Experimental Details

HPLC Method A:

LC column: Xterra C_{18} 2.1 x 50 mm, 3.5 μM.

Gradient: 5-100% acetonitrile with 0.2% ammonium hydroxide in 7.0 min, then held at 100% acetonitrile for 1.0 min.

Column Temperature: 50 °C +/- 10 °C.

AS Temperature: ambient.

Flow Rate: 1 ml/min.

Sample: 5 μL.

HPLC Method B:

LC column: YMC C_{18} 2.0 x 50 mm, 3 μM.

Gradient: 5-100% 1:1 acetonitrile/water with 0.2% ammonium formate in 7.0 min, then held at 100% 1:1 acetonitrile/water for 1.0 min.

Column Temperature: 50 °C +/- 10 °C.

AS Temperature: ambient.

Flow Rate: 1 ml/min.

Sample: 5 μL.

Compounds **1** and **3** were purchased from Peakdale Fine Chemicals.

Representative procedure for the preparation of 4-quinoline-substituted pyrazoles: synthesis of 4-[3-(6-methylpyridin-2-yl)-1H-pyrazol-4-yl]quinoline (2).

To a solution of lepidine (1.4 g, 10 mmol) in tetrahydrofuran (20 mL) was added a solution of 1.0 M lithium bis(trimethylsilyl)amide in tetrahydrofuran (30 mL, 30 mmol). After stirring for 10 min the mixture was treated with a solution of 6-methylpyridine-2-

carboxylic acid methyl ester (1.7 g, 11 mmol) in tetrahydrofuran (10 mL). The mixture was stirred for 18 h, concentrated in vacuo, and diluted with saturated ammonium chloride solution. The solution was extracted with ethyl acetate and the organic layer washed once with saturated sodium chloride solution. The organic layer was dried (Hydromatrix™), treated with silica gel for 30 min, and filtered through Celite®. The solution was concentrated in vacuo and the intermediate ketone was dissolved in tetrahydrofuran (20 mL) and treated with N,N-dimethylformamide-dimethyl acetal (11 mL, 80 mmol). The reaction mixture was stirred for 48 h, concentrated in vacuo, and the residue concentrated in ethanol (20 mL) and treated with hydrazine monohydrate (11.6 mL, 240 mmol). The mixture was stirred for 18 h and concentrated in vacuo. Chromatography (silica gel, 50% ethyl acetate/49% hexanes/1% triethylamine) of the residue provided 572 mg (19%) of the title compound.

^{1}H NMR ($CDCl_3$): δ 8.94 (d, J = 4 Hz, 1H), 8.20 (d, J = 8 Hz, 1H), 7.79 (d, J = 8 Hz, 1H), 7.72 (m, 2H), 7.43 (m, 2H), 7.21 (t, J = 8 Hz, 1H), 6.97 (d, J = 8 Hz, 1H), 6.58 (d, J = 8 Hz, 1H), 2.53 (s, 3H); TOF MS ES+ exact mass calculated for $C_{18}H_{15}N_4$ (p + 1): m/z = 287.1297, Found: 287.1295; HPLC Method A: >99%; HPLC Method B: >99%.

Representative procedure for the preparation of 5-alkyl-4-quinoline-substituted pyrazoles: synthesis of 5-cyclopropyl-3-(pyridin-2-yl)-4-(quinolin-4-yl)-pyrazole (10). A solution of 2-(quinolin-4-yl)-1-(pyridin-2-yl)-ethanone (0.50 g, 2.01 mmol), cyclopropanecarboxylic acid hydrazide (0.20 g, 2.01 mmol), and concentrated hydrochloric acid (3 drops) in tetrahydrofuran (10 mL) was heated under nitrogen at 40 °C for 18 h. The precipitate formed was collected via vacuum filtration. The solid

hydrazone hydrochloride was dried in vacuo, then heated in an oil bath at 185 °C for 15 min. The resulting residue was partitioned between chloroform and aqueous sodium bicarbonate solution. The organic layer was dried (sodium sulfate), filtered, and concentrate in vacuo. Chromatography (silica gel, 2% methanol/chloroform plus a trace of concentrated ammonium hydroxide solution) of the residue afforded 100 mg (16%) of the title compound as an off-white solid.

^{1}H NMR ($CDCl_3$): δ 9.01 (d, J = 4 Hz, 1H), 8.55 (d, J = 4 Hz, 1H), 8.24 (d, J = 8 Hz, 1H), 7.79 (t, J = 8 Hz, 1H), 7.75 (td, J = 8, 2 Hz, 1H), 7.48 (m, 2H), 7.30 (td, J = 8, 2 Hz, 1H), 7.10 (ddd, J = 8, 7, 2 Hz, 1H), 6.64 (m, 1H), 1.45 (m, 1H), 0.94 (m, 2H), 0.75 (m, 2H); TOF MS ES+ exact mass calculated for $C_{20}H_{17}N_4$ (p + 1): m/z = 313.1453, Found: 313.1445; HPLC Method A: >99%; HPLC Method B: >99%.

Representative procedure for the preparation of 4-phenyl-substituted pyrazoles: synthesis of 3-(6-methylpyridin-2-yl)-4-(4-fluorophenyl)-1H-pyrazole (16).

2-(4-Fluorophenyl)-1-(6-methylpyridin-2-yl)-1-ethanone. A solution of methyl-4-fluorophenylacetate (2.5 g, 15 mmol), potassium t-butoxide (5.0 g, 45 mmol), and 6-methylpyridine-2-carboxylic acid methyl ester (2.3 g, 15 mmol) in tetrahydrofuran (75 mL) was heated at 65 °C for 48 h. The mixture was concentrated in vacuo and treated carefully with concentrated hydrochloric acid (10 mL). The resulting mixture was heated at 100 °C for 12 h. The mixture was cooled to room temperature and the pH adjusted to ~9 with 6 N sodium hydroxide solution. The mixture was extracted with ethyl acetate, and the organic layer dried (magnesium sulfate), filtered, and concentrated in vacuo.

Chromatography (silica gel, hexanes/ethyl acetate) of the residue provided 1.12 g (27%) of the title compound.

MS CI m/e 231 (M + 1).

3-(6-Methylpyridin-2-yl)-4-(4-fluorophenyl)-1H-pyrazole (16). A solution of 2-(4-fluorophenyl)-1-(6-methylpyridin-2-yl)-1-ethanone (1.12 g, 4.88 mmol) in tetrahydrofuran (20 mL) was treated with N,N-dimethyformamide-dimethylacetal (5.82 g, 48.8 mmol) and stirred for 40 h. The mixture was concentrated in vacuo, diluted with ethanol (10 mL), and treated with hydrazine monohydrate (7.43 g, 148 mmol). The resulting mixture was stirred for 18 h, concentrated in vacuo, diluted with ethyl acetate, and washed once with water. The aqueous layer was extracted once with a fresh portion of ethyl acetate. The combined organic layers were concentrated in vacuo. Chromatography (silica gel, hexanes/ethyl acetate to ethyl acetate methanol) of the residue provided 65 mg (5%) of the title compound.

^{1}H NMR ($CDCl_3$): δ 7.61 (s, 1H), 7.44 (t, J = 8 Hz, 1H), 7.33-7.37 (m, 2H), 7.02-7.10 (m, 4H), 2.58 (s, 3H); TOF MS ES+ exact mass calculated for $C_{15}H_{13}FN_3$ (p + 1): m/z = 254.1093, Found: 254.1090; HPLC Method A: >99%; HPLC Method B: >99%.

Representative procedure for the preparation of 5-methyl-substituted pyrazoles: 6-methyl-2-[4-(4-methoxyphenyl)-5-methyl-1H-pyrazol-3-yl]pyridine (20). To a solution of (4-methoxyphenyl)acetone (820 mg, 5.0 mmol) and 6-methyl-2-pyridinecarboxaldehyde (610 mg, 5.0 mmol) in toluene (15 mL) was added a 18/1/1 (v/v/v) solution of toluene/acetic acid/piperidine. The resulting mixture was heated at

100 °C for 48 h. The mixture was concentrated in vacuo, diluted with methylene chloride (20 mL), and treated with zinc triflate (240 mg) and polystyrene sulfonylhydrazide resin (2.7 g, Argonaut). The resulting mixture was placed on a shaker table and shaken for 7 days. The resin was washed with methanol and tetrahydrofuran. The resin was suspended in tetrahydrofuran (30 mL), treated with a solution of 1 M potassium t-butoxide in tetrahydrofuran (15 mL), and heated at 75 °C for 48 h. The mixture was cooled to room temperature, filtered, and the resin washed with tetrahydrofuran. The resulting solution was diluted with ethyl acetate and saturated aqueous sodium bicarbonate solution. The organic layer was separated, washed with saturated sodium chloride solution, dried (magnesium sulfate), filtered, and concentrated in vacuo. Chromatography (silica gel, ethyl acetate) of the residue provided 42 mg (3%) of the title compound.

^{1}H NMR ($CDCl_3$): δ 7.37 (t, J = 8 Hz, 1H), 7.25 (d, J = 8 Hz, 2H), 7.01 (d, J = 8 Hz, 1H), 6.99 (d, J = 9 Hz, 2H), 6.94 (d, J = 8 Hz, 1H), 3.88 (s, 3H), 2.58 (s, 3H), 2.23 (s, 3H); TOF MS ES+ exact mass calculated for $C_{17}H_{18}N_3O$ (p + 1): m/z = 280.1450, Found: 280.1431; HPLC Method A: >99%; HPLC Method B: >99%.

3-(3-Trifluoromethylphenyl)-4-quinolin-4-yl-pyrazole (4). ^{1}H NMR ($CDCl_3$): δ 9.14 (d, J = 4 Hz, 1H), 8.36-8.32 (m, 2H), 8.05-7.95 (m, 2H), 7.84 (t, J = 8 Hz, 1H), 7.75-7.62 (m, 3 H), 7.50-7.41 (m, 2 H); TOF MS ES+ exact mass calculated for $C_{19}H_{13}F_3N_3$ (p + 1): m/z = 340.1061, Found: 340.1064; HPLC Method A: >99%; HPLC Method B: >99%.

4-[3-(5-Fluoropyridin-2-yl)-1H-pyrazol-4-yl]quinoline (5). TOF MS ES+ exact mass calculated for $C_{17}H_{12}FN_4$ (p + 1): m/z = 291.1046, Found: 291.1040; HPLC Method A: >99%; HPLC Method B: 95%.

4-[3-(5-Chloropyridin-2-yl)-1H-pyrazol-4-yl]quinoline (6). ^{1}H NMR (DMSO-d_6): δ 13.70 (bs), 8.86 (d, J = 4 Hz, 1H), 8.23 (bs, 1H), 8.06 (m, 2H), 7.87 (dd, J = 8, 3 Hz, 1H), 7.70 (m, 3H), 7.43 (t, J = 7 Hz, 1H), 7.38 (d, J = 4 Hz, 1H); IR (KBr, cm^{-1}) 2759 (b), 1474, 1111, 845; TOF MS ES+ exact mass calculated for $C_{17}H_{12}ClN_4$ (p + 1): m/z = 307.0750, Found: 307.0738. Anal. Calcd for $C_{17}H_{11}ClN_4$: C, 66.56; H, 3.61; N, 18.26. Found: C, 66.56; H, 3.51; N, 18.11.

4-[3-(6-Bromopyridin-2-yl)-1H-pyrazol-4-yl]quinoline (7). TOF MS ES+ exact mass calculated for $C_{17}H_{12}BrN_4$ (p + 1): m/z = 351.0245, Found: 351.0241; HPLC Method A: >95%; HPLC Method B: >94%.

4-(4-Pyridin-2-yl-1H-pyrazol-3-yl)quinoline (8). ^{1}H NMR ($CDCl_3$): δ 8.94 (d, J = 4 Hz, 1H), 8.48 (d, J = 4 Hz, 1H), 8.37 (s, 1H), 8.24 (d, J = 8 Hz, 1H), 7.82 (d, J = 8 Hz, 1H), 7.71 (t, J = 8 Hz, 1H), 7.30-7.50 (m, 3H), 7.06 (t, J = 6 Hz, 1H), 6.81 (d, J = 8 Hz, 1H); IR (KBr, cm^{-1}) 3233 (b), 1589; TOF MS ES+ exact mass calculated for $C_{17}H_{13}N_4$ (p + 1): m/z = 273.1140, Found: 273.1130; HPLC Method A: >99%; HPLC Method B: >99%.

4-[5-Methyl-3-(6-methylpyridin-2-yl)-1H-pyrazol-4-yl]quinoline (9). ^{1}H NMR ($CDCl_3$): δ 9.01 (d, J = 4 Hz, 1H), 8.23 (d, J = 8 Hz, 1H), 7.75 (t, J = 8 Hz, 1H), 7.70 (d, J = 8 Hz, 1H), 7.46 (t, J = 8 Hz, 1H), 7.41 (d, J = 4 Hz, 1H), 7.19 (t, J = 8 Hz, 1H), 6.97 (d, J = 8 Hz, 1H), 6.47 (d, J = 8 Hz, 1H), 2.55 (s, 3H), 2.12 (s, 3H); TOF MS ES+ exact mass calculated for $C_{19}H_{17}N_4$ (p + 1): m/z = 301.1453, Found: 301.1451; HPLC Method A: >99%; HPLC Method B: >99%.

4-(3-Thiophen-2-yl-1H-pyrazol-4-yl)quinoline (11). ^{1}H NMR ($CDCl_3$): δ 9.18 (s, 1H), 9.13 (d, J = 5 Hz, 1H), 9.06 (d, J = 8 Hz, 1H), 8.15 (m, 2H), 7.97 (d, J = 5 Hz, 1H), 7.89 (t, J = 7 Hz, 1H), 7.39 (d, J = 5 Hz, 1H), 6.92, (dd, J = 4, 1 Hz, 1H), 6.79 (dd, J = 4, 1 Hz, 1H); TOF MS ES+ exact mass calculated for $C_{16}H_{12}N_3S$ (p + 1): m/z = 278.0752, Found: 278.0742. Anal. Calcd for $C_{16}H_{11}N_3S$: C, 69.29; H, 4.00; N, 15.15. Found: C, 69.25; H, 4.07; N, 15.10.

1-Benzyl-3-(2-pyridyl)-4-(4-quinolyl)pyrazole dihydrochloride (12). ^{1}H NMR (DMSO-d_6): δ 9.20 (d, J = 6 Hz, 1H), 8.56 (s, 1H), 8.44 (d, J = 8 Hz, 1H), 8.10 (m, 2H), 8.02 (d, J = 8 Hz, 1H), 7.90 (m, 3H), 7.75 (t, J = 7 Hz, 1H), 7.28-7.33 (m, 6H), 5.60 (s, 2H); TOF MS ES+ exact mass calculated for $C_{24}H_{19}N_4$ (p + 1): m/z = 363.1609, Found: 363.1601; HPLC Method A: >99%; HPLC Method B: >99%.

2-(4-Phenyl-1H-pyrazol-3-yl)pyridine (13). TOF MS ES+ exact mass calculated for $C_{14}H_{12}N_3$ (p + 1): m/z = 222.1031, Found: 222.1033; HPLC Method A: >99%; HPLC Method B: >99%.

2-[4-(4-Fluorophenyl)-1H-pyrazol-3-yl]pyridine (14). ^{1}H NMR ($CDCl_3$): δ 11.76 (bs, 1H), 8.65 (d, J = 5 Hz, 1H), 9.63 (s, 1H), 7.56 (dt, J = 8, 2 Hz, 1H), 7.39 (m, 2H), 7.31 (d, J = 8 Hz, 1H), 7.21 (m, 1H), 7.11 (t, J = 9 Hz, 2H); TOF MS ES+ exact mass calculated for $C_{14}H_{11}FN_3$ (p + 1): m/z = 240.0937, Found: 240.0939; HPLC Method A: >99%; HPLC Method B: >99%.

6-Methyl-2-(4-phenyl-1H-pyrazol-3-yl)pyridine (15). TOF MS ES+ exact mass calculated for $C_{15}H_{14}N_3$ (p + 1): m/z = 236.1187, Found: 236.1188; HPLC Method A: >99%; HPLC Method B: >99%.

6-Methyl-2-[4-(4-chlorophenyl)-1H-pyrazol-3-yl]pyridine (17). ^{1}H NMR ($CDCl_3$): δ 11.20 (bs, 1H), 7.61 (s, 1H), 7.45 (t, J = 8 Hz, 1H), 7.37 (m, 4H), 7.12 (d, J = 8 Hz, 1H), 7.07 (d, J = 8 Hz, 1H), 2.58 (s, 3H); TOF MS ES+ exact mass calculated for $C_{15}H_{13}ClN_3$ (p + 1): m/z = 270.0798, Found: 270.0801; HPLC Method A: >99%; HPLC Method B: >99%.

6-Methyl-2-[4-(4-hydroxyphenyl)-1H-pyrazol-3-yl]pyridine (18). ^{1}H NMR ($CDCl_3$): δ 7.55 (s, 1H), 7.39 (t, J = 8 Hz, 1H), 7.20 (dt, J = 8, 2 Hz, 2H), 7.16 (d, J = 8 Hz, 1H), 7.01 (d, J = 8 Hz, 1H), 6.87 (dt, J = 8, 2 Hz, 2H), 2.55 (s, 3H); TOF MS ES+ exact mass calculated for $C_{15}H_{14}N_3O$ (p + 1): m/z = 252.1137, Found: 252.1134; HPLC Method A: >99%; HPLC Method B: >99%.

6-Methyl-2-[4-(3,4-difluorophenyl)-1H-pyrazol-3-yl]pyridine (19). ^{1}H NMR ($CDCl_3$): δ 7.61 (s, 1H), 7.49 (t, J = 8 Hz, 1H), 7.10-7.30 (m, 4H), 7.08 (d, J = 8 Hz, 1H), 2.58 (s, 3H); TOF MS ES+ exact mass calculated for $C_{15}H_{12}F_2N_3$ (p + 1): m/z = 272.0999, Found: 272.0991); HPLC Method A: >99%; HPLC Method B: >99%.

In vitro assays:

Cell lines. Mv1Lu and NIH3T3 cells were obtained from ATCC and cultured as recommended. The Mv1Lu p3TP-Lux stable transfectant was generated by co-transfection with pPURO (Clontech), selection in 2 μg/ml puromycin and is maintained in 0.5 μg/ml puromycin. Recombinant human TGF-β was obtained from R&D Systems.

Enzyme assays. p38α (Activated form) was purchased from UBI. The reference compound for the p38 assay was SB203580 (IC_{50} = 59 nM, n = 1, literature K_i = 100 nM).[1] Based on repeat data of other compounds tested in this assay (n = 255), a 95% confidence interval for a single IC_{50} determination is 2.8-fold above and below the stated IC_{50}. The intracellular kinase domain of ALK5(T204D) TGF-β type I receptor was cloned and expressed in Sf9 cells by standard procedures. ALK5(T204D) kinase domain was purified from Sf9 cells on a single Ni-affinity column, eluted in a linear gradient of imidazole and desalted prior to protein concentration determination and freezing at –80 °C. Reactions were performed under optimized kinetic conditions with a final DMSO concentration of 4% as follows: p38α, 10 nM enzyme, 62.5 μM peptide substrate (KRELVEPLTPSGEAPNQALLR)[2] in 1X kinase buffer (Cell Signaling #9802), 1 μM microcystin, 100 μM ATP and 1 μCi ^{33}P-γ-ATP for 40 min at 30 °C. Reactions were

stopped with 10% phosphoric acid and captured on MAPH filter plates (Millipore). ALK5(T204D) autophosphorylation, 200 nM enzyme in 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 4 mM $MgCl_2$, 1 mM NaF, 2 mM β-mercaptoethanol, 4 uM ATP and 1 uCi ^{33}P-γ-ATP for 60 min at 30 °C. Reactions were stopped with 20% TCA. BSA was added to 250 μg/ml final concentration and captured on MAFB filter plates (Millipore).

Reporter and proliferation assays. Mv1Lu p3TP-Lux stable transfectants were plated in parallel into white Isoplates™ for the ^{3}H-thy assay or black Isoplates™ (Wallac) for the luciferase reporter assay at $1.5x10^4$ cells/well. After overnight attachment of the cells, media was changed to 0.5% FBS and compound dilutions were added (20 μM to 0.1 nM final concentration). After 2 h, TGF-β was added to 10 pM final concentration (0.25 ng/ml) for Mv1Lu cells and 5 pM for NIH3T3 cells for 24 h. In proliferation assays ^{3}H-thymidine (Amersham) was added for the final 6 hours. The ^{3}H-thy assays were harvested by rinsing once with PBS and adding 200 μL OptiPhase Supermix (Wallac). The p3TP-Lux assay was harvested by rinsing once with PBS and lysing cells in 20 μL passive lysis buffer (Promega) for 15 min before addition of 50 μL luciferase assay substrate (Promega). Both assays were quantitated on a Wallac MicrobetaJET.

References:

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2) Young, P. R.; McLaughlin, M. M.; Kumar, S.; Kassis, S.; Doyle, M. L.; McNulty, D.; Gallagher, T. F.; Fisher, S.; McDonnell, P. C.; Carr, S. A.; Huddleston, M. J.; Seibel, G.; Porter, T. G.; Livi, G. P.; Adams, J. L.; Lee, J. C. Pyridinyl imidazole inhibitors of p38 mitogen-activated protein kinase bind in the ATP site. *J. Biol. Chem.* **1997**, *272(18)*, 12116-21.