Supporting Information

Identification of *N*-{*cis*-3-[Methyl(7*H*-pyrrolo[2,3-*d*]pyrimidin-4-yl)amino]cyclobutyl}propane-1-sulfonamide (PF-04965842): A Selective JAK1 Clinical Candidate for the Treatment of Autoimmune Diseases

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Table of Contents

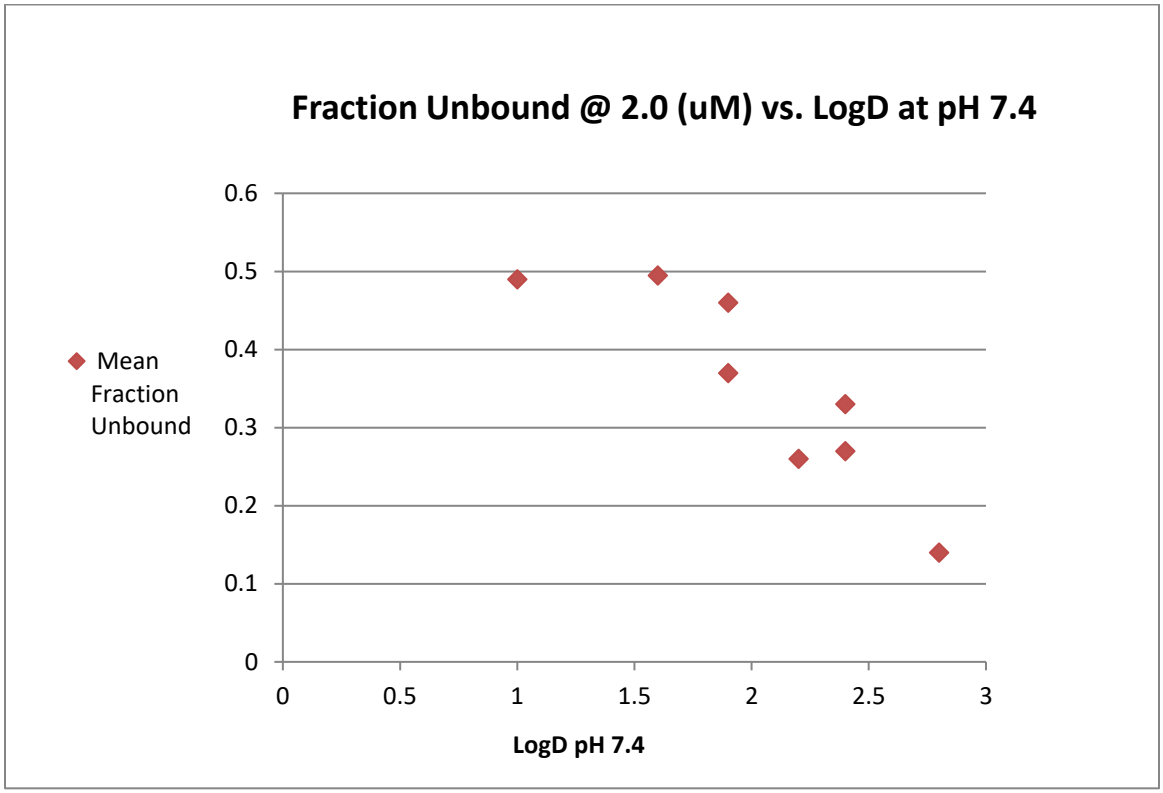
Table S1: Table of JAK3 and TYK2 activity for compounds **1-43**.

Compound	JAK3[a] IC50 (uM)	TYK2[a] IC50 (uM)	RRCK[b] Papp AB (10-6 cm/sec)
1	0.055	0.489	12.389
2	0.787	0.061	6.6
3	0.487	0.03	28.053
4	>10.00	0.266	16.841
5	>10.00	2.865	6.6
6	>10.00	>4.796	17.8
7	>10.00	0.479	24.079
8	>9.219	1.021	13.774
9	8.103	0.217	25.306
10	>10.00	6.518	18.563
11	>10.00	3.131	5.219
12	>10.00	2.715	9.173
13	>7.809	2.235	6.781
14	>10.00	1.782	9.649
15	>10.00	8.385	1.489
16	>10.00	1.352	22.107
17	>10.00	1.068	20.607
18	>10.00	1.124	18.647
19	>6.7	0.553	23
20	>10.0	0.263	24.3
21	>10.00	0.71	16.553
22	>10.00	0.736	22.083
23	>10.00	2.44	20.239
24	8.6	0.482	23.273
25	>10.00	1.253	16
26	>10.00	9.477	21.031

