SUPPLEMENT

DRUG DISCOVERY; CHEMICAL BIOLOGY; BIOCHEMISTRY

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Structural Basis and Biological Consequences for JNK2/3 Isoform Selective Aminopyrazoles

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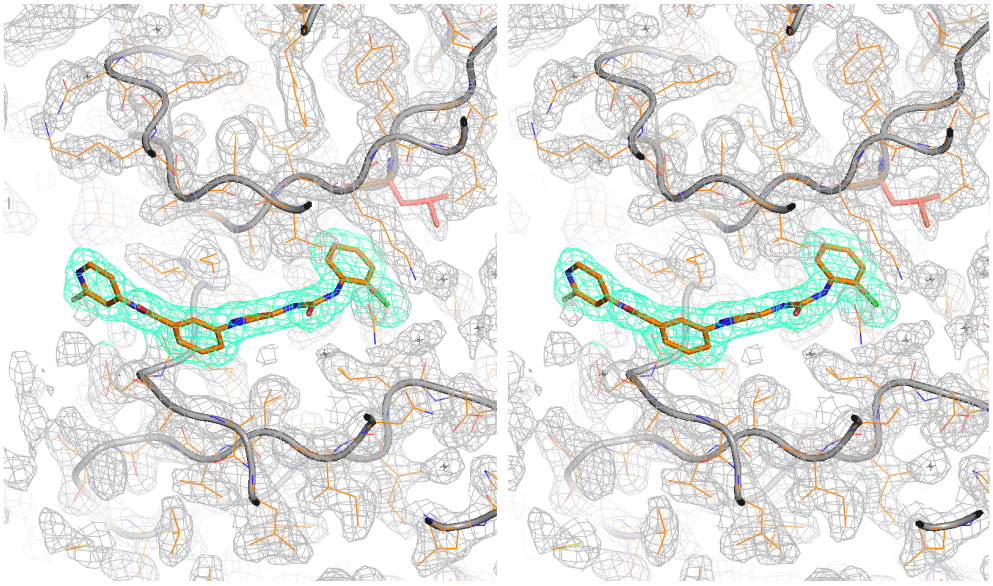
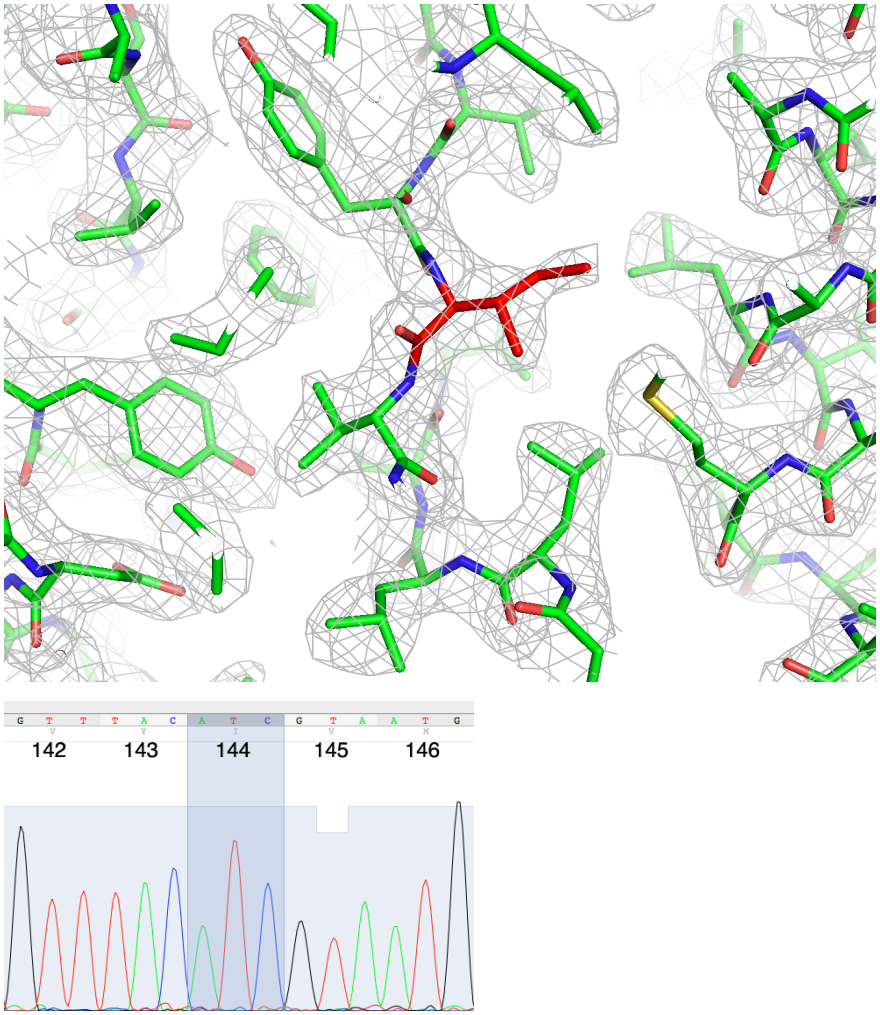
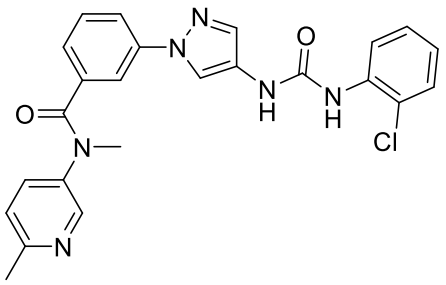
Supplemental Figure 1. Stereo view of 2Fo-Fc electron density map of JNK3 39-402 and SR-11165 complex. The electron density is contoured at 1.2σ. SR-11165 and L144 are shown in sticks. Map region corresponding to SR-11165 is highlighted in green.



Supplemental Figure 2. Crystal structure for JNK3 L144I solved at 2.4Å. The isoleucine side chain is shown in red. Electron density map (upper panel) and electropherogram of sequencing reaction (lower panel) are shown.



Supplemental Figure 3: Structure of SR-11404



Supplemental Table 1: Data collection and refinement statistics

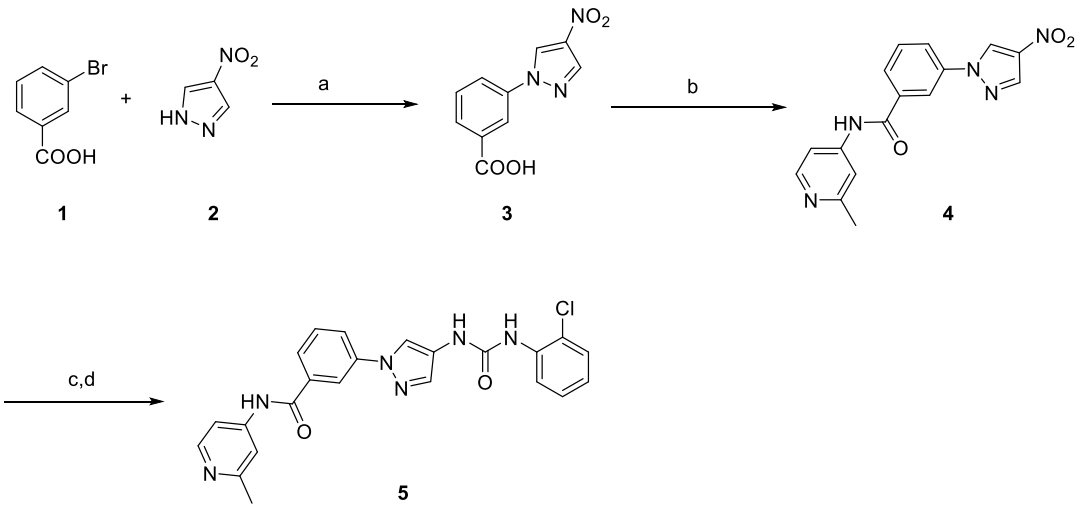
Crystal	JNK3-SR11165	JNK3-SR12326	JNK3-SR12327	JNK3-SR12130
Data collection	LS-CAT 21-ID-G	SSRL BL11-1	In-house	SSRL BL11-1
Space group	P $2_12_12_1$	P $2_12_12_1$	P $2_12_12_1$	P $2_12_12_1$
Unit cell dimensions				
a, *b*, *c* (Å)	52.02, 71.33, 107.90	52.36, 71.04, 107.8	52.5, 71.44, 107.6	53.30, 71.57, 107.55
α, β, γ (°)	90, 90, 90	90, 90, 90	90, 90, 90	90, 90, 90
Resolution (Å) [a]	75.08-2.01 (2.07-2.01)	42.94-1.9 (1.94-1.9)	47.19-2.65 (2.78-2.65)	59.58-2.21 (2.27-2.21)
Total measured reflections	120,918	234,702	50,706	137,862
Unique reflections	27,374	32,380	12,217	20,779
R_{merge} [%][a]	6.3 (49.3)	5.9 (50.2)	10.9 (49.6)	6.5 (27.7)
$I/\sigma(I)$ [a]	8.9 (2.3)	18.1 (3.7)	9.8 (2.4)	12.3 (5.7)
Completeness (%)[a]	99.9 (100)	99.8 (99.9)	99.4 (100)	97.9 (95.2)
Redundancy [a]	4.4 (4.5)	7.2 (7.3)	4.2 (4.3)	6.6 (5.8)
Refinement				
Resolution (Å) [a]	21.76-2.01 (2.1-2.01)	33.20-1.9 (1.96-1.9)	47.9-2.65 (2.9-2.65)	42.75-2.3 (2.44-2.3)
Number of reflections	23,665	32,321	12,151	18,463
R_{work} / R_{free} [a, b]	19.78/23.64 (20.97/26.88)	20.42/23.6 (25.11-23.67)	20.12/28.06 (22.94 /32.73)	19.41/25.65 (20.88/30.24)
Number of atoms				
Protein	2,838	2815	2,838	2838
