Supporting Information

Discovery of a JAK1/3 Inhibitor and the Use of a Prodrug to Demonstrate Efficacy in a Model of Rheumatoid Arthritis

Steven H. Spergel, Michael E. Mertzman, James Kempson, Junqing Guo, Sylwia Stachura, Lauren Haque, Jonathan S. Lippy, Rosemary F. Zhang, Michael Galella, Sidney Pitt, Guoxiang Shen, Aberra Fura, Kathleen Gillooly, Kim W. McIntyre, Vicky Tang, John Tokarski, Jack Sack, Percy H. Carter, Joel C. Barrish, Steven G. Nadler, Luisa M. Salter-Cid, Gary L. Schieven, Stephen T. Wrobleski, and William J. Pitts.*

Research and Development, Bristol-Myers Squibb Company, Route 206 & Provinceline Road, Princeton, NJ 08543-4000, United States

steven.spergel@bms.com

Table of Contents

I. General Methods

Proton magnetic resonance (^{1}H) spectra were recorded on either a Bruker Avance 400 or a JEOL Eclipse 500 spectrometer and are reported in *ppm* relative to the reference solvent of the sample in which they were run. HPLC and LCMS analyses were conducted using a Shimadzu SCL-10A liquid chromatograph and a SPD UV-Vis detector at 220 or 254 nm with the MS detection performed on a Waters Micromass ZQ spectrometer, unless otherwise shown. All flash column chromatography was performed on EM Science silica gel 60 (particle size of 40 – 60 μm). All reagents were purchased from commercial sources and used without further purification unless otherwise noted. All reactions were performed under an inert atmosphere.

HPLC analyses were performed using the following conditions. All final compounds had an HPLC purity of ≥ 95%.

Analytical HPLC

Method A: A linear gradient using 5% acetonitrile, 95% water, and 0.05% TFA (Solvent A) and 95% acetonitrile, 5% water, and 0.05% TFA (Solvent B); *t* = 0 min., 10% B, *t* = 15 min., 100% B (20 min.) was employed on a SunFire C18 3.5u 4.6 x 150 mm column. Flow rate was 1.0 ml/min and UV detection was set to 220/254 nm. The LC column was maintained at ambient temperature.

Method B: A linear gradient using 5% acetonitrile, 95% water, and 0.05% TFA (Solvent A) and 95% acetonitrile, 5% water, and 0.05% TFA (Solvent B); *t* = 0 min., 10% B, *t* = 15 min., 100% B (20 min.) was employed on a XBridge Ph 3.5u 4.6 x 150 mm column. Flow rate was 1.0 ml/min and UV detection was set to 220/254 nm. The LC column was maintained at ambient temperature.

Method C. A linear gradient using 10% methanol, 90% water, and 0.2% H_3PO_4 (Solvent A) and 90% methanol, 10% water, and 0.2% H_3PO_4 (Solvent B); *t* = 0 min., 0% B, *t* = 4 min., 100% B (5 min.) was

employed on a Chromolith SpeedROD 4.6 x 50 mm column. Flow rate was 4.0 ml/min and UV detection was set to 220 nm. The LC column was maintained at ambient temperature.

Method D. Column: Supelco Ascentis Express C18, 4.6 x 50 mm, 2.7-µm particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 90:10 acetonitrile:water with 10 mM ammonium acetate; Temperature: 35 °C; Gradient: 0-100% B over 4 minutes, then a 1-minute hold at 100% B; Flow: 4 mL/min.

Method E. Waters Acquity UPLC BEH C18, 2.1 x 50 mm, 1.7-µm particles; Mobile Phase A: 5:95 acetonitrile:water with 10 mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10 mM ammonium acetate; Temperature: 50 °C; Gradient: 0-100% B over 3 minutes, then a 0.75-minute hold at 100% B; Flow: 1.11 mL/min.

Preparative HPLC (This method was used unless otherwise noted)

Waters XBridge C18, 19 x 250 mm, 5-µm particles; Guard Column: Waters XBridge C18, 19 x 10 mm, 5-µm particles; Mobile Phase A: 5:95 acetonitrile:water with 10-mM ammonium acetate; Mobile Phase B: 95:5 acetonitrile:water with 10-mM ammonium acetate; Gradient: 25-100% B over 25 minutes, then a 5-minute hold at 100% B; Flow: 20 mL/min. Fractions containing the desired product were combined and dried via centrifugal evaporation. (This method was used unless otherwise noted.)

II. Chemistry Experimental

Methyl 1-amino-4-bromo-1*H*-pyrrole-2-carboxylate, methanesulfonic acid salt (4)[2]: To a 5 L 3-neck round bottom flask was added conc. NH_4OH (2.0 L) and the solution was cooled to – 20 °C. Ammonia gas was purged into the solution until the volume doubled. In a separate 10 L 3-neck round bottom flask was added solid NH_4Cl (87 g) and MTBE (5.0 L) and the mixture was cooled to -5 °C. At this time, 555 mL of the previously prepared concentrated NH_4OH solution was added to this mixture followed by a slow addition of commercial grade sodium hypochlorite solution (2.0 L) over 60 minutes.

After the addition was complete, the reaction was stirred at -5 °C for additional 30 minutes. The MTBE layer was separated, washed with brine (720 mL), dried over Na_2SO_4 and decanted. To a separate 20 L round bottom flask was added methyl 4-bromo-1*H*-pyrrole-2-carboxylate (**3**) (100 g, 0.49 mol) and DMF (2.0 L) under nitrogen. Then sodium hydride (60% dispersion in mineral oil, 24 g, 0.58 mol) was added to the reaction portion-wise at room temperature (**CAUTION**: use of sodium hydride in DMF has been known to produce significant exothermic reactions that have resulted in catastrophic fires[3]). After the addition was complete, the reaction mixture was stirred at room temperature for 45 minutes, cooled to -20 ^oC, and then the previously prepared chloramine solution was added in one portion to the reaction mixture. The resulting mixture was allowed to warm to ambient temperature and stir for 30 minutes. The reaction mixture was washed with 10 % aq. $Na_2S_2O_3$ solution (720 mL) and the organic layer was separated and washed again with water (720 mL) and brine (720 mL) before drying over Na_2SO_4, filtering and concentrating under vacuum to afford a semi-solid as the crude product. To this material was added toluene (1.2 L) to give a homogeneous mixture. Methane sulfonic acid (60 g, 0.62 mol) was added dropwise and stirred for 30 minutes. The resulting precipitated solid was collected by vacuum filtration, rinsed with additional toluene and dried to yield 139 g (90%) of the methane sulfonic acid salt of the title compound. H^1 NMR (400MHz, DMSO-d_6) δ ppm: 7.21 (d, *J*= 2.0 Hz, 1H), 6.77 (d, *J*= 2.0 Hz, 1H), 3.75 (s, 3H), 2.50 (s, 3H).

6-Bromo-4-chloropyrrolo[1,2-*b*]pyridazine-3-carbonitrile (5)[2]: To a round bottom flask was added the methyl 1-amino-4-bromo-1*H*-pyrrole-2-carboxylate, methane sulfonic acid salt (**5**) (140 g, 0.444 mol), isopropanol (700 mL) and 3,3- diethoxypropionitrile (128 g, 0.888 mol). The reaction mixture was slowly brought to 85 °C over 1 h and was then stirred at 85 °C for 2 hours. At this time, the ethanol, that was generated, and the isopropanol were removed under vacuum. The resulting residue was dissolved in CH_2Cl_2 and washed with water and brine solution. The organic layer was separated, dried over Na_2SO_4,

filtered and concentrated. The resulting residue was transferred into a 2L round bottom flask and dichloroethane (900 mL) and DBU (210 gm, 1.36 mol) were successively added to the reaction mixture. After stirring at 85 °C for 5 hours, the reaction mixture was cooled to rt and diluted with CH_2Cl_2. The resulting solution was washed with water, followed by brine solution. The organic layer was separated, dried over Na_2SO4, filtered and concentrated. The resulting residue was purified by flash silica gel column chromatography to yield 58 g of 6-bromo-4-hydroxypyrrolo[1,2-*b*]pyridazine-3-carbonitrile as the crude product containing residual DBU. This material was used as is in the next transformation. To a 25 mL round bottom flask was added 6-bromo-4-hydroxypyrrolo[1,2-*b*]pyridazine-3-carbonitrile (16.5 g, 69.47 mmol) and $POCl_3$ (85 mL, 0.88 mol) and the reaction mixture was heated with stirring to 75 °C for 3 hours. The $POCl_3$ was removed under vacuum and the resulting residue was dissolved in CH_2Cl_2. The solution was cooled to 0 °C and saturated aq. $NaHCO_3$ solution was added. The biphasic mixture was stirred vigorously while warming to rt. The organic layer was separated and concentrated and the obtained residue was purified by flash silica gel column chromatography to yield 8.5 g (29%) of the title compound as a yellow solid. H^1 NMR (400MHz, $CDCl_3$) δ ppm: 8.09 (s, 1H), 7.94 (d, *J*= 2.0 Hz, 1H), 7.06 (d, *J*= 2.0 Hz, 1H).

6-Bromo-4-chloropyrrolo[1,2-*b*]pyridazine-3-carboxamide (6)[2]: To a 50 mL round bottom flask was added 6-bromo-4-chloropyrrolo[1,2-*b*]pyridazine-3-carbonitrile (12 g, 0.046 mol) and concentrated H_2SO_4 (60 mL). The reaction mixture was heated at 55 °C for 2 hours then cooled to room temperature and slowly diluted with ice water to precipitate the product which was collected by vacuum filtration, rinsed with water and dried to yield 11.2 g (89%) of 6-Bromo-4-chloropyrrolo[1,2-*b*]pyridazine-3-carboxamide (**6**) as a yellow solid. LCMS (ESI) *m/z* Calcd for $C_8H_6BrClN_3O$ $[M + H]^+$ 273.9. Found: 274.0 (276.0); H^1 NMR (400MHz, DMSO-d_6) δ ppm: 8.32 (s, 2H), 8.05 (s, 1H), 7.90 (s, 1H), 7.01 (s, 1H).

4-(Ethylsulfinyl)-6-phenylpyrrolo[l,2-b]pyridazine-3 –carboxamide (9a)[2]: A mixture of 6-bromo-4-chloropyrrolo[l,2-b]pyridazine- 3-carboxamide **(6)[1]**, 5 g, 18.21 mmol), ethanethiol (1.48 mL, 20.04 mmol) and potassium carbonate (5.0 g, 36.4 mmol) in NMP (60 mL) was stirred at rt for 18 h. Water (500 ml) was added and the resulting suspension was stirred at rt for 1 h. The suspension was filtered and the filter cake was air dried. The solid obtained was then washed with hexane and dried to afford a dark yellow solid. This solid was dissolved in warm ethyl acetate (200 ml) and decolorizing carbon was added. The mixture was allowed to stand for 10 minutes. The mixture was filtered through Celite® and the filter cake was washed thoroughly with warm ethyl acetate. The filtrate was concentrated and dried under high vacuum to afford 6-bromo-4-(ethylthio)pyrrolo[l,2-b]pyridazine-3 -carboxamide (3.6 g, 11.9 mmol, 65% yield) as a yellow solid. LCMS (ESI) *m/z* Calcd for $C_{10}H_{10}BrN_3OS$ $[M + H]^+$ 300.0. Found: 300.0 (302.0); ^1H NMR (400 MHz, $CDCl_3$) δ ppm 7.95 (brs, 1 H) 8.66 (s, 1 H), 7.83 (d, *J*=1.8 Hz, 1 H), 7.03 (d, *J*=1.8 Hz, 1 H), 5.98 (brs, 1 H), 3.11 (q, *J*=7.4 Hz, 2 H), 1.29 (t, *J*=7.5 Hz, 3 H).

A mixture of 6-bromo-4-(ethylthio)pyrrolo[l,2-b]pyridazine-3-carboxamide (1.2 g, 4.0 mmol), phenylboronic acid (0.73 g, 6.0 mmol), palladium (II) acetate (0.090 g, 0.4 mmol), 2M potassium phosphate, tribasic (7.6 mL, 15.2 mmol) and 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbyphenyl (0.38 g, 0.8 mmol) in dioxane (30 mL) was heated to 125° C. in a pressure vessel, behind a blast shield for 4 h. Additional phenylboronic acid (0.37 g, 3.00 mmol), palladium (II) acetate (0.045 g, 0.2 mmol), 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbiphenyl (0.19 g, 0.4 mmol) and 2M potassium phosphate, tribasic (7.6 mL, 15.2 mmol) were added and heating was continued for 1 h. The reaction mixture was partitioned between ethyl acetate (250 mL) and water (250 mL). The organic layer was washed with brine (100 mL), dried (MgSO4) and concentrated to a yellow solid that was triturated with ethyl ether. Filtration, washing with ethyl ether/hexane, 1:1 and drying afforded the 4-(ethylthio)-6-phenylpyrrolo[l,2-b]pyridazine-3 -carboxamide (0.91 g, 3.1 mmol, 77% yield) (**11a)** as a yellow solid

that was used in the next step without further purification. LCMS (ESI) *m/z* Calcd for $C_{16}H_{16}N_3OS$ $[M + H]^+$ 298.1. Found: 298.1; ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.21 (d, *J*=1.8 Hz, 1 H), 8.16 (s, I H), 7.74 (d, *J*=7.3 Hz, 2 H), 7.40 (t, *J*=7.7 Hz, 2 H), 7.28 (d, *J*=7.5 Hz, 1 H), 7.23 (d, *J*=1.8 Hz, 1 H), 3.19 (q, *J*=7.5 Hz, 2 H), 1.28 (t, *J*=7.4 Hz, 3 H).

Oxone (4.1 g, 6.7 mmol) as a solution in water (40 mL) was added to a stirred suspension of 4-(ethylthio)-6-phenylpyrrolo[l,2-b]pyridazine-3-carboxamide (910 mg, 3.1 mmol) in acetone (40 mL) at rt. The reaction mixture was stirred at rt for 3 h. After the reaction mixture was diluted with water (5 ml), the acetone was removed by rotovap and the resulting suspension was filtered and the filter cake was washed with water. Drying afforded 4-(Ethylsulfinyl)-6-phenylpyrrolo[l,2-b]pyridazine-3 – carboxamide (**9a)** (890 mg, 2.84 mmol, 93% yield) as a yellow solid. The material was used as is in the next step. $C_{16}H_{16}N_3O_2S$ $[M + H]^+$ 314.1. Found: 314.1; ^{1}H NMR (400 MHz, DMSO-d^6) δ ppm 8.67 (d, *J*=1.8 Hz, 1 H), 8.49 (s, 1 H), 8.19-8.30 (m, 1 H), 7.98 (d, *J*=1.7 Hz, 1 H), 7.80 (d, *J*=7.3 Hz, 2 H), 7.74 (s, 1 H), 7.40-7.48 (m, 2 H), 7.31 (t, *J*=7.4 Hz, 1 H), 3.31-3.39 (m, 2 H), 1.34 (t, *J*=7.5 Hz, 3 H).

4-(ethylsulfinyl)-6-(6-methoxypyridin-3-yl)pyrrolo[1,2-b]pyridazine-3-carboxamide (9b)[2]: This compound was prepared by the same method as **9a**. $C_{17}H_{17}N_4O_3S$ $[M + H]^+$ 345.1. Found: 345.1; D_2O Exchange: ^{1}H NMR (400 MHz, DMSO-D_6) δ ppm 8.62 (s, 1 H), 8.59 (d, J=1.5 Hz, 1 H), 8.45 (s, 1 H), 8.09 (dd, J=8.7, 2.5 Hz, 1 H), 7.90 (s, 1 H), 6.89 (d, J=8.6 Hz, 1 H), 3.86 (s, 3 H), 3.26 - 3.37 (m, 2 H), 1.32 (t, J=7.5 Hz, 3 H).

General Library Procedure: Into reaction vessel was added amine (0.600 mmol) followed by 4-(ethylsulfinyl)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide (0.030 mmol) or 4-(ethylsulfinyl)-6-(6-methoxypyridin-3-yl)pyrrolo[1,2-b]pyridazine-3-carboxamide (0.030 mmol) in NMP (330 μL). Reaction

was agitated on Innovo Platform Shaker at 80 °C for 24h. To crude reaction was added 1.5mL of DMF and material was purified via preparative HPLC.

Library Detailed Examples:

(*S*)-4-(1-hydroxy-4-methylpentan-2-ylamino)-6-(6-methoxypyridin-3-yl)pyrrolo[1,2-b]pyridazine-3-carboxamide (16): A mixture of 4-(ethylsulfinyl)-6-(6-methoxypyridin-3-yl)pyrrolo[1,2-b]pyridazine-3-carboxamide (28 mg, 0.081 mmol) **(9b)**, (S)-2-amino-4-methylpentan-1-ol (47.6 mg, 0.407 mmol) and in NMP (Volume: 0.4 mL) was stirred at 80 °C for 2 h. The reaction mixture was diluted with 1.5 ml of DMF and was subjected to preparative HPLC (Phenominex 30 x 100 mm S-5 column; eluting with 40-90% aqueous methanol + 0.1% TFA over a 12 minute gradient). The appropriate fractions were concentrated to afford (S)-4-(1-hydroxy-4-methylpentan-2-ylamino)-6-(6-methoxypyridin-3-yl)pyrrolo[1,2-b]pyridazine-3-carboxamide **(16)** (23 mg, 0.060 mmol, 73.6 % yield). HPLC purity 99.9% (Method B); LCMS (ESI) *m/z* Calcd for $C_{20}H_{26}N_5O_3$ $[M + H]^+$ 384.2. Found: 384.1. ^{1}H NMR (400 MHz, MeOD) δ ppm 8.45 (d, *J*=2.0 Hz, 1 H), 8.12 (s, 1 H), 7.99 (dd, *J*=8.6, 2.4 Hz, 1 H), 7.91 (d, *J*=1.5 Hz, 1 H), 7.26 (d, *J*=1.5 Hz, 1 H), 6.84 (d, *J*=8.6 Hz, 1 H), 4.43 (dd, *J*=9.7, 5.5 Hz, 1 H), 3.93 (s, 3 H), 3.81 (dd, *J*=11.1, 4.3 Hz, 1 H), 3.66 (dd, *J*=11.2, 6.2 Hz, 1 H), 1.76 - 1.87 (m, 1 H), 1.56 (m, 1 H) 1.70 (m, 1 H), 1.01 (d, J=6.6 Hz, 3 H), 0.90 (d, *J*=6.4 Hz, 3 H).

(*S*)-4-(1-cyclopropyl-3-hydroxypropan-2-ylamino)-6-(6-methoxypyridin-3-yl)pyrrolo[1,2-b]pyridazine-3-carboxamide (17): A mixture of 4-(ethylsulfinyl)-6-(6-methoxypyridin-3-yl)pyrrolo[1,2-b]pyridazine-3-carboxamide **(9b)** (14 mg, 0.041 mmol), (S)-2-amino-3-cyclopropylpropan-1-ol (23.41 mg, 0.203 mmol) and in NMP (0.2 mL) was heated to 80 °C for 2.5 h. The reaction mixture was diluted with 1.5 ml of DMF and was subjected to preparative HPLC (Phenominex 30 x 100 mm S-5 column; eluting with 35-90% aqueous methanol + 0.1% TFA over a 10 minute gradient). The pure fraction was concentrated to afford (S)-4-(1-cyclopropyl-3-hydroxypropan-

2-ylamino)-6-(6-methoxypyridin-3-yl)pyrrolo[1,2-b]pyridazine-3-carboxamide, **(17)** (13 mg, 0.025 mmol, 61.8 % yield). HPLC purity 95.8% (Method B); LCMS (ESI) *m/z* Calcd for $C_{20}H_{24}N_5O_3$ $[M + H]^+$ 383.2. Found: 382.0. ^{1}H NMR (400 MHz, CD_3OD) δ ppm 8.48 (d, *J*=2.6 Hz, 1 H), 0.10 - 8.13 (s, 1 H), 8.08 (dd, *J*=8.7, 2.5 Hz, 1 H), 7.93 (d, *J*=1.8 Hz, 1 H), 7.28 (d, *J*=1.8 Hz, 1 H), 6.93 (d, *J*=8.8 Hz, 1 H), 4.48 (dd, *J*=6.9, 5.4 Hz, 1 H), 3.96 (s, 3 H), 3.81 - 3.89 (m, 1 H), 3.71 - 3.80 (m, 1 H), 1.62 - 1.73 (m, 2 H), 0.78 - 0.90 (m, 1 H), 0.41 - 0.52 (m, 2 H), 0.17 - 0.23 (m, 1 H), 0.16 (m, 1 H).