27	>10.00	1.32	18.679
28	>10.00	0.837	13.207
29	>10.00	1.873	11.377
30	>10.00	1.117	22.563
31	>10.00	1.694	10.552
32	>10.00	2.674	17.282
33	>10.00	5.569	8.163
34	>10.00	5.588	9.076
35	>10.00	0.819	4
36	>10.00	2.384	3
37	>10.00	0.935	15.201
38	>9.306	0.389	16.928
39	>10.00	0.841	17.386
40	>10.00	1.189	5.237
41	>10.00	2.017	24.141
42	>10.00	0.303	25.719
43	>10.00	0.616	32.613

[a]All compounds were assayed at least twice at 1 mM ATP and the IC_{50}'s averaged.

Figure S2: Plot of $LogD_{7.4}$ vs. mean fraction unbound.



Compound ID	LogD pH 7.4	Mean Fraction Unbound
4	2.8	0.14
19	2.2	0.26
27	2.4	0.27
7	2.4	0.33
8	1.9	0.37
25	1.9	0.46
36	1	0.49
28	1.6	0.50

Table S3: Results of the kinase selectivity panel for **25**.

Kinase (Invitrogen)	**% Inhibition**	**Dose [μM]**
ABL1	3.20 % (n=4)	1
STK6	19.8 % (n=4)	1
BTK	6.27 % (n=4)	1
CDK2/cyclinA	4.62 % (n=4)	1
CHEK1 (CHK1)	7.02 % (n=4)	1
CHEK2 (CHK2)	6.12 % (n=4)	1
SRC	17.3 % (n=4)	1
EGFR	12.3 % (n=4)	1
EPHA2	6.10 % (n=4)	1
FGFR1	5.84 % (n=4)	1
GSK3B	1.98 % (n=4)	1
INSR	8.24 % (n=4)	1
JAK3	60.6 % (n=4)	1
KDR	17.9 % (n=4)	1
LCK	4.59 % (n=4)	1
MAPK1 (ERK2)	2.02 % (n=4)	1
MAPKAPK2	-0.599 % (n=4)	1
MARK1	23.5 % (n=4)	1
MET	20.2 % (n=4)	1
STK3 (MST2)	8.55 % (n=4)	1
NEK2	-1.80 % (n=4)	1
PAK4	3.29 % (n=4)	1

PIM2	6.23 % (n=4)	1
PRKACA (PKACa)	25.9 % (n=4)	1
AKT1	3.61 % (n=4)	1
ROCK1	22.3 % (n=4)	1
SGK	-1.91 % (n=4)	1
TEK (Tie2)	-2.55 % (n=4)	1
NTRK1 (TRK-A)	30.2 % (n=4)	1
ZAP70	3.15 % (n=4)	1
TAOK2	3.39 % (n=4)	1
CAMK2A	10.1 % (n=4)	1
CSNK1A1 (CK1a1)	5.00 % (n=4)	1
CSNK2A2 (CK2a')	5.48 % (n=4)	1
MAP4K4	10.1 % (n=4)	1
MST4	-8.21 % (n=4)	1
MYLK2	7.68 % (n=4)	1
PRKCB2	7.93 % (n=4)	1
PDK1	12.5 % (n=4)	1
p38	-0.235 % (n=4)	1

The inhibitory activity of **25** was assessed against a panel of 40 kinases from the Invitrogen panel at a concentration of 1 μM of compound and an ATP concentration around the K_m value for each kinase.

Table S4: Mean (± SD) Pharmacokinetic Parameters of **25** in Mice, Rats, Monkeys, and Dogs Following a Single Intravenous Administration.

Species	Sex/N	Formulation	Dose (mg/kg)	CL (mL/min/kg)	Vss (L/kg)	t1/2 (h)	AUC(0-∞) (ng•h/mL)
Mouse (C57/B6)	M/3	5% DMSO/95% of 30% SBECD	3	188 ± 17	1.4 ± 0.1	0.2 ± 0.08	268 ± 26
Rat (Sprague-Dawley)	M/2	5% DMSO/95% of 30% SBECD	1	27	1.0	0.8	632
Monkey (Cynomolgus)	M/1	10% SBECD	1	29	0.7	0.5	574
	F/1	10% SBECD	1	32	1.0	0.6	522
Dog (Beagle)	M/2	5% DMSO/95% of 20% SBECD	1	4	1.2	4.0	4140

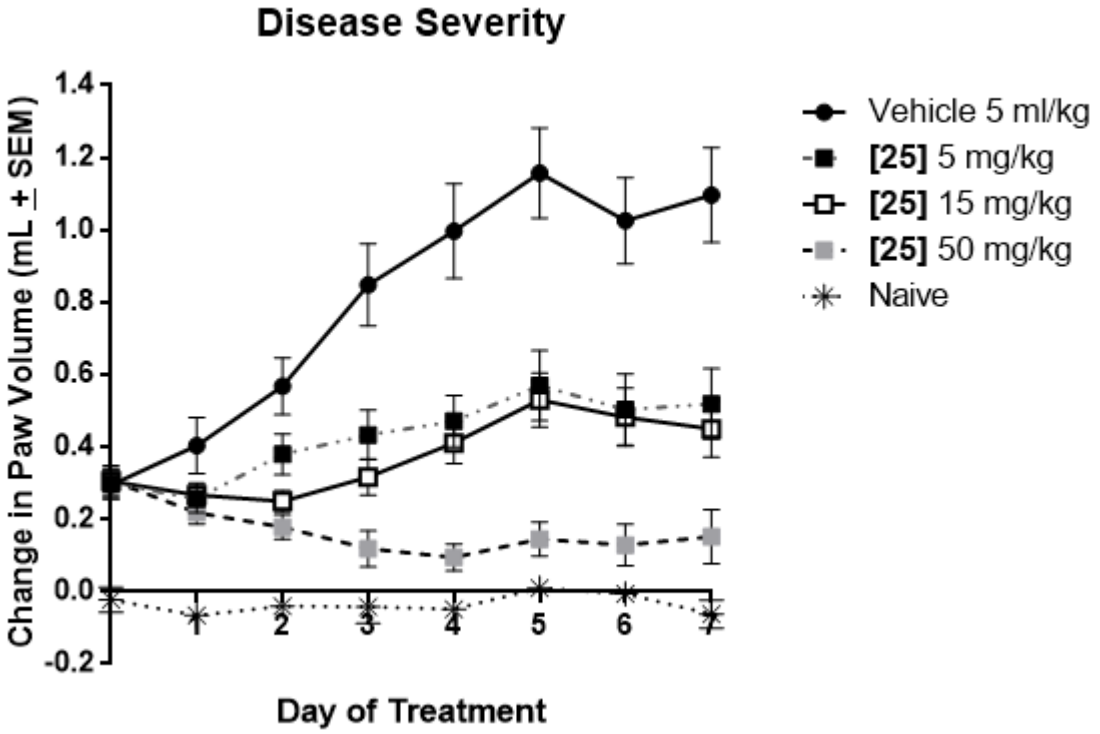
SD = Standard deviation; CL = Systemic plasma clearance; Vss = Apparent volume of distribution at steady state; t1/2 = Apparent terminal elimination half-life; $AUC_{(0-\infty)}$ = Area under the concentration-time curve from time 0 to infinity; M = Male F = Female; DMSO = Dimethylsulfoxide; SBECD = Sulfobutylether-β-cylcodextrin.

Table S5: Mean ± SD Pharmacokinetic Parameters of **25** in Mice, Rats, Monkeys, and Dogs Following a Single Oral Administration.

Species	Sex/N	Dose (mg/kg)	Formulation	C_{max} (ng/mL)	Tmax (h)	$AUC_{(0-\infty)}$ (ng•h/mL)	t1/2 (h)	F (%)
Mouse (C57/B6)	M/3	30	0.5% MC	1090 ± 194	1.2 ± 0.3	755 ± 58	1.2 ± 0.3	28 ± 0
Rat (Sprague-Dawley)	M/2	3	0.5% MC	849	0.5	1810	1.1	96
Monkey (Cynomolgus)	M/1	3	0.5% MC/ 0.1% Polysorbate 80	137	0.5	166	0.5	10
	F/1	3	0.5% MC/ 0.1% Polysorbate 80	95	0.5	160	1.1	10
Dog (Beagle)	M/2	3	0.5% MC/ 0.1% Tween 80	1080	1.5	8100	4.1	65

SD = Standard deviation; N = Number of animals; C_{max} = Maximum plasma concentration; Tmax = Time to reach C_{max}; $AUC_{(0-\infty)}$ = Area under the concentration-time curve from time 0 to infinity; t1/2 = Apparent terminal elimination half-life; F = Bioavailability; M = Male; ND = Not determined; F = Female; MC = methