4.68 – 4.60 (m, 2H), 4.19 (t, *J* = 5.9 Hz, 1H). ^{13}C NMR (151 MHz, Acetone-d_6) δ 167.0, 160.2, 158.3, 157.6, 146.2, 133.7, 128.8, 120.1, 119.5, 107.3, 104.2, 83.9, 59.1. HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{14}N_7O$ 308.1262, found 308.1270.

***N*-(3-(6-amino-2-((4-cyanophenyl)amino)pyrimidin-4-yl)phenyl)methanesulfonamide (9).** A solution of **7** (0.20 mmol), phenylboronic acid (0.40 mmol), $Pd(PPh_3)_4$ (0.02 mmol) and Cs_2CO_3 (0.60 mmol) were added in a microwave vial. The vial was sealed and purged with N_2. A degassed mixture of DMF/H_2O (3.2:1, 2 mL) was added and the reaction stirred at 85 °C for 18 hours. The reaction mixture was cooled to room temperature and the desired final compound purified by flash chromatography (hexanes/EtOAc) (30 mg, 39%). ^{1}H NMR (400 MHz, Methanol-d_4) δ 8.08 (s, 1H), 8.03 (d, *J* = 8.8 Hz, 2H), 7.75 (d, *J* = 7.8 Hz, 1H), 7.62 (d, *J* = 8.9 Hz, 2H), 7.44 (t, *J* = 7.8 Hz, 1H), 7.29 (d, *J* = 8.7 Hz, 1H), 6.55 (s, 1H), 3.02 (s, 3H). ^{13}C NMR (151 MHz, Methanol-d_4) δ 166.9, 163.9, 161.2, 147.1, 140.7, 140.1, 134.1, 130.7, 123.7, 122.7, 120.7, 119.5, 119.4, 103.6, 95.0, 39.3. HRMS (ESI) calcd for $[M+H]^+$ $C_{18}H_{17}N_6O_2S$ 381.1134, found 381.1139.



Scheme S4. Synthesis of **10** and **12**.

4-((4,6-dichloro-1,3,5-triazin-2-yl)amino)benzonitrile (27). To a solution of cyanuric chloride (500 mg, 2.71 mmol) in acetone (10 mL), potassium carbonate (320 mg, 2.71 mmol) was added at 0 °C and the solution stirred for 20 minutes. Then, 4-aminobenzonitrile (373 mg, 2.71 mmol) was added. The reaction was allowed to warm at room temperature and stirred overnight. The solvent was removed under reduced pressure and the product collected by filtration, washed with H_2O and dried under vacuum to yield **27** as a clean white solid (578 mg, 81%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 10.28 (s, 1H), 8.01 (d, J = 8.8 Hz, 2H), 7.85 (d, J = 8.9 Hz, 2H). MS (ESI) calcd for $[M+H]^+$ $C_{10}H_6Cl_2N_5$ 266.0, found 266.0.

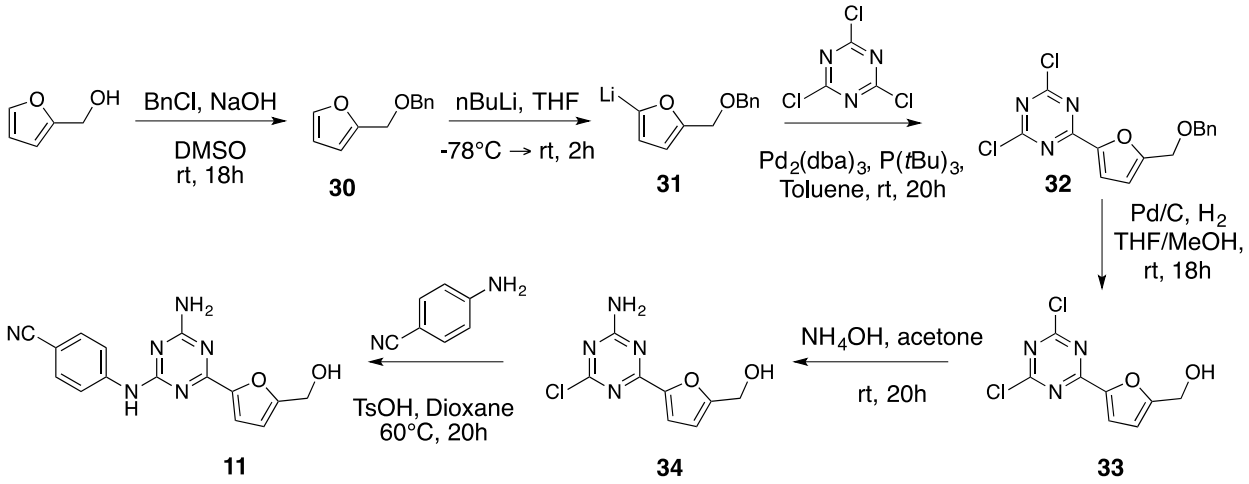
4-((4-amino-6-chloro-1,3,5-triazin-2-yl)amino)benzonitrile (28). To a solution of **27** (3.83 g, 14.5 mmol) in acetone (100 mL), NH_4OH (1 mL) was added at 0 °C. The reaction was allowed to warm at room temperature and stirred overnight. The product was collected by filtration and washed with H_2O to yield **28** as a pure white solid (3.03 g, 85%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 1H), 7.95 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 8.4 Hz, 2H), 7.21 (bs, 2H). MS (ESI) calcd for $[M+H]^+$ $C_{10}H_8ClN_6$ 247.0, found 247.0.[4]

***N*-(3-(4-amino-6-((4-cyanophenyl)amino)-1,3,5-triazin-2-yl)phenyl)methanesulfonamide (10).** A solution of **28** (0.20 mmol), phenylboronic acid (0.40 mmol), $Pd(PPh_3)_4$ (0.02 mmol) and Cs_2CO_3 (0.60 mmol) were added in a microwave vial. The vial was sealed and purged with N_2. A degassed mixture of DMF/H_2O (3.2:1, 2 mL) was added and the reaction was stirred at 85 °C for 18 hours. The reaction mixture was cooled to room temperature and the desired final compound purified by flash chromatography (hexanes/EtOAc) (38 mg, 50%). ^{1}H NMR (600 MHz, Acetone-d_6) δ 9.16 (s, 1H), 8.70 (s, 1H), 8.47 (s, 1H), 8.21 – 8.17 (m, 3H), 7.72 (d, J = 8.7 Hz, 2H), 7.51 – 7.47 (m, 2H), 6.76 (bs, 2H), 3.05 (s, 3H). ^{13}C NMR (151 MHz, Acetone-d_6) δ 171.8, 168.8, 166.1, 145.3, 139.6, 139.0, 133.7, 130.1, 125.1, 124.3, 120.8, 120.6, 119.8, 105.4, 39.5. HRMS (ESI) calcd for $[M+H]^+$ $C_{17}H_{16}N_7O_2S$ 382.1087, found 382.1091.

4-((4-amino-6-azido-1,3,5-triazin-2-yl)amino)benzonitrile (29). To a solution of **28** (300 mg, 1.22 mmol) in DMF (8 mL), NaN_3 (318 mg, 4.90 mmol) was added and the reaction stirred for 4 hours at 70 °C. The reaction mixture was cooled to room temperature, the excess of NaN_3 filtered off, the DMF removed under reduced pressure and the desired intermediate purified by flash chromatography (hexanes/EtOAc) (73 mg, 24%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.14 (s, 1H), 7.95 (d, J = 8.4 Hz, 2H), 7.75 (d, J = 8.4 Hz, 2H), 7.51 (bs, 2H). MS (ESI) calcd for $[M+H]^+$ $C_{10}H_8N_9$ 254.1, found 254.1.

***N*-((1-(4-amino-6-((4-cyanophenyl)amino)-1,3,5-triazin-2-yl)-1*H*-1,2,3-triazol-4-yl)methyl)methanesulfonamide (12**). A solution of **29** (35 mg, 0.14 mmol), *Ms*-protected propargylamine (28 mg, 0.21 mmol), $CuSO_4$ (0.0056 mmol, 1.4 mg), sodium ascorbate (5.5 mg, 0.028 mmol), in *t*-BuOH/ H_2O (1:1, 1.5 mL), was stirred at room temperature for 18 hours. The

solvent was removed under reduced pressure. The product was purified by flash chromatography (hexanes/EtOAc) to afford **12** (9 mg, 17%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.53 (bs, 1H), 8.54 (bs, 1H), 8.06 (d, J = 8.4 Hz, 2H), 7.92 (bs, 1H), 7.85 (bs, 1H), 7.77 (d, J = 8.1 Hz, 2H), 7.69 (t, J = 5.4 Hz, 1H), 4.35 (d, J = 5.4 Hz, 2H), 2.95 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 167.5, 164.7, 160.4, 145.4, 143.9, 133.0, 132.7, 121.6, 119.8, 119.3, 104.1, 37.6. HRMS (ESI) calcd for $[M+H]^+$ $C_{14}H_{15}N_{10}O_2S$ 387.1100, found 387.1104.[5]



Scheme S5. Synthesis of **11**.

2-((benzyloxy)methyl)furan (30). To a solution of furan-2-ylmethanol (1.0 g, 10.2 mmol) in DMSO (3 mL), NaOH (0.612 g, 15.3 mmol) was added and the solution stirred 1 hour. Then, benzyl chloride (1.413 g, 11.2 mmol) was added and the reaction stirred overnight. The mixture was poured into water, extracted with EtOAc and the organic layer dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure and **30** was used in the next step without further purification (1.82 g, 93%). ^{1}H NMR (400 MHz, Chloroform-d) δ 7.44 – 7.42 (m, 1H), 7.38 – 7.34 (m, 4H), 7.32 – 7.28 (m, 1H), 6.37 – 6.32 (m, 2H), 4.56 (s, 2H), 4.49 (s, 2H). MS (ESI) calcd for $[M+H]^+$ $C_{12}H_{13}O_2$ 189.1, found 189.1.

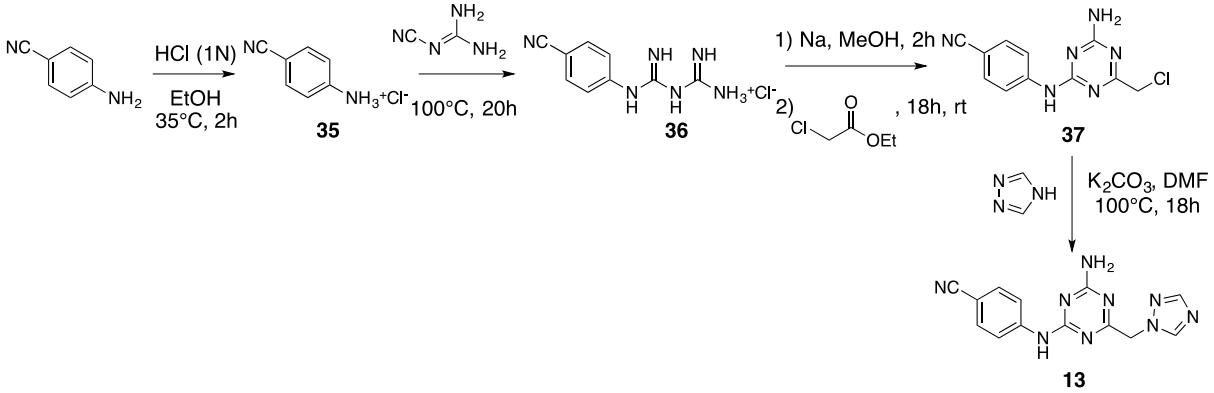
2-(5-((benzyloxy)methyl)furan-2-yl)-4,6-dichloro-1,3,5-triazine (**32).** To a solution of **30** (0.5 g, 2.6 mmol) in dry THF (6.5 mL), *n*BuLi (2.5 M in hexane, 1.144 mL) was added at -78 °C under N_2. The reaction was stirred for 2 hours. This solution was added dropwise for 1 hour, at 0 °C, to a solution of cyanuric chloride (1.43 g, 7.8 mmol), $Pd_2(dba)_3$ (59 mg, 0.065 mmol) and P(*t*Bu)$_3$ in toluene (17 mL) and stirred for 20 hours at room temperature. The reaction was quenched with NH_4Cl and extracted with EtOAc. The organic layer was dried over anhydrous Na_2SO_4 and the solvent removed under reduced pressure. The crude reaction mixture was purified by flash chromatography (hexanes/EtOAc) to afford **32** (0.11 g, 13%). ^{1}H NMR (400 MHz, Acetone-d_6) δ 7.72 (d, *J* = 3.6 Hz, 1H), 7.41 – 7.29 (m, 5H), 6.80 (d, *J* = 3.6 Hz, 1H), 4.68 (s, 2H), 4.64 (s, 2H). MS (ESI) calcd for $[M+H]^+$ $C_{15}H_{12}Cl_2N_3O_2$ 336.0, found 336.0.[6]

(5-(4,6-dichloro-1,3,5-triazin-2-yl)furan-2-yl)methanol (33). A solution of **32** (111 mg, 0.33 mmol) and 10% Pd/C (57 mg) in THF/ MeOH (1:1, 5 mL) was stirred under H_2 for 18 hours. The reaction was filtered through celite and the solvent removed under reduced pressure to afford **33** (80 mg, 0.33 mmol), used in the next step without further purification.

(5-(4-amino-6-chloro-1,3,5-triazin-2-yl)furan-2-yl)methanol (34). A solution of **33** (80 mg, 0.33 mmol) was stirred with NH_4OH (22 µL) in acetone (5 mL) for 20 hours. The solvent was removed under reduced pressure and the product used in the next step without further purification.

4-((4-amino-6-(5-(hydroxymethyl)furan-2-yl)-1,3,5-triazin-2-yl)amino)benzonitrile (11). A solution of **34** (74 mg, 0.33 mmol) in dioxane (4 mL) was stirred with *p*-toluenesulfonic acid (57 mg, 0.33 mmol) for 30 minutes. Then, 4-aminobenzonitrile (39 mg, 0.33 mmol) was added and the solution stirred at 60 °C for 20 hours. The reaction mixture was cooled to room temperature

and the product purified by flash chromatography (hexanes/EtOAc) (12 mg, 12%). ^{1}H NMR (600 MHz, Acetone-d_6) δ 9.07 (s, 1H), 8.19 (d, J = 8.3 Hz, 2H), 7.70 (d, J = 8.4 Hz, 2H), 7.24 (d, J = 2.2 Hz, 1H), 6.68 (s, 2H), 6.48 (d, J = 2.2 Hz, 1H), 4.64 (d, J = 6.3 Hz, 2H), 4.42 (t, J = 5.8 Hz, 1H). ^{13}C NMR (151 MHz, Acetone-d_6) δ 168.5, 165.7, 165.0, 159.8, 151.6, 145.4, 133.7, 120.4, 119.8, 116.5, 109.9, 105.3, 57.6. HRMS (ESI) calcd for $[M+H]^+$ $C_{15}H_{13}N_6O_2$ 309.1100, found 309.1099.



Scheme S6. Synthesis of **13**.

1-(4-cyanophenyl)phenylbiguanide monohydrochloride (36). A solution of 4-aminobenzonitrile (500 mg, 4.23 mmol) in EtOH (2 mL) and HCl (1N, 4.23 mL) was stirred at 35 °C for 2 hours. Dicyandiamide (355 mg, 4.23 mmol) was added directly to the solution and the reaction was stirred overnight at 100 °C. After cooling to room temperature the precipitated was collected by filtration and washed with THF, hexanes and acetone to yield **36** as a clean solid (481 mg, 56%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.15 (s, 1H), 7.74 (d, J = 8.2 Hz, 2H), 7.59 (d, J = 8.6 Hz, 2H), 7.54 (bs, 4H), 7.15 (s, 2H). MS (ESI) calcd for $[M+H]^+$ $C_9H_{12}ClN_6$ 239.1, found 239.1.[7]

4-((4-amino-6-(chloromethyl)-1,3,5-triazin-2-yl)amino)benzonitrile (37). Sodium (11 mg, 0.49 mmol) was added to methanol (2 mL) and stirred for 10 minutes. Then, **36** (100 mg, 0.49 mmol) was added and the reaction stirred for 2 hours. Ethyl-2-chloroacetate (119 mg, 0.98 mmol) was added to the solution and the reaction stirred overnight. The solvent was removed under reduced pressure, and the compound used in the next step without further purification (114 mg, 90%) ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.17 (s, 1H), 8.02 (d, J = 8.7 Hz, 2H), 7.72 (d, J = 8.7 Hz, 2H), 5.93 (bs, 2H), 4.39 (s, 2H). MS (ESI) calcd for $[M+H]^+$ $C_{11}H_{10}ClN_6$ 261.1, found 261.1.[8]

4-((4-((1*H*-1,2,4-triazol-1-yl)methyl)-6-amino-1,3,5-triazin-2-yl)amino)benzonitrile (13). A solution of **37** (30 mg, 0.12 mmol), 1,2,4-triazole (16 mg, 0.23 mmol) and K_2CO_3 (23 mg, 0.23 mmol) in DMF (2 mL) was stirred at 100 °C for 18 hours. The reaction mixture was cooled to room temperature, the solvent evaporated under reduced pressure, and the final product purified by flash chromatography (hexanes/EtOAc) (12 mg, 37%). ^{1}H NMR (600 MHz, Acetone-d_6) δ 9.07 (s, 1H), 8.46 (s, 1H), 7.99 – 7.93 (m, 2H), 7.91 (s, 1H), 7.63 (d, J = 8.6 Hz, 2H), 6.77 (bs, 2H), 5.28 (s, 2H). ^{13}C NMR (151 MHz, Acetone-d_6) δ 174.0, 168.4, 165.6, 152.2, 146.1, 144.9, 133.5, 120.6, 119.7, 105.7, 54.5. HRMS (ESI) calcd for $[M+H]^+$ $C_{13}H_{12}N_9$ 294.1216, found 294.1204.

4. FP assays

Determination of the affinities of ligands 5 and 6. The expression and purification of JAK2 JH2 are described in the accompanying paper.[9] In a flat black bottom 96 well plate (Corning), the buffer (20 mM Tris-Cl pH 8.0, 150 mM NaCl, 20% Glycerol, 0.5 mM TCEP, 10 mM $MgCl_2$, 0.01% Brij) is added - 200 µL to column 1 (blank), 290 µL to column 2, 150 µL to columns 3-12. 6 µL of protein (6.4 nM JAK2-JH2 WT) were added to column 2. 150 µL was transferred, using a multichannel pipette, from column 2 to 3, 3 to 4, 4 to 5, until reaching the last column to make a serial dilutions (1:2). 50 µl of 6.0 pM tracer were added from columns 2-12 and fluorescence polarization was measured at λ_{exc} = 485 ± 20 nm, λ_{em} = 535 ± 25 nm using an Infinite F500 plate reader until no FP variation was observed. From the lowest and highest FP values (tracer free and tracer fully bound to JAK) fraction of ligand bound to the protein to ligand total (L_b/L_t) was calculated for each concentration of JAK2-JH2 (Figure 1B). Experiments were carried out by quadruplicates in three independent experiments. The data provided a typical saturation-binding curve and K_d was calculated fitting the results to the Hill equation using Prism 7.

Competitive FP assay. In a flat black bottom 96 well plate (Corning), 200 µL of FP buffer were added to column 1 (blank), 150 µL to column 2, and 140 µL to columns 3-12. 10 µL of 4.0 µM JAK2-JH2 WT were added to columns 3-12, followed by the addition of 2 µL of DMSO to columns 1-3. 2 µL of inhibitor in DMSO at different concentrations were added from column 4 to 12. 50 µL of 6 pM of **5** were added to columns 2-12. Fluorescence polarization was measured at λ_{exc} = 485 ± 20 nm, λ_{em} = 535 ± 25 nm for 1 hour. Experiments were carried out by quadruplicates in three independent experiments. Data were analyzed by a least-squares non-

linear fit, generated using Prism 7 in order to determine the compound's IC_{50}. K_d values for each inhibitor are calculated using the following equation based on the IC_{50}, K_d of the tracer (K_d^t), total (L_t) and bound (L_b) tracer, as well as total JAK2-JH2 concentration (P_t).[10]

$$K_d^I = \frac{L_b IC_{50} K_d^t}{P_t L_t + L_b(P_t - L_t + L_b - K_d^t)}$$

5. Protein crystallography

Compounds

NVP-BSK805 and filgotinib (GLPG0634) were purchased from Selleckchem. Compound **8** was synthesized as above.

Crystallization and Structure Solution for Complexes with JAK2 JH2.

The protein crystallization and structure solution were performed as described elsewhere.[9] X-ray diffraction data for the JAK2 JH2/NVP-BSK805 and JAK2 JH2/filgotinib complex crystals were collected at Yale University on a Rigaku Micromax 007 HF+ rotating anode X-ray source and Pilatus 200k detectors. Data were scaled and merged using HKL-2000. Data for the JAK2 JH2/**8** complex crystal were collected at Argonne National Laboratory beam line 24-ID-E and were scaled and merged using the automated XDS[11] at APS. The PyMOL[12] molecular viewer was used to generate Figures.

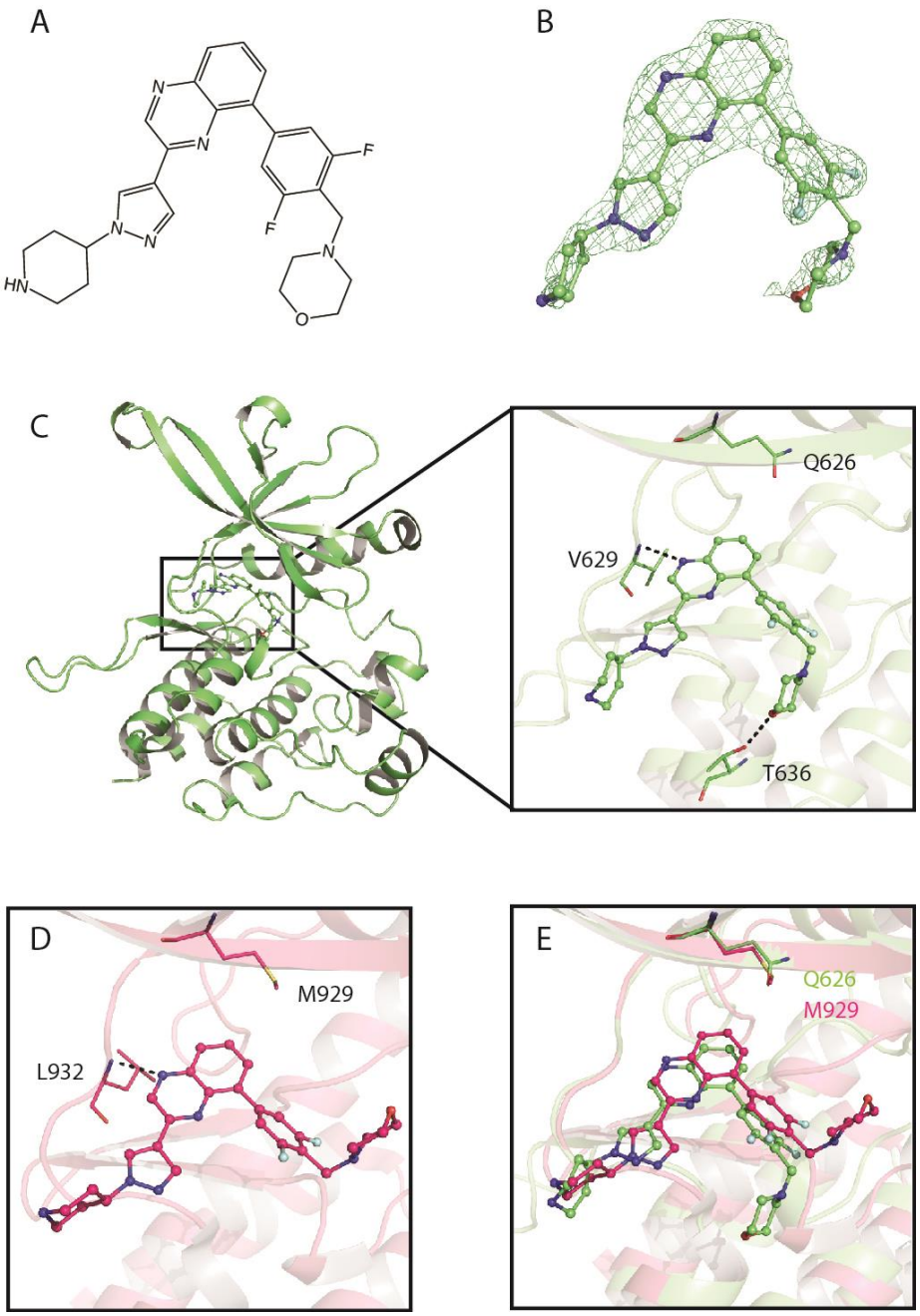


Figure S1. Characterization of NVP-BSK805 binding to the JAK2 JH2 domain. (A) Chemical structure of NVP-BSK805. (B) Electron density map (F_o-F_c contoured at 3σ) of NVP-BSK805 at the JH2 ATP-binding site. The map was calculated without NVP-BSK805 in the model but present during refinements. (C) Structure of JAK2 JH2 in complex with NVP-BSK805. Shown is the overall JH2 domain structure and magnified view of the ATP-binding pocket. (D) Structure of JAK2 JH1 in complex with NVP-BSK805 (PDB ID 3KRR). Shown is a magnified view of the ATP-binding pocket. (E) Structural alignment of JAK2 JH1 (pink) and JH2 (green) domains in complex with NVP-BSK805. The hinge regions of JH1 (chain A, residues 929-935) and JH2 (residues 626-632) were used for the alignment.

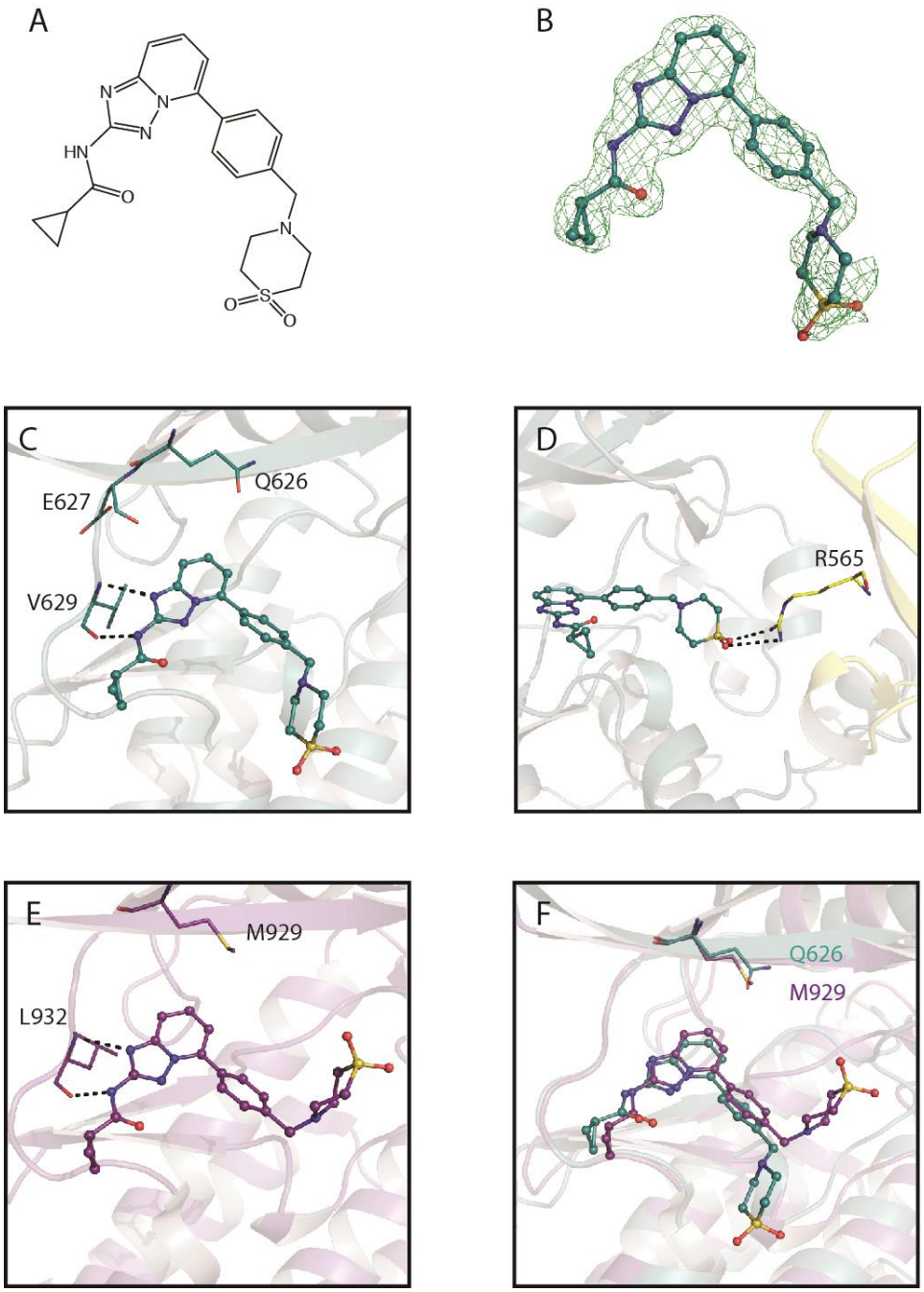


Figure S2. Characterization of filgotinib (GLPG0634) binding to the JAK2 JH2 domain. (A) Chemical structure of filgotinib. (B) Electron density map (F_o-F_c contoured at 3σ) of filgotinib at the JH2 ATP-binding site. The map was calculated without filgotinib in the model but present during refinements. (C) Structure of JAK2 JH2 in complex with filgotinib. Shown is a magnified view of the ATP-binding pocket. (D) Interaction of filgotinib with R565 from a symmetry mate (yellow). (E) Structure of JAK2 JH1 in complex with filgotinib (PDB ID 4P7E). Shown is a magnified view of the ATP-binding pocket. (F) Structural alignment of JAK2 JH1 (magenta) and

JH2 (teal) domains in complex with filgotinib. The hinge regions of JH1 (chain A, residues 929-935) and JH2 (residues 626-632) were used for the alignment.

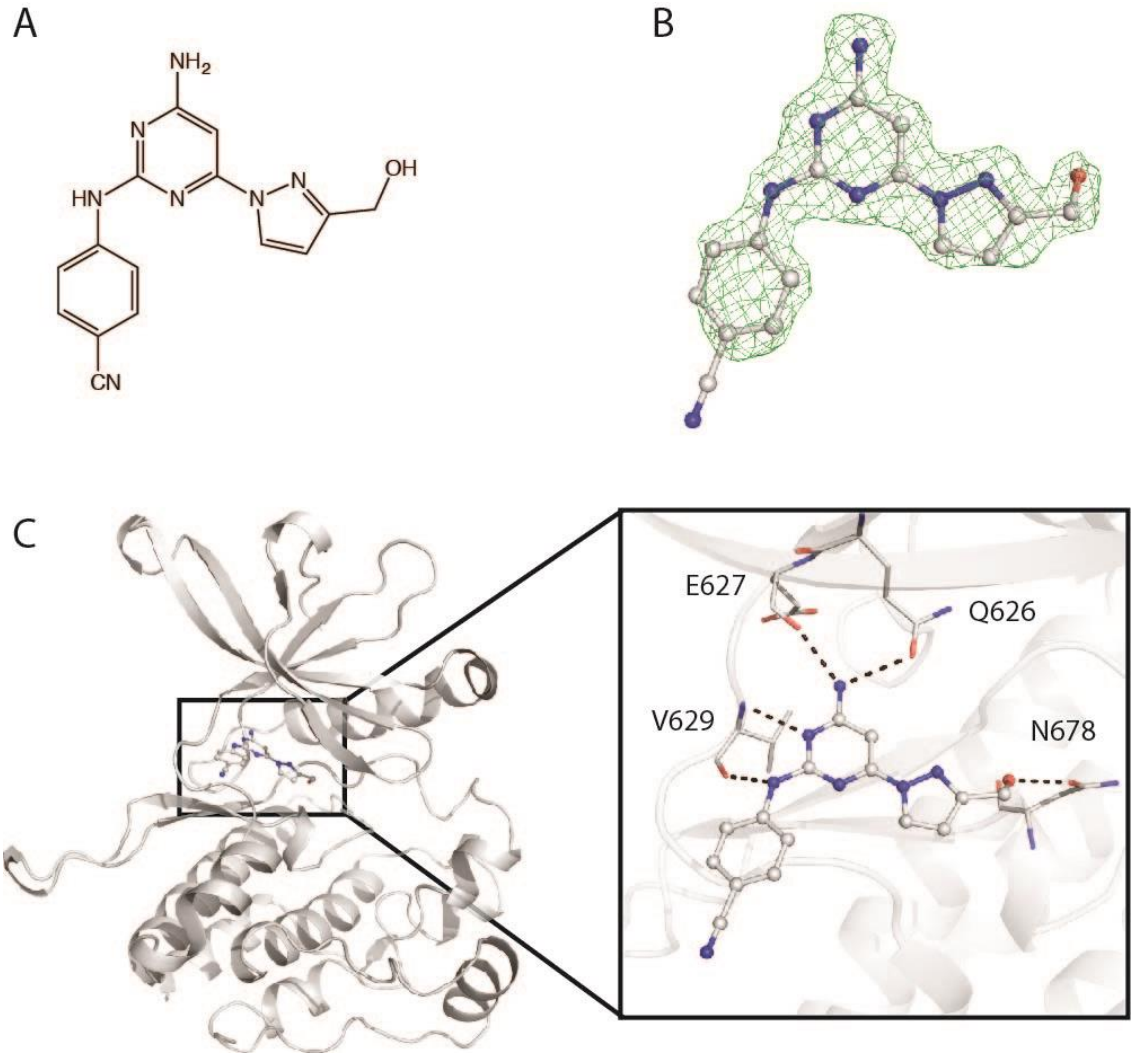


Figure S3. Characterization of compound **8** binding to the JAK2 JH2 domain. (A) Chemical structure of Compound **8**. (B) Electron density map (F_o-F_c contoured at 3σ) of **8** at the JAK2 JH2 ATP-binding site. The map was calculated without **8** in the model but present during refinements. (C) Structure of JAK2 JH2 in complex with **8**. Shown is the overall JH2 structure and magnified view of the ATP-binding pocket.

Table S1. Data Collection and Refinement Statistics.

Protein/Small Molecule	**JAK2 JH2/NVP-BSK805**	**JAK2 JH2/Filgotinib (GLPG0634)**
PDB ID	5UT4	5UT5
Data Collection		
X-ray Source	Rigaku 007 HF+	Rigaku 007 HF+
Wavelength (Å)	1.54	1.54
Space Group	$P2_1$	$P2_1$
Unit Cell Dimensions		
a, *b*, *c* (Å)	45.3, 57.1, 60.7	44.4, 57.3, 60.5
α, β, γ (°)	90.0, 110.2, 90.0	90.0, 110.0, 90.0
Resolution (Å)*	50 – 2.0 (2.07 – 2.00)	50 – 1.9 (1.97 – 1.90)
No. Total Reflections	131,045	132,236
No. Unique Reflections	19,632	22,747
R_{sym} or R_{meas} (%)*	8.9 (37.3)	12.9 (69.1)
$I/\sigma I$*	24.2 (3.3)	19.1 (3.2)
Completeness (%)*	98.9 (93.9)	100.0 (100.0)
Redundancy*	6.7 (5.8)	5.8 (5.4)
Refinement		
Molecules/ASU	1	1
Resolution (Å)	34.1 – 2.0	41.7 – 1.9
R_{work}/R_{free}	17.8/21.9	15.9/20.0
Number of Atoms		
Protein, Solvent, Ion/Small Molecule	2078, 111, 52	2121, 262, 62
B Factors (Å^2)		
Protein, Solvent, Ion/Small Molecule	43.6, 44.8, 51.0	29.0, 39.8, 44.5
RMS Deviations		
Bond (Å)	0.003	0.007
Angle (°)	0.66	0.83

*Values in parentheses refer to the outer resolution shell.

Each data set was collected from a single crystal.

Table S2. Data Collection and Refinement Statistics.

Protein/Small Molecule	**JAK2 JH2/Compound 8**
PDB ID	5UT6
Data Collection	
X-ray Source	APS 24-ID-E
Wavelength (Å)	0.98
Space Group	$P2_1$
Unit Cell Dimensions	
a, *b*, *c* (Å)	44.3, 57.6, 60.6
α, β, γ (°)	90.0, 110.2, 90.0
Resolution (Å)*	41.6 – 1.6 (1.70 – 1.64)
No. Total Reflections	116,787
No. Unique Reflections	34,041
R_{sym} or R_{meas} (%)*	10.1 (101)
$I/\sigma I$*	11.7 (0.8)
Completeness (%)*	97.1 (73.1)
Redundancy*	3.4 (1.4)
Refinement	
Molecules/ASU	1
Resolution (Å)	56.9 – 1.6
R_{work}/R_{free}	16.6/20.5
Number of Atoms	
Protein, Solvent, Ion/Small Molecule	2125, 281, 61
B Factors (Å^2)	
Protein, Solvent, Ion/Small Molecule	21.9, 35.2, 40.5
RMS Deviations	
Bond (Å)	0.012
Angle (°)	1.19

*Values in parentheses refer to the outer resolution shell.

Each data set was collected from a single crystal.

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