Library Examples:

4-(Isopropylamino)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide (11): Yield 57%; Purity 99.9%

4-(isobutylamino)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide (12): Yield 51%; Purity 99.9%

4-(isopentylamino)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide (13): Yield 45%; Purity 99.9%

4-(neopentylamino)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide (14): Yield 48%; Purity 99.9%

4-(cyclopentylamino)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide (15): Yield 57%; Purity 99.9%

4-((1,3-dihydroxypropan-2-yl)amino)-6-(6-methoxypyridin-3-yl)pyrrolo[1,2-b]pyridazine-3-carboxamide (20): Yield 12%; Purity 99.0%

4-(((2R,3R)-1,3-dihydroxybutan-2-yl)amino)-6-(6-methoxypyridin-3-yl)pyrrolo[1,2-b]pyridazine-3-carboxamide (21): Yield 88%; Purity 99.2%

Examples

(*R*)-4-((1-cyclopropyl-1,3-dihydroxypropan-2-yl)amino)-6-(6-methoxypyridin-3-yl)pyrrolo[1,2-b]pyridazine-3-carboxamide, Isomer A (18) and (*R*)-4-((1-cyclopropyl-1,3-dihydroxypropan-2-yl)amino)-6-(6-methoxypyridin-3-yl)pyrrolo[1,2-b]pyridazine-3-carboxamide, Isomer B (19): To a solution of (*R*)-tert-butyl 4-formyl-2,2-dimethyloxazolidine-3-carboxylate (Garner's aldehyde) (0.46 g, 2.006 mmol) in THF (8 mL) at 0 °C was added 0.5 M cyclopropylmagnesium bromide in THF (12.04 mL, 6.02 mmol) over 5 minutes. The reaction mixture was stirred at 0 °C for one hour, at which time it was quenched with saturated NH_4Cl solution (50 mL). The resulting mixture was extracted with ethyl acetate (50 mL). The organic layer was washed with water (50 mL) and brine (50 mL). Drying ($MgSO_4$) and concentration afforded an oil that was chromatographed on a 24 gm ISCO silica gel cartridge, eluting with a 0-100% ethyl acetate /hexane gradient. The product containing fractions were concentrated to afford (4*R*)-tert-butyl 4-(cyclopropyl(hydroxy)methyl)-2,2-dimethyloxazolidine-3-carboxylate (0.45 g, 1.658 mmol, 83% yield) as a colorless oil. The material was a mixture of diasteriomers and was used as is in the next step.

To a solution of (4*R*)-tert-butyl 4-(cyclopropyl(hydroxy)methyl)-2,2-dimethyloxazolidine-3-carboxylate (0.44 g, 1.622 mmol) in DCM (6 mL) at rt was added HCl, 4N in dioxane (8.11 mL, 32.4 mmol) and the resulting mixture was allowed to stand at rt for 24 h. The volatiles were removed *in*

vacuo and the residue was triturated with ether and ethyl acetate. Drying afforded (2*R*)-2-amino-1-cyclopropylpropane-1,3-diol, HCl (270 mg, 1.611 mmol, 99% yield) as a tan solid. The material was a mixture of diasteriomers and was used as is in the next step.

A mixture of 4-(ethylsulfinyl)-6-(6-methoxypyridin-3-yl)pyrrolo[1,2-b]pyridazine-3-carboxamide **(9b)** (25 mg, 0.073 mmol), (*R*)-2-amino-1-cyclopropylpropane-1,3-diol, HCl (mixture of diasteriomers) (36.5 mg, 0.218 mmol) and Hunig'sBase (0.076 mL, 0.436 mmol) in NMP (0.2 mL) was stirred at 80 °C for 3 h and 100 °C for 4 h. After cooling to rt, the reaction mixture was subjected to preparative HPLC. The pure fraction of the first eluting diasteriomer was concentrated to afford (*R*)-4-((1-cyclopropyl-1,3-dihydroxypropan-2-yl)amino)-6-(6-methoxypyridin-3-yl)pyrrolo[1,2-b]pyridazine-3-carboxamide, Isomer A **(18)** (5 mg, 0.013 mmol, 17% yield). HPLC purity 99.9% (Method E); LCMS (ESI) *m/z* Calcd for $C_{20}H_{24}N_4O_4$ $[M + H]^+$ 398.2. Found: 398.2. ^{1}H NMR (500MHz, DMSO-d_6) δ 10.79 (d, *J*=9.4 Hz, 1H), 8.60 (d, *J*=2.0 Hz, 1H), 8.20 (s, 1H), 8.16 (d, *J*=1.5 Hz, 1H), 8.08 (dd, *J*=8.9, 2.5 Hz, 1H), 7.95 (s, 1H), 7.31 (d, *J*=2.0 Hz, 1H), 6.88 (d, *J*=8.9 Hz, 1H), 4.99 - 4.86 (m, 2H), 4.41 (dd, *J*=9.4, 5.0 Hz, 1H), 3.87 (s, 3H), 3.82 - 3.69 (m, 2H), 3.25 - 3.15 (m, 1H), 1.04 - 0.94 (m, 1H), 0.44 - 0.33 (m, 2H), 0.29 (d, *J*=4.5 Hz, 2H) one exchangeable proton missing. The pure fraction of the second eluting diasteriomer was concentrated to afford (*R*)-4-((1-cyclopropyl-1,3-dihydroxypropan-2-yl)amino)-6-(6-methoxypyridin-3-yl)pyrrolo[1,2-b]pyridazine-3-carboxamide, Isomer B **(19)** (2 mg, 0.005 mmol, 7% yield). HPLC purity 99.0% (Method E); LCMS (ESI) *m/z* Calcd for $C_{20}H_{24}N_5O_4$ $[M + H]^+$ 398.2. Found: 398.2. ^{1}H NMR (500MHz, CD_3OD-d_4) δ 8.41 (d, *J*=2.0 Hz, 1H), 8.07 (s, 1H), 7.93 (dd, *J*=8.4, 2.5 Hz, 1H), 7.82 (d, *J*=2.0 Hz, 1H), 7.24 (d, *J*=1.5 Hz, 1H), 6.82 (d, *J*=8.4 Hz, 1H), 4.49 - 4.39 (m, 1H), 3.93 (s, 3H), 3.90 - 3.84 (m, 2H), 3.27 (dd, *J*=8.9, 2.0 Hz, 1H), 1.13 - 0.97 (m, 1H), 0.63 - 0.50 (m, 1H), 0.48 - 0.36 (m, 2H), 0.31 - 0.20 (m, 1H).

4-((2*R*,3*R*)-l,3-Dihydroxybutan-2-ylamino)-6-phenylpyrrolo [1,2-b]pyridazine-3 – carboxamide (22)[2]: A mixture of 4-(ethylsulfinyl)-6-phenylpyrrolo[l,2-b]pyridazine-3-carboxamide (50 mg, 0.160 mmol) and (2*R*,3*R*)- 2-aminobutane-l,3-diol (84 mg, 0.798 mmol) in DMF (0.75 mL) was stirred at 80° C. for 1.5 h. The DMF was removed under high vacuum on the rotovap to afford a yellow solid residue. This residue was triturated with water and was allowed to stand overnight. The suspension was filtered and the filtercake was dried to afford a yellow solid. Trituration 2 times with ethyl ether and drying afforded 43 mg of a yellow solid. The yellow solid was suspended in 10 ml of EtOAc: MeOH, 1: 1 . The suspension was heated to reflux and was subsequently allowed to cool to rt. Filtration and drying afforded the title compound (**22**) (29 mg, 0.085 mmol, 69% yield) as a tan solid. HPLC purity 99.6%; t_r = 7.78 min. (Method A); 99.6%; t_r = 7.46 min. (Method B); LCMS (ESI) *m/z* Calcd for $C_{18}H_{21}N_4O_3$ [M + H]$^+$ 341.2. Found: 341.1. ^{1}H NMR (400 MHz, DMSO-d^6) δ 10.68 (d, *J*=8.8 Hz, 1H), 8.19 (s, 1H), 8.15 (d, *J*=1.5 Hz, 1H), 7.77 (d, *J*=7.3 Hz, 2H), 7.40 (t, *J*=7.7 Hz, 2H), 7.31 (d, *J*=1.3 Hz, 1H), 7.28-7.22 (m, 1H), 5.09-4.85 (m, 2H), 4.25-4.01 (m, 2H), 3.77-3.53 (m, 2H), 1.12 (d, *J*=6.4 Hz, 3H).

4-(((2*S*,3*S*)-1,3-Dihydroxybutan-2-yl)amino)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide (23): A mixture of 4-(ethylsulfinyl)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide (21mg, 0.067 mmol) and (2*S*,3*S*)-2-aminobutane-1,3-diol (21.14 mg, 0.201 mmol) in DMA (0.3 mL) was stirred at 70 °C for 2.5 hours. The reaction mixture was then diluted with methanol (~2mL) and purified by prep HPLC using two injections. The reaction mixture was subjected to preparative HPLC (Phenominex 30 x 100 mm S-5 column; eluting with 25-90% aqueous methanol + 0.1% TFA over a 10 minute gradient). The pure fraction was concentrated to afford 4-(((2*S*,3*S*)-1,3-dihydroxybutan-2-yl)amino)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide (**23**) (10mg, 0.028 mmol, 42% yield) as an off-white solid. HPLC purity 96.2%; t_r = 7.95 min. (Method A); 96.1%; t_r = 7.67 min. (Method B); LCMS (ESI) *m/z* Calcd for $C_{18}H_{21}N_4O_3$ [M + H]$^+$ 341.2. Found: 341.2. ^{1}H NMR (400MHz, DMSO-d_6) δ 10.68 (d, *J*=8.8 Hz, 1H), 8.19 (s, 1H), 8.15 (d, *J*=1.5 Hz, 1H), 7.77 (d, *J*=7.3 Hz, 2H), 7.40 (t, *J*=7.7

Hz, 3H), 7.31 (d, *J*=1.5 Hz, 1H), 7.28 - 7.21 (m, 1H), 5.03 - 4.94 (m, 2H), 4.22 - 4.04 (m, 2H), 3.75 - 3.57 (m, 2H), 1.12 (d, *J*=6.2 Hz, 3H) exchangeable amide protons are are not detected.

.

4-(((2*R*,3*S*)-1,3-Dihydroxybutan-2-yl)amino)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide (24): A mixture of 4-(ethylsulfinyl)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide (40 mg, 0.128 mmol), (2*R*,3*S*)-2-aminobutane-1,3-diol (40.3 mg, 0.383 mmol) and in DMA (0.5 mL) was stirred at 90 °C for 1.5 h. The reaction mixture was subjected to preparative HPLC (Phenominex 30 x 100 mm S-5 column; eluting with 35-90% aqueous methanol + 0.1% TFA over a 12 minute gradient). The pure fraction was concentrated to afford 4-(((2*R*,3*S*)-1,3-dihydroxybutan-2-yl)amino)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide (**24**) (30 mg, 0.088 mmol, 69 % yield) as an off-white solid. HPLC purity 99.5%; t_r = 7.84 min. (Method A); 99.5%; t_r = 7.58 min. (Method B); LCMS (ESI) *m/z* Calcd for $C_{18}H_{21}N_4O_3$ $[M + H]^+$ 341.2. Found: 341.1. ^{1}H NMR (400MHz, DMSO-d_6) δ 10.73 (d, *J*=8.8 Hz, 1H), 8.28 - 8.12 (m, 2H), 7.76 (d, *J*=7.5 Hz, 2H), 7.40 (t, *J*=7.4 Hz, 2H), 7.32 (br. s., 1H), 7.29 - 7.20 (m, 1H), 4.29 (d, *J*=4.2 Hz, 1H), 3.95 (d, *J*=5.5 Hz, 1H), 3.69 (d, *J*=4.0 Hz, 2H), 1.19 (d, *J*=6.2 Hz, 3H) none of the five exchangeable protons are seen due to the addition of D_2O to the NMR tube before acquisition of the data.

4-(((2*S*,3*R*)-1,3-Dihydroxybutan-2-yl)amino)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide (25): A mixture of 4-(ethylsulfinyl)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide (25 mg, 0.080 mmol) and (2*S*,3*R*)-2-aminobutane-1,3-diol (25.2 mg, 0.239 mmol) in DMA (0.3 mL) was shaken at 80 °C for 1.5h. The reaction solution was then diluted with methanol (1.7mL) and purified by preparative HPLC (Phenominex 30 x 100 mm S-5 column; eluting with 25-90% aqueous methanol + 0.1% TFA over a 10 minute gradient). The pure fraction was concentrated to afford 4-(((2*S*,3*R*)-1,3-dihydroxybutan-2-yl)amino)-6-phenylpyrrolo[1,2-b]pyridazine-3-carboxamide **(25)** (16mg, 0.046 mmol, 58 % yield) as an off-white solid. HPLC purity 98.5%; t_r = 7.84 min. (Method A); 98.6%; t_r = 7.58 min.

(Method B); LCMS (ESI) *m/z* Calcd for $C_{18}H_{21}N_4O_3$ $[M + H]^+$ 341.2. Found: 341.1. ^{1}H NMR (400MHz, CD_3OD) δ 8.14 (s, 1H), 7.94 (d, *J*=1.8 Hz, 1H), 7.70 (dd, *J*=8.3, 1.2 Hz, 2H), 7.42 - 7.31 (m, 3H), 7.28 - 7.21 (m, 1H), 4.43 (q, *J*=5.3 Hz, 1H), 4.16 - 4.05 (m, 1H), 3.95 - 3.83 (m, 2H), 1.33 (d, *J*=6.6 Hz, 3H).

***Tert*-butyl((2*R*,3*R*)-3-(benzyloxy)-l-hydroxybutan-2-yl)carbamate (27)[2]:** To a suspension of lithium aluminum hydride (2.94 g, 78 mmol) in 50 mL of diethyl ether at 0° C. was slowly added a solution of (2S,3R)-3-(benzyloxy)-2-((tert-butoxycarbonyl) amino)butanoic acid (**26)** (8.0 g, 25.9 mmol) in 25 mL of diethyl ether. The reaction was allowed to warm to rt and stir 1 h. Water (3 mL) was added with caution, to the reaction mixture. This addition was followed by an addition of 15% NaOH (3 ml) and water (9 mL). These additions were made with extreme caution to control the rate of gas evolution. After stirring 1h at rt, $MgSO_4$ was added and the mixture was filtered. The filtrate was concentrated to afford an oil, which was chromatographed on a 120 gm Isco cartridge using a 0-75% ethyl acetate/hexane gradient. The pure fractions were concentrated to afford tert-butyl((2*R*,3*R*)-3-(benzyloxy)-l-hydroxybutan-2-yl)carbamate **(27)** (5.44 g, 18.42 mmol, 71.2% yield) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.32 (s, 5H), 5.07 (br. s, 1H), 4.64 (d, *J*=11.4 Hz, 1H), 4.38 (d, *J*=11.7 Hz, 1H), 3.83 (d, *J*=6.2 Hz, 1H), 3.79-3.71 (m, 1H), 3.70-3.59 (m, 2H), 2.60-2.38 (m, 1H), 1.48-1.42 (m, 9H), 1.27-1.25 (m, 3H).

(2*R*,3*R*)-2-Amino-3-(benzyloxy)butan-l-ol, HCl (28)[2]: HC1, 4N in dioxane (28.3 mL, 113 mmol) was added to a solution of tert-butyl((2*R*,3*R*)-3-(benzyloxy)-l-hydroxybutan-2-yl)carbamate (2.79 g, 9.45 mmol) in DCM (30 mL) at rt. The reaction mixture was allowed to stand at rt for 18h. The volatiles were removed *in vacuo* and the residue was dried to afford (2*R*,3*R*)-2-amino-3-(benzyloxy)butan-l-ol, HCl **(28)** (2.18 g, 9.41 mmol, 100% yield) as an off-white solid. ^{1}H NMR (400 MHz, CD_3OD) δ 7.47-7.25 (m, 5H), 4.70 (d, *J*=11.7 Hz, 1H), 4.52 (d, *J*=11.4 Hz, 1H), 3.85-3.57 (m, 3H), 3.21-3.02 (m, 1H), 1.30 (d, *J*=6.2 Hz, 3H).

4-(((2*R*,3*R*)-3- (Benzyloxy)-l-hydroxybutan-2-yl)amino)-6-bromopyrrolo [l,2-b]pyridazine-3-carboxamide (29)[2]: A mixture of 6-bromo-4-chloropyrrolo[l,2-b]pyridazine-3-carboxamide (4) (1.9 g, 6.92 mmol), (2*R*,3*R*)-2-amino-3-(benzyloxy)butan-l-ol, HCl (2.18 g, 9.41 mmol) and diisopropylethylamine (4.84 mL, 27.7 mmol) in DMA (20 mL) was heated to 100° C. for 5 h. After cooling to rt, the reaction mixture was partitioned between ethyl acetate (150 mL) and water (150 mL). The organic layer was washed with water (2 x 150 mL) and brine (150 mL). After drying (MgSO4) and filtration, the organic layer was concentrated to afford 4-(((2*R*,3*R*)-3- (benzyloxy)-l-hydroxybutan-2-yl)amino)-6-bromopyrrolo [l,2-b]pyridazine-3-carboxamide **(29)** (2.98 g, 6.88 mmol, 99% yield) as a brown solid. The material was used directly as is in the next step. LCMS (ESI) *m/z* Calcd for $C_{19}H_{22}BrN_4O_3$ $[M + H]^+$ 433.1. Found: 433.0 (435.0).

4-(((2*R*,3*R*)-3-(benzyloxy)-l-hydroxybutan-2-yl)amino)-6-phenylpyrrolo[l,2-b]pyridazine- 3-carboxamide (30)[2]: A mixture of 4-(((2*R*,3*R*)-3-(benzyloxy)-1 -hydroxybutan- 2-yl)amino)-6-bromopyrrolo[l,2-b]pyridazine-3-carboxamide (2.98 g, 6.88 mmol), phenylboronic acid (1.68 g, 13.75 mmol), palladium (II) acetate (0.31 g, 1.38 mmol), 2-(dicyclohexylphosphino)-2',4',6'-triisopropylbyphenyl (1.31 g, 2.75 mmol) and potassium phosphate, tribasic, 2M (20.6 mL, 41.3 mmol) in dioxane (50 mL) was heated to 100° C. for 1 h. After cooling to rt, the reaction mixture was filtered through a Celite® and the filtrate was partitioned between ethyl acetate (200 mL) and water (200 mL). The organic layer was washed with brine (150 mL), dried ($MgSO_4$) and concentrated to afford a brown solid that was triturated with ether. Filtration and drying afforded 4-(((2*R*,3*R*)-3-(benzyloxy)-l-hydroxybutan-2-yl)amino)-6-phenylpyrrolo[l,2-b]pyridazine- 3-carboxamide **(30)** (2.17 g, 5.04 mmol, 73.3% yield) as a tan solid. ^{1}H NMR (400 MHz, CD_3OD) δ 8.16-8.11 (m, 1H), 7.94 (d, *J*=1.8 Hz, 1H), 7.73-7.67 (m, 2H), 7.45-7.21 (m, 9H), 4.76 (d, *J*=11.9 Hz, 1H), 4.56 (d, *J*=11.9 Hz, 1H), 4.44-4.33 (m, 1H), 4.09 (qd, *J*=6.3,2.8 Hz, 1H), 3.96-3.83 (m, 2H), 1.31 (d, *J*=6.4 Hz, 3H). LCMS (ESI) *m/z* Calcd for $C_{25}H_{27}N_4O_3$ $[M + H]^+$ 431.2. Found: 431.1.

Dibenzyl((2*R*,3*R*)-3-(benzyloxy)-2-((3-carbamoyl-6-phenylpyrrolo[l,2-b] pyridazin-4-yl)amino)butyl)phosphate (31)[2]: To a solution of 4-((2*R*,3*R*)-3-(benzyloxy)-l-hydroxybutan-2-yl)amino)-6-phenylpyrrolo[l,2-b]pyridazine-3-carboxamide (2.15 g, 4.99 mmol) and dibenzyl diisopropylphosphoramidite (3.69 mL, 10.99 mmol) in DCM (10 mL) and THF (30mL) at 0° C. was added l-H-tetrazole (0.77 g, 10.99 55 mmol). After stirring 1.5 h at 0° C., hydrogen peroxide, 30% (4.08 mL, 40.0 mmol) was added and stirring was continued for 30 minutes. The reaction mixture was partitioned between ethyl acetate (300 mL) and 5% NaHSO3 solution (250 mL). The organic layer was washed with 1N HCl (200 mL), saturated 60 $NaHCO_3$ solution (200 mL) and brine (200 mL). After drying (MgSO4) and filtration, the organic layer was concentrated to a yellow oil that was chromatographed on silica gel column, eluting with a 0-3% methanol/DCM gradient. The pure fractions were concentrated to afford 2.7 g of partially purified material that was re-chromatographed on a silica column eluting with a 0-100% ethyl acetate/hexane gradient. The pure fractions were concentrated **31** (2.51 g, 3.63 mmol, 72.8% yield) as a light yellow foam. LCMS (ESI) *m/z* Calcd for $C_{39}H_{40}N_4O_6P$ $[M + H]^+$ 691.3. Found: 691.3. ^1H NMR (400 MHz, CCl3D) δ 10.56 (d, *J*=8.8 Hz, 1H), 7.90 (d, *J*=1.8 Hz, 1H), 7.85 (s, 1H), 7.71-7.66 (m, 2H), 7.41-7.15 (m, 19H), 5.48 (s, 2H), 4.99-4.91 (m, 4H), 4.73 (d, *J*=12.1 Hz, 1H), 4.55-4.43 (m, 2H), 4.33-4.25 (m, 2H), 3.94 (qd, *J*=6.2, 2.0 Hz, 1H), 1.32-1.14 (m, 3H),

(2*R*,3*R*)-2-((3-Carbamoyl-6-phenylpyrrolo[l,2-b] pyridazin-4-yl)amino)-3-hydroxybutyl dihydrogen phosphate, disodium (32)[2]: A mixture of dibenzyl((2*R*,3*R*)-3-(benzyloxy)-2-((3-carbamoyl-6-phenylpyrrolo[l,2-b]pyridazin-4-yl)amino)butyl) phosphate (2.50 g, 3.62 mmol) and Pearlman's catalyst (1.017 g, 1.448 mmol) in ethanol (100 mL) was stirred at rt under an atmosphere of hydrogen for 48 h. The reaction mixture was filtered through Celite® and the filter cake was washed thoroughly with ethanol, followed by methanol. The filtrate was concentrated and the residue (1.75 gm) was subjected to preparative HPLC; 6-injections of between 250 and 320 mg of crude (Phenominex 30x100 mm S-5 column; eluting with 40-90% aqueous methanol+0.1% TFA over an 12 minute

gradient; Flow rate=40 ml/min; UV detection at 254 nM wavelength). The pure fractions of fully deprotected phosphate were concentrated to afford (2*R*,3*R*)-2-((3-carbamoyl-6-phenylpyrrolo[l,2-b]pyridazin-4-yl)amino)-3-hydroxybutyl dihydrogen phosphate (**32)** (1.14 g, 2.71 mmol, 74.9% yield) as an off white solid. LCMS (ESI) *m/z* Calcd for $C_{18}H_{22}N_4O_6P$ $[M + H]^+$ 421.1. Found: 421.1. ^{1}H NMR (400 MHz, CD_3OD) δ 8.18-8.12 (m, 1H), 8.01-7.94 (m, 1H), 7.75 (d, *J*=7.3 Hz, 2H), 7.46-7.33 (m, 3H), 7.29-7.19 (m, 1H), 4.50 (d, *J*=5.5 Hz, 1H), 4.40-4.18 (m, 3H), 1.29-1.22 (m, 3H).

(2*R*,3*R*)-2-((3-carbamoyl-6-phenylpyrrolo[l,2-b]pyridazin-4-yl)amino)-3-hydroxybutyl dihydrogen phosphate, 0.75-methanol (3.86 g, 8.69 mmol) was dissolved in methanol (500 mL) and sodium bicarbonate (1.459 g, 17.37 mmol) was added as a solution in 40 ml of water. The solution was concentrated to an off-white solid that was dissolved in water and lyophilized to afford (2*R*,3*R*)-2-((3-carbamoyl-6-phenylpyrrolo[l,2-b]pyridazin-4-yl)amino)-3-hydroxybutyl dihydrogen phosphate, 2 Na+ (**32**) (4.05 g, 8.68 mmol, 100% yield) as an off-white solid. HPLC purity 99.9%; t_r = 6.83 min. (Method A); 99.9%; t_r = 6.23 min. (Method B); LCMS (ESI) *m/z* Calcd for $C_{18}H_{22}N_4O_6P$ $[M + H]^+$ 421.1. Found: 421.1. 1H NMR (400 MHz, CD_3OD) δ 8.11 (s, 1H), 7.91 (d, *J*=I .5 Hz, 1H), 7.78-7.59 (m, 2H), 7.40-7.32 (m, 3H), 7.26-7.20 (m, 1H), 4.58-4.47 (m, 1H), 4.43-4.31 (m, 1H), 4.22-4.03 (m, 2H), 1.23 (d, *J*=6.6Hz, 3H).

III. Single Crystal x-ray Structure of 22

Crystallographically the structure is neat with one independent conformation in the asymmetric unit. The substituent phenyl ring is out of plane with the pyrrolopyridazine (torsion C5-C6-C19-C20=161°; see **Figure 1**). The molecules of **22** are bound together via numerous hydrogen bond interactions forming 'molecular bi-layers' which are stacked along the direct c axis (or the [001] direction). The near orthogonal relationship of the threonol and pyrrolopyridazine groups facilitates a two-dimensional hydrogen bond network parallel to the (001) plane forming a 'molecular layer'. Further, independent layers which are related to one another by the symmetry axis parallel to b are bound together via hydrogen

bonding involving the carboxamide groups thus forming the aforementioned ‘molecular bi-layer’ (See Figure 2.).

Figure 1. The asymmetric unit of **22** form N-1, with the hydrogen bond scheme noted on the right.

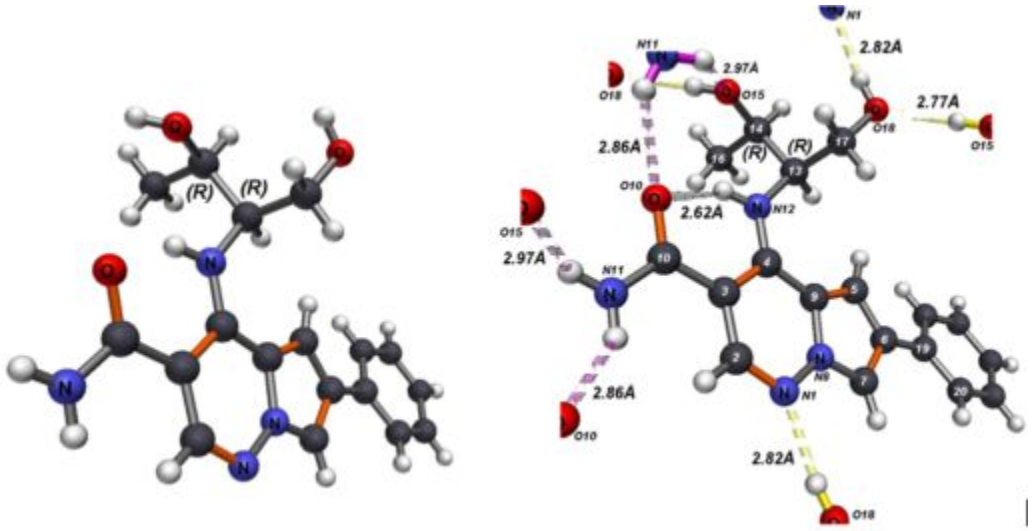
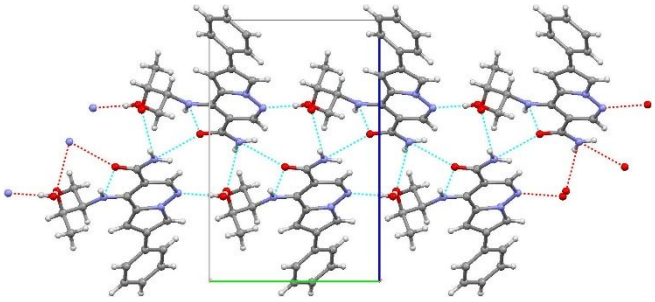
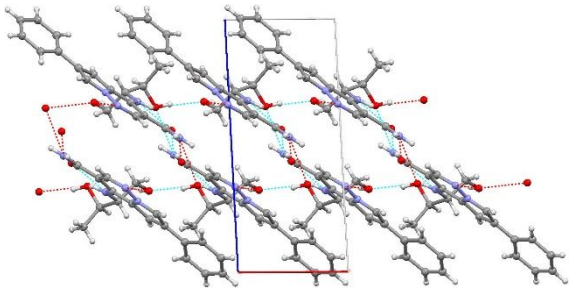


Figure 2. Views along each of the crystallographic directions showing the stacking of the bi-layers.

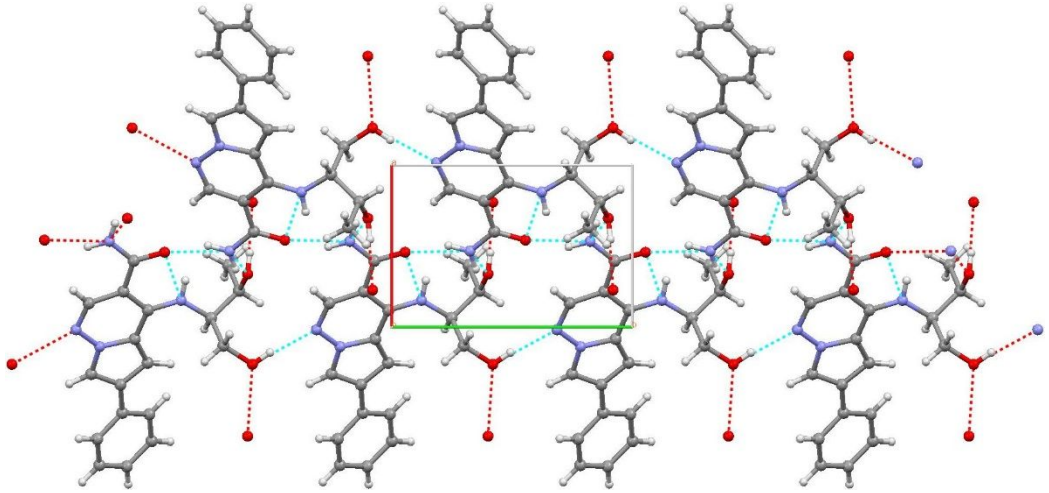
View along a:



View along b:

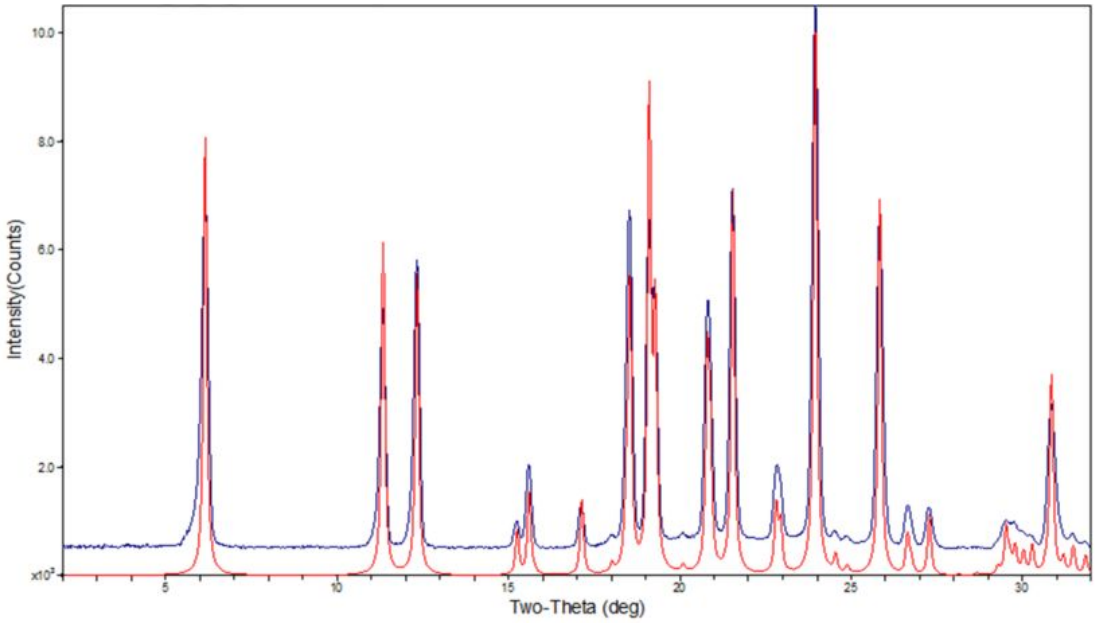


View along c:

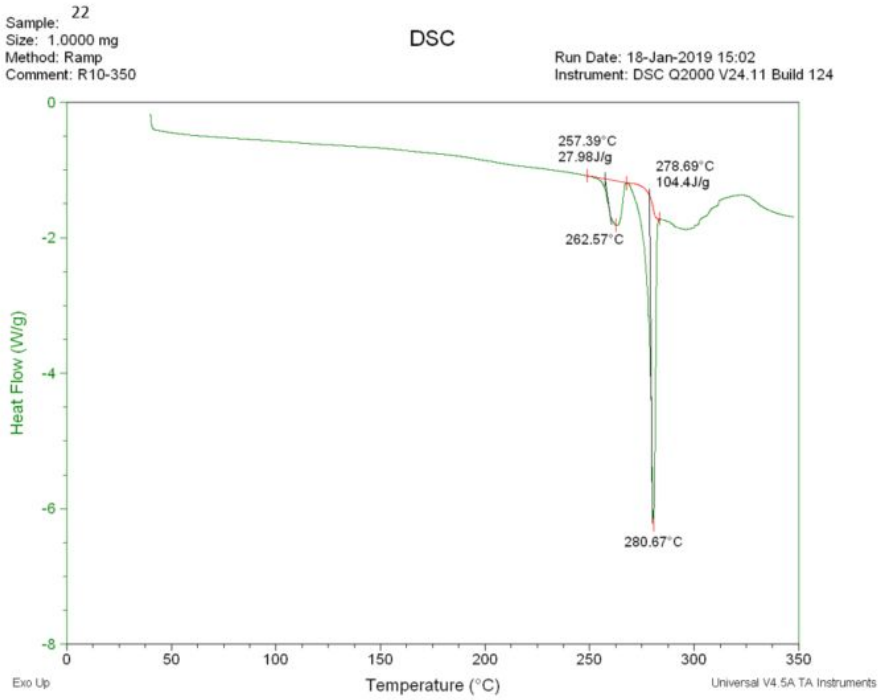


IV. Compound 22 PXRD and DSC

Sample used for single crystal x-ray in red, Sample on repreparation for DSC determination in blue.



Differential Scanning Calorimetry data for 22.



IV. Profiling Data for Compound 22

Compound	22
JAK1 (IC_{50}, nM) JAK2 (IC_{50}, nM) JAK3 (IC_{50}, nM) TYK2 (IC_{50}, nM)	5±2 (n=3) 70±18 (n=2) 3±1 (n=2) 34±13 (n=2)
IL-2 pSTAT3 (IC_{50}, nM) SET2 (IC_{50}, nM) EPO pSTAT5 (IC_{50}, nM) EPO/IL-2 ratio IFNα pSTAT3 (IC_{50}, nM) IL23 KIT225 IFNα pSTAT3/ IL23 KIT225 ratio	7±2 (n=3) 870±120 (n=2) 2200±740 (n=3) 310 20±3 (n=3) 1800±290 (n=2) 90
IL2 hWB (IC_{50}, nM) IL15 hWB (IC_{50}, nM) IL15 mWB (IC_{50}, nM)	510±210 (n=2) 1900±540 (n=6) 810±320 (n=4)
Met stab (h/r/m, %rem) PAMPA (nm/s) Caco-2 (a to b / efflux ration)	96 / 74 / 84 448 / 494 26 / 14
hERG / Ca / Na (flux IC_{50}, uM)	>80 / 14 / >83

Mouse PK (10 mpk, PO) C_{max} / AUC (5% EtOH; 5% d-α-tocopheryl polyethelene glycol 1000 succinate (TPGS); 90% PEG 300)	3.2 μM/6.8 μM
Mouse PK (50 mpk, PO) C_{max} / AUC (2% polyvinyl pyrrolidinone-K30; 0.15 % sodium lauryl sulfate; water)	0.1 μM/0.4 μM

V. Kinome Profile of 22 Kinases < 10% of control at 1 μM

Rho kinase 2 have been reported to have anti-inflammatory activity and also to block IL-17 and IL-21 secretion in T cells.[4] Compound **22** was evaluated in a ROCK1 assay as a surrogate enzyme (IC_{50} of 180 nM caliper format) and had an IC_{50} of 1.0 μM in a phosphomyosin light chain assay.[5]

Kinase	**% control at 1 μM**
"MST2 (% Control, 1uM)"	0.0
"JAK1_D2, (% Control, 1uM)"	0.0
"JAK1 Pseudokinase (% Control, 1uM)"	0.2
"ULK3 (% Control, 1uM)"	0.2
"ROCK2 (% Control, 1uM)"	0.4
"ULK2 (% Control, 1uM)"	0.4
"JAK3 (% Control, 1uM)"	0.4
"GCK/MAP4K2 (% Control, 1uM)"	0.4
"ROCK1 (% Control, 1uM)"	0.8
"ULK1 (% Control, 1uM)"	0.9
"RIPK5/DSTYK (% Control, 1uM)"	1.1
"PKCE (% Control, 1uM)"	1.4
"DMPK 1, (% Control, 1uM)"	1.9
"MAP3K2 (% Control, 1uM)"	2.0
"JAK2 (% Control, 1uM)"	2.2
"RSK4 (% Control, 1uM)"	2.7
"TYK2_D1 (% Control, 1uM)"	3.1
"TYK2 (% Control, 1uM)"	3.3
"MST4 (% Control, 1uM)"	3.4
"NUAK2 (% Control, 1uM)"	3.4
"NUAK1 (% Control, 1uM)"	4.2
"YSK1 (% Control, 1uM)"	4.6

"PKN1(% Control, 1uM)"	5.1
"SBK1 (% Control, 1uM)"	5.8
"BIKE, (% Control, 1uM)"	6.2
"MAP3K3 (% Control, 1uM)"	6.8
"LRRK2 (G2019S) (% Control, 1uM)"	6.8
"MARK4 (% Control, 1uM)"	6.9
"LRRK2 (% Control, 1uM)"	7.4
"HPK1, (% Control, 1uM)"	8.1
"MST1 (% Control, 1uM)"	10.0

VI. Biological Assays

Assay variability was measured using internal standards as controls. Individual assay variability ±SD against a standard is highlighted in the table below:

JAK3 1.9 ± 1.3 nM, n = 111

JAK1 3.5 ± 5.7 nM, n = 128

Tyk2 82 ± 41 nM, n = 61

JAK2 4.9 ± 2.2 nM, n = 135

EPO STAT5 67 ± 35 nM, n = 124

IL2 STAT3 3.1 ± 1.4 nM, n = 134

hWB 41 ± 24 nM, n = 214

Biology Experimental

JAK3 Kinase Assay Protocol: The assay reactions were performed in U-bottom 384-well plates. The final assay volume was 30 μl prepared from 15 μL additions of enzyme and substrates (fluoresceinated peptide and ATP) and test compounds in assay buffer (100 mM HEPES pH 7.4, 10 mM $MgCl_2$, 25 mM beta-glycerol phosphate 0.015% Brij35 and 4 mM DTT). The reaction was initiated by the combination of GST-JAK3 enzyme with substrates and test compounds. The reactions were incubated at room temperature for 3 hours and terminated by adding 60 μL of 35 mM EDTA to each sample. Each reaction mixture was analyzed on the Caliper LabChip 3000 (Caliper, Hopkinton, MA) by electrophoretic separation of the fluorescent substrate and phosphorylated product. Inhibition data were calculated by comparison with no enzyme control reactions for 100% inhibition, and vehicle-only treated reactions for 0% inhibition. The final concentration of reagents in the assay was: ATP, 8 μM; fluoresceinated peptide, 1.5 μM; GST-JAK3, 4.5 nM; and DMSO, 1.6%. Dose response curves were generated to determine the concentration required inhibiting 50% of kinase activity (IC_{50}). Compounds were dissolved at 10 mM in dimethylsulfoxide (DMSO) and evaluated at eleven concentrations. IC_{50} values were derived by non-linear regression analysis.

JAK2 Kinase Assay Protocol: The assay reactions were performed in U-bottom 384-well plates. The final assay volume was 30 μL prepared from 15 μL additions of enzyme and substrates (fluoresceinated peptide and ATP) and test compounds in assay buffer (100 mM HEPES pH 7.4, 10 mM $MgCl_2$, 25 mM beta-glycerol phosphate, 0.015% Brij35 and 4 mM DTT). The reaction was initiated by the combination of GST-JAK2 enzyme with substrates and test compounds. The reactions were incubated at room temperature for 3 hours and terminated by adding 60 μL of 35 mM EDTA to each sample. Each reaction mixture was analyzed on the Caliper LabChip 3000 (Caliper, Hopkinton, MA) by electrophoretic separation of the fluorescent substrate and phosphorylated product. Inhibition data were calculated by comparison with no enzyme control reactions for 100% inhibition, and vehicle-only treated reactions for

0% inhibition. The final concentration of reagents in the assay was: ATP, 30 μM; fluoresceinated peptide, 1.5 μM; GST-JAK2, 1.5 nM; and DMSO, 1.6%. Dose response curves were generated to determine the concentration required inhibiting 50% of kinase activity (IC_{50}). Compounds were dissolved at 10 mM in dimethylsulfoxide (DMSO) and evaluated at eleven concentrations. IC_{50} values were derived by non-linear regression analysis.

JAK1 Kinase Assay Protocol: The assay reactions were performed in U-bottom 384-well plates. The final assay volume was 30 μL prepared from 15 μL additions of enzyme and substrates (fluoresceinated peptide and ATP) and test compounds in assay buffer (20 mM HEPES pH 7.4, 10 mM $MgCl_2$, 25 mM beta-glycerol phosphate, 0.015% Brij35 and 4 mM DTT). The reaction was initiated by the combination of GST-JAK1 enzyme with substrates and test compounds. The reactions were incubated at room temperature for 3 hours and terminated by adding 60 μL of 35 mM EDTA to each sample. Each reaction mixture was analyzed on the Caliper LabChip 3000 (Caliper, Hopkinton, MA) by electrophoretic separation of the fluorescent substrate and phosphorylated product. Inhibition data were calculated by comparison with no enzyme control reactions for 100% inhibition, and vehicle-only treated reactions for 0% inhibition. The final concentration of reagents in the assays was: ATP, 100 μM; fluoresceinated peptide, 1.5 μM; GST-JAK1, 12.5 nM; and DMSO, 1.6%. Dose response curves were generated to determine the concentration required inhibiting 50% of kinase activity (IC_{50}). Compounds were dissolved at 10 mM in dimethylsulfoxide (DMSO) and evaluated at eleven concentrations. IC_{50} values were derived by non-linear regression analysis.

TYK2 Kinase Assay Protocol: The assay reactions were performed in U-bottom 384-well plates. The final assay volume was 30 μL prepared from 15 μL additions of enzyme and substrates (fluoresceinated peptide and ATP) and test compounds in assay buffer (20 mM HEPES pH 7.4, 10 mM $MgCl_2$, 25 mM beta-glycerol phosphate, 0.015% Brij35 and 4 mM DTT). The reaction was initiated by the combination

of HIS-TYK2 enzyme with substrates and test compounds. The reactions were incubated at room temperature for 3 hours and terminated by adding 60 μL of 35 mM EDTA to each sample. Each reaction mixture was analyzed on the Caliper LabChip 3000 (Caliper, Hopkinton, MA) by electrophoretic separation of the fluorescent substrate and phosphorylated product. Inhibition data were calculated by comparison with no enzyme control reactions for 100% inhibition, and vehicle-only treated reactions for 0% inhibition. The final concentration of reagents in the assay was: ATP, 70 μM; fluoresceinated peptide, 1.5 μM; HIS-TYK2, 2.25 nM; and DMSO, 1.6%. Dose response curves were generated to determine the concentration required inhibiting 50% of kinase activity (IC_{50}). Compounds were dissolved at 10 mM in dimethylsulfoxide (DMSO) and evaluated at eleven concentrations. IC_{50} values were derived by non-linear regression analysis.

IL-2 Dependent T Cell Proliferation Assay Protocol: IL-2 Expanded PHA Blasts (Activated T cells) were prepared from peripheral blood mononuclear cells (PBMC). PBMCs were prepared from human whole blood. 15 ml blood was mixed with 15 ml RPMI (Gibco# 61870) in a 50 ml centrifuge tube and under laid with 1 ml lymphocyte separation media (LSM) (MC Biomedicals # 1492254). Tubes were centrifuged at 1800 rpm for 25 minutes and allowed to stop without braking. Red blood cells pelleted under the separation media and the PBMCs were trapped at the interface between the LSM and the serum/RPMI layers. The serum/RPMI mix was pipetted from above the PBMC layer and discarded. The PBMCs from 2 tubes were collected in a pipette along with some of the LSM layer and combined into a single tube. Each tube was brought to 50 ml and centrifuged at 1400 rpm for 10 minutes. Cell pellets were resuspended in RPMI, combined into 1 tube and centrifuged for 5 minutes at 1200 rpm. Cells were resuspended in cell culture media (RPMI w/10% fetal bovine serum (Summit Biotechnology # RS-50-05), 100 U/ml penicillin and 100 μg/ml streptomycin (Gibco # 14140-122)) with 5 μg/ml PHA (Sigma #L1668) at 2 10 6 cells/ml and incubated for 3 days at 37 °C in 5% CO2. Cells were washed 3X and resuspended at 5 10 5 cells/ml and 25 units/ml IL-2 (BD Bioscience # 356043) was added. After 4 days

incubation at 37 °C in 5% CO2 the cells were washed 3X and resuspended at 2X106 cells/ml and rested 2 hours at 37 °C in 5% CO2 before use. Compounds were diluted in DMSO (in triplicate) to 800X final high concentration in the assay and 3-fold serial dilutions were performed to give six concentrations. Each concentration was diluted 1:20 in media to give the intermediate concentration. DMSO was diluted 1:20 in media for use in wells without compound. 45 µĭ media plus 5 µĭ of the intermediate dilution of compound or DMSO was added to each test well in the assay plate. 100 µĭ of cells at 3X105 cells/ml were added to each well. Plates were Incubated 60 minutes at 37 °C in 5% CO2 and 50 µĭ of IL-2 at 200 units/ml to each well. Negative control wells received 100 µĭ media. The plates were incubated 3 days at 37 °C in 5% C0 2 . 0.5 µ H^3 Thymidine in 20 µĭ media was added to each well and the plates incubated 6 hours at 37 °C in 5% C0 2 . The plates were harvested onto a Unifilter-96 GF/C Filter Plate (Perkin Elmer 6005 174) using a Packard Filtermate Harvester. The bottom of each dried filter plate was sealed, 50 µĭ Microscint 20 (Perkin Elmer # 6013621) added to each well and the top of the plate sealed. Proliferation as measured by H-Thymidine incorporation was determined by counting on a Packard TopCount-NXT.

IL-2 Induced STAT3 Phosphorylation in PHA Blasts Assay: IL-2 Expanded PHA Blasts were prepared (see IL-2 Dependent T Cell Proliferation Assay Protocol for preparation of IL-2 expanded PHA blasts). Compounds were diluted in DMSO (in duplicate) to 333.3X final high concentration in the assay and 3-fold serial dilutions were performed to give six concentrations. Each concentration was diluted 1:20 in media to give the intermediate concentration. DMSO was diluted 1:20 in RPMI media (Gibco# 61870) for use in wells without compound.

173 µL/well of a PHA blast cell suspension at 5.78X10^6 cells/mL was added to a round bottom tissue culture plate (Falcon # 353077) followed by 12 µL of the Intermediate compound dilution or DMSO for wells without compound. The plate was incubated at 37°C in 5% CO_2 for 30 minutes. 15 µL of 266.7 ng/mL IL-2 (R&D # 202-IL-050) in media was added to each well or media only to negative control wells. The plates were mixed on an orbital shaker for 1 minute and incubated at 37 °C in 5% CO_2 for a total 10

minutes. Plates were centrifuged 4 minutes at 1500 rpm and 150 μL of culture supernatant was removed from each well leaving the pellet intact. Plates were mixed on an orbital shaker for 1 minute and 50 μL of 2X lysis buffer was added (20 mM Tris, pH7.5, 0.2 M NaCl, 2 mM EDTA, 2 mM EGTA, 2 mM NaF, 2 mM β-Glycerophosphate, 40 mM Sodium Pyrophosphate, 4 mM Na_3VO_4 (add at time of use), 2% Triton X-100 (Sigma T9284), 20% Glycerol (EM Science GX0185-6), 0.2% SDS (Bio-Rad # 161-0416), 1.0% Deoxycholate (Sigma D5670), 2x Protease Inhibitor Cocktail (Sigma P2714, dissolved in 10 mL PBS to give 10X concentration, add at time of use)). The plates were incubated on ice for 1 hour and the lysate in each well was mixed by pipetting up and down 5–6 times. STAT3 phosphorylation levels were determined by ELISA (PathScan Phospho-STAT3 ELISA Antibody Pair, Cell Signaling # 7146).

ELISA plates were coated with 100 μL/well of a 1:100 dilution of Capture antibody in PBS and incubated at least overnight at 4 °C. On day of use plates were washed 3X with wash buffer (PBS (Gibco # 14190) + 0.05% Tween 20). Plates were blocked with 200 μL/well of Assay Buffer 1 (AB1) (PBS + 1% BSA + 0.1% Tween 20(Bio-Rad # 161-0416)) for 2 hours at room temperature. Plates were washed 3X and 90 μL/well AB1 buffer added. 10 μL/well of assay sample or standards were added followed by 100 μL/well of a 1:100 dilution of Detection Antibody in AB1 Buffer. Plates were incubated overnight at 4 °C and then washed 6X. 100 μL/well of a 1:1000 dilution of anti-mouse IgG HRP-Linked Antibody in AB1 Buffer was added to each well and plates were incubated 1 hour at room temperature. Plates were washed 6X. 100 μL of a 1:1 mix of TMB Peroxidase Substrate (KPL # 50-76-01) and Peroxidase Substrate Solution (KPL # 50-65-00) was added and the plates were incubated in the dark at room temperature for 10–30 min. The reaction was stopped with 100 μL/well of 1N H_2SO_4 and the plates were read at 450 nm with correction at 650 nm within 30 min.

pSTAT3 Standards were prepared from IL-6 stimulated TF-1 cells. TF-1 cells were washed 3X and resuspended at $1x10^6$ cells/mL and rested overnight without GM-CSF at 37 °C in 5% CO_2. The cells were washed 1X and resuspended at $5x10^6$ cells/mL. IL-6 was added to 20 ng/mL and cells incubated at

37 °C in 5% CO_2 for 15 minutes, spun 4 minutes at 1500 rpm and the supernatant removed. 150 µL lysis buffer was added for every $5X10^6$ cells and incubated on ice for 60 minutes. Using a syringe fitted with a 21 gauge needle, lysates were passed 6 times through the needle. The lysates were transferred to microfuge tubes and centrifuged at 14K rpm for 10 minutes and the supernatants were combined and aliquots frozen on dry ice and stored at -80 °C. The lysate was assigned a value of 100 units/mL of pSTAT3 and used as a standard in the pSTAT3 ELISA assay. Standards were diluted in a 1:2 dilution series using a 1:1 mix of 2X lysis buffer and Media.

EPO Induced STAT5A Phosphorylation in TF-1 Cells: TF-1 Cells were carried in cell culture media (RPMI w/10% fetal bovine serum (Summit Biotechnology # RS-50-05), 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco # 14140-122)) + 2 ng/ml GM-CSF (R&D #215GM). On the day before use the cells were washed 3X, resuspended at $1X10^6$ cells/mL in media without GM-CSF and rested overnight at 37 °C in 5% CO_2. The cells were washed 1X and resuspended in media at $2.78X10^6$ cells/mL. Compounds were prepared as in the IL-2 Induced STAT3 phosphorylation in PHA blasts assay.

173 µL/well of a TF-1 cell suspension at $2.78X10^6$ cells/mL was added to each well of a round bottom tissue culture plate (Falcon # 353077) followed by 12 µL of the Intermediate compound dilution or DMSO for wells without compound. The plate was incubated at 37 °C in 5% CO_2 for 30 minutes. 15 µl of 13.33 units/mL recombinant human EPO (R&D # 287-TC) in media was added to each well or media only to negative control wells. The plates were mixed on an orbital shaker for 1 minute and incubated at 37 °C in 5% CO_2 for a total 10 minutes. Plates were centrifuged 4 minutes at 1500 rpm and 150 µL of culture supernatant was removed from each well leaving the pellet intact. Plates were mixed on an orbital shaker for 1 minute and 50 µL of 2X lysis buffer was added (20 mM Tris, pH7.5, 0.2 M NaCl, 2 mM EDTA, 2 mM EGTA, 2 mM NaF, 2 mM β-Glycerophosphate, 40 mM Sodium Pyrophosphate, 4 mM Na_3VO_4 (add at time of use), 2% Triton X-100 (Sigma T9284), 20% Glycerol (EM Science GX0185-6), 0.2% SDS (Bio-Rad # 161-0416), 1.0% Deoxycholate (Sigma D5670), 2x Protease Inhibitor Cocktail

(Sigma P2714, dissolved in 10 mL PBS to give 10X concentration, add at time of use)). The plates were incubated on ice for 1 hour and the lysate in each well was mixed by pipetting up and down 5–6 times. STAT5A phosphorylation levels were determined by ELISA.

ELISA plates (NUNC # 439454) were coated with 100 µL/well of a 1:500 dilution of Capture antibody (Invitrogen # 13-3600) in carbonate/bicarbonate buffer (Sigma # C3041) and incubated at least overnight at 4 °C. On day of use plates were washed 3X with wash buffer (PBS (Gibco # 14190) + 0.05% Tween 20 (Bio-Rad # 170-6531)). Plates were blocked with 200 µL/well of Assay Buffer 2 (AB2) (PBS + 2% BSA (Sigma # A-9576) + 0.1% Tween 20 (Bio-Rad # 161-0416)) for 2 hours at room temperature. Plates were washed 3X and 90 µL/well AB2 buffer added. 10 µL/well of assay sample or standards were added followed by 100 µL/well of a 1:4000 dilution of Detection Antibody (Genway # 18-785-210434) in AB2 Buffer. Plates were incubated overnight at 4 °C and then washed 6X. 100 µL/well of a 1:3000 dilution of HRP-Goat anti-rabbit IgG (Invitrogen # 65-6120 in AB2 Buffer was added to each well and plates were incubated 1 hour at room temperature. Plates were washed 6X. 100 µL of a 1:1 mix of TMB Peroxidase Substrate (KPL # 50-76-01) and Peroxidase Substrate Solution (KPL # 50-65-00) was added and the plates were incubated in the dark at room temperature for 10–30 minutes. The reaction was stopped with 100 µL/well of 1N H_2SO_4 and the plates were read at 450 nm with correction at 650 nm within 30 min. pSTAT5A Standards were prepared from GM-CSF stimulated TF-1 cells. TF-1 cells were washed 3X and resuspended at $1x10^6$ cells/mL and rested overnight without GM-CSF at 37 °C in 5% CO_2. The cells were washed 1X and resuspended at $5x10^6$ cells/mL. GM-CSF was added to 50 ng/mL and cells incubated at 37 °C in 5% CO_2 for 15 minutes, spun 4 minutes at 1500 rpm and the supernatant removed. 150 µL lysis buffer was added for every $5X10^6$ cells and incubated on ice for 60 minutes. Using a syringe fitted with a 21 gauge needle, lysates were passed 6 times through the needle. The lysates were transferred to microfuge tubes and centrifuged at 14K rpm for 10 minutes and the supernatants were combined and aliquots frozen on dry ice and stored at -80 °C. The lysate was assigned a value of 100 units/mL of

pSTAT5A and used as a standard in the pSTAT5A ELISA assay. Standards were diluted in a 1:2 dilution series using a 1:1 mix of 2X lysis buffer and Media.

IFNα Induced STAT3 Phosphorylation in PHA Blasts: IFNα induced STAT3 phosphorylation in PHA blasts was performed exactly as the IL-2 Induced STAT3 phosphorylation in PHA blasts assay except the cells were stimulated with 15 μL/well of 13,333 units/mL IFNα2a (R&D #11105-1) in media.

SET-2 Cell Proliferation Inhibition Assay: The antiproliferative effects of compounds on tumor cell lines were monitored by [3H] thymidine incorporation. Cells were incubated with stepwise dilutions of compound for 72 h in RPMI media supplemented with 10% fetal bovine serum. On day 4, 0.022 mCi/mL of [3H] thymidine was added to each well and allowed to incubate for 3–4 h. Cells were harvested onto filter plates, washed and processed for incorporated radioactivity on a scintillation counter.

Kit225 T Cell Assay: Kit225 T cells with a stably-integrated STAT-dependent luciferase reporter were plated in RPMI (GIBCO) containing 10% heat-inactivated FBS (GIBCO) and 100 U/mL PenStrep (GIBCO). The cells were then stimulated with either 20 ng/mL human recombinant IL-23 or 200 U/mL human recombinant IFN-α (PBL InterferonSource) for 5-6 hours. Luciferase expression was measured using the STEADY-GLO® Luciferase Assay System (PROMEGA®) according to the manufacturer's instructions. Inhibition data were calculated by comparison to no inhibitor control wells for 0% inhibition and non-stimulated control wells for 100% inhibition. Dose response curves were generated to determine the concentration required to inhibit 50% of cellular response (IC50) as derived by non-linear regression analysis.

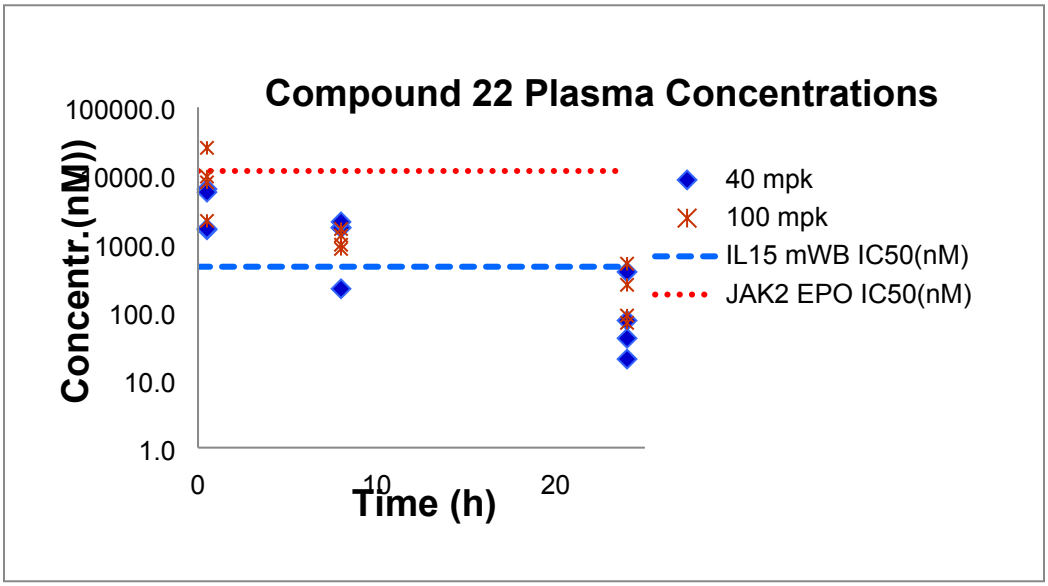
Whole Blood IFN-γ: The whole blood assay was carried out in 96-well flat bottom tissue cultureplates. Human whole blood was stimulated, in the presence or absence of test compounds, with 1

μg/mL soluble anti-CD3 (R&D Systems), 10 ng/mL anti-CD28 (R&D systems) and 10 ng/mL IL-2 (BD Biosciences). The final volume of the assay was 200 μL with 50% whole blood in RPMI 1640 (Gibco). The cells were incubated at 37 °C in 5% CO_2 in a humidified incubator for 18 h. The plates were centrifuged and supernatant was removed. IFN-γ levels in the supernatant were determined using a human IFN- γ ELISA set (BD Biosciences). Compounds were dissolved at 10 mM in dimethylsufoxide (DMSO) and tested at 6 concentrations in triplicate. IC_{50} values were derived by nonlinear regression analysis.

Collagen-induced arthritis in mice: DBA/1 male mice (8-10 wk of age; Harlan, n = 11–15/treatment group) were immunized subcutaneously at the base of the tail on Day 0 and again on Day 21 with 200 □g bovine type II collagen admixed with reconstituted Sigma Adjuvant System (SAS; Sigma-Aldrich). Mice were dosed daily (beginning on Day 21) by oral gavage with vehicle (100% PEG300) or compound. Following the booster immunization, mice were monitored 3 times per week for the development and severity of paw inflammation. Each paw was visually scored by the following scheme: +0 = normal. +1 = one (or more) joints inflamed on digits. +2 = mild-moderate inflammation of plantar surface of paw and paw thickness modestly increased. +3 = moderate-severe inflammation of plantar surface of paw and paw thickness significantly increased. +4 = ankylosis of ankle joint (significantly reduced hock joint motion on flexion/extension). Clinical paw scores for all four paws were summed for each mouse, and mean ± SEM was calculated for each treatment group.

For histological evaluation, rear paws were fixed, decalcified and embedded in paraffin. Sections were cut in the sagittal plane, stained in H & E and evaluated microscopically without knowledge of treatment group. Lesions were scored on a severity scale of 0 (normal) to 4 in two separate categories, inflammation (cellular infiltration and pannus formation) and bone resorption.

VII. Terminal plasma concentrations of compound 22 from CIA study



VIII. References

1. US patent #8,921,368
2. US patent #9,221,826
3. Buckley, J.; Webb, R. L.; Laird, T.; Ward, R. J. "Report on thermal reaction" *Chem. Eng. News*, **1982**, *60*,5.
4. Zanin-Zhorov, A.; Weiss, J.M.; Nyuydzefe, M. S.; Chen, W.; Scher, J. U.; Mo, R.; Depoil, D.; Rao, N.; Liu, B.; Wei, J.; Lucas, S.; Koslow, M.; Roche, M.; Schueller, O.; Weiss, S.; Poyurovski, M. V.; Tonra, J.; Hippen, K. L.; Dustin, M. L.; Blazar, B. R.; Liu, C-J.; Waksal, S. D. " Selective oral ROCK2 inhibitor down-regulates IL-21 and IL-17 secretion in human T cells via STAT3-dependent mechanism." *Proc. Nat. Acad Sci.* **2014**, *111*, 16814.
5. Schroeter, T.; Griffin, E.; Weiser, A.; Feng, Y. LoGrasso, P. "Detection of myosin light chain phosphorylation- A cell-based assay for screening Rho-kinase inhibitors." *Biochem. Biophys. Res. Comm.* **2008**, *374*, 356.