Ligand	51	51	51	54
Solvent	305	159	40	151
Average *B*-factors				
Protein	44.9	46.54	54.96	69.16
Ligand	-	-	-	-
Solvent	-	-	-	-
R.m.s. deviations from ideal geometry				
Bond lengths (Å)	0.01	0.01	0.009	0.01
Bond angles (°)	1.18	1.09	1.15	1.17

[a] Values in parentheses are for the highest resolution shell.

[b] R_{free} was calculated as R_{work} using the 5% of reflections which were selected randomly and omitted from refinement.

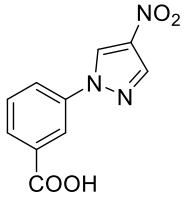
Supplemental Methods

Scheme 1. Synthesis of SR-11165



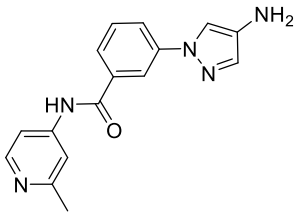
Reagents and Conditions: a. CuI, *trans-N,N*-dimethylcyclohexane-1,2-diamine, Cs_2CO_3, DMF, 100 ^oC; b. EDC, HOBt, DIEA, CH_2Cl_2, 25 ^oC; c. Pt/C, MeOH, H_2; d. 1-chloro-2-isocyanatobenzene, CH_2Cl_2, 25 ^oC.

3-(4-nitro-1H-pyrazol-1-yl)benzoic acid



A mixture of 4-nitro-1H-pyrazole (10 mmol), 3-bromobenzoic acid (20 mmol), CuI (2.0 mmol), *trans-N,N*-dimethylcyclohexane -1,2-diamine (4.0 mmol) and Cs_2CO_3 (30 mmol) in DMF (20 mL) was purged with argon and stirred for 12 h at 100 ^oC in a sealed tube. The reaction mixture was cooled, and filtered through a pad of silica gel and rinsed with EtOAc. The resulting solution was concentrated *in vacuo* to yield a crude residue which was purified by chromatography on silica gel (EtOAc/hexane) to provide 3-(4-nitro-1H-pyrazol-1-yl)benzoic acid, 75% yield. ^1H NMR (400 MHz, DMSO) δ 13.21 (s, 1H), 9.50 (s, 1H), 8.53 (s, 1H), 8.20 (dd, J = 8.3, 1.5 Hz, 1H), 8.06 (ddd, J = 8.2, 2.4, 1.0 Hz, 1H), 7.89 – 7.80 (m, 2H).

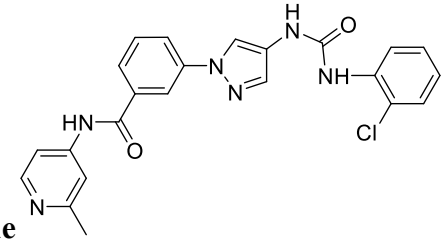
3-(4-amino-1H-pyrazol-1-yl)-N-(2-methylpyridin-4-yl)benzamide



A solution of 3-(4-nitro-1H-pyrazol-1-yl)benzoic acid (5.0 mmol) in CH_2Cl_2 (20 mL) was added EDC (10 mmol), HOBt (10 mmol) and diisopropyl ethyl amine (15 mmol) and stirred for 30 min. Then the 6-methylpyridin-3-amine (5.5 mmol) was added and the resulting mixture was stirred over night. Water (50 ml) was added to the reaction mixture and extracted with EtOAc (2x100 mL). The resulting solution was concentrated *in vacuo* to yield a crude product.

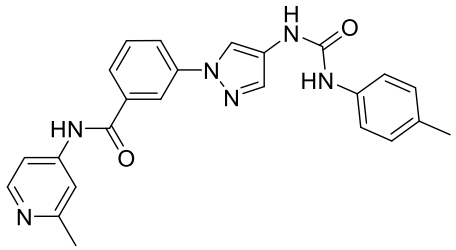
This intermediate was hydrogenated in anhydrous methanol (100 mL) in the presence of 5% Pt-C (1.0 g) under a balloon of hydrogen for 3 hour. The mixture was filtered through a Celite pad and evaporated. The residue was purified by chromatography on silica gel (dichloromethane/methanol) to give the 3-(4-amino-1H-pyrazol -1-yl)-N-(6-methylpyridin -3-yl)benzamide , 80% yield.

3-(4-(3-(2-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-(2-methylpyridin-4-yl)benzamide



1-chloro-2-isocyanatobenzene (0.12 mmol) was added to 3-(4-amino-1H-pyrazol -1-yl)-N-(6-methylpyridin-3-yl)benzamide (0.1 mmol) in CH_2Cl_2 (1.0 mL) at RT and stirred for 1 h. The solvent was evaporated and the residue was purified by reverse-phase preparative HPLC to give 3-(4-(3-(2-chlorophenyl) ureido)-1H-pyrazol-1-yl)-N-(6-methylpyridin-3-yl) benzamide, 70% yield. LC-MS: 447 (M + H). ^{1}H NMR (400 MHz, DMSO) δ 10.88 (s, 1H), 9.45 (s, 1H), 9.04 (s, 1H), 8.62 (s, 1H), 8.40 (s, 3H), 8.20 (dd, *J* = 8.3, 1.5 Hz, 1H), 8.09 (d, *J* = 8.2 Hz, 1H), 7.94 – 7.81 (m, 2H), 7.69 (t, *J* = 8.0 Hz, 1H), 7.63 (s, 1H), 7.47 (dd, *J* = 8.0, 1.4 Hz, 1H), 7.38 – 7.25 (m, 1H), 7.10 – 6.96 (m, 1H), 2.59 (s, 3H).

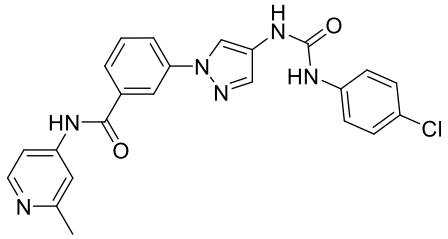
N-(2-methylpyridin-4-yl)-3-(4-(3-(p-tolyl)ureido)-1H-pyrazol-1-yl)benzamide



Procedures in **Scheme 1** were utilized to synthesize this compound SR-12130. LC-MS: 427 (M + H). ^{1}H NMR (400 MHz, DMSO) δ 11.43 (s, 1H), 8.72 (s, 1H), 8.70 – 8.61 (m,

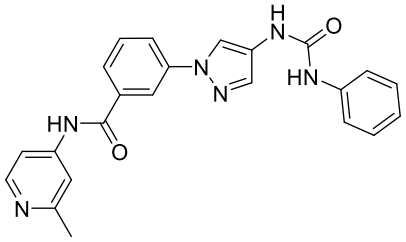
2H), 8.60 (s, 1H), 8.39 (s, 1H), 8.12 (d, *J* = 8.1 Hz, 2H), 8.05 (s, 1H), 7.88 (d, *J* = 7.8 Hz, 1H), 7.83 (s, 1H), 7.71 (t, *J* = 8.0 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 2H), 7.09 (d, *J* = 8.3 Hz, 2H), 2.67 (s, 3H), 2.25 (s, 3H).

3-(4-(3-(4-chlorophenyl)ureido)-1H-pyrazol-1-yl)-N-(2-methylpyridin-4-yl)benzamide



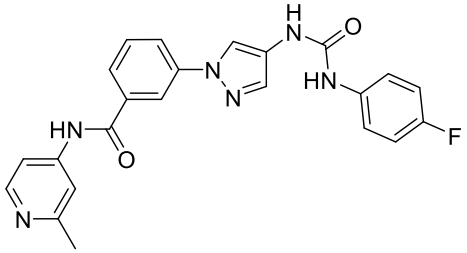
Procedures in **Scheme 1** were utilized to synthesize this compound SR-12103. LC-MS: 447 (M + H). ^{1}H NMR (400 MHz, DMSO) δ 11.43 (s, 1H), 9.04 (s, 1H), 8.82 (s, 1H), 8.63 (d, *J* = 6.6 Hz, 1H), 8.60 (s, 1H), 8.40 (d, *J* = 1.8 Hz, 1H), 8.15 – 8.10 (m, 2H), 8.05 (d, *J* = 5.3 Hz, 1H), 7.88 (d, *J* = 8.3 Hz, 1H), 7.84 (s, 1H), 7.71 (t, *J* = 8.0 Hz, 1H), 7.52 (d, *J* = 8.9 Hz, 2H), 7.37 – 7.31 (m, 2H), 2.67 (s, 3H).

N-(2-methylpyridin-4-yl)-3-(4-(3-phenylureido)-1H-pyrazol-1-yl)benzamide



Procedures in **Scheme 1** were utilized to synthesize this compound SR-12326. LC-MS: 413 (M + H). ^{1}H NMR (400 MHz, DMSO) δ 11.47 (s, 1H), 8.89 (s, 1H), 8.76 (s, 1H), 8.65 (d, *J* = 6.7 Hz, 1H), 8.61 (s, 1H), 8.40 (t, *J* = 1.8 Hz, 1H), 8.19 – 8.11 (m, 2H), 8.07 (d, *J* = 6.5 Hz, 1H), 7.88 (d, *J* = 8.3 Hz, 1H), 7.85 (s, 1H), 7.71 (t, *J* = 7.9 Hz, 1H), 7.49 (d, *J* = 7.7 Hz, 2H), 7.33 – 7.25 (m, 2H), 6.97 (t, *J* = 7.3 Hz, 1H), 2.68 (s, 3H).

3-(4-(3-(4-fluorophenyl)ureido)-1H-pyrazol-1-yl)-N-(2-methylpyridin-4-yl)benzamide



Procedures in **Scheme 1** were utilized to synthesize this compound SR-12327. LC-MS: 431 (M + H). ^{1}H NMR (400 MHz, DMSO) δ 11.44 (s, 1H), 8.95 (s, 1H), 8.80 (s, 1H), 8.64 (d, *J* = 6.7 Hz, 1H), 8.59 (s, 1H), 8.40 (t, *J* = 1.9 Hz, 1H), 8.15 – 8.10 (m, 2H), 8.05

(d, J = 6.5 Hz, 1H), 7.88 (d, J = 8.3 Hz, 1H), 7.84 (s, 1H), 7.71 (t, J = 8.0 Hz, 1H), 7.54 – 7.45 (m, 2H), 7.17 – 7.07 (m, 2H), 2.67 (s, 3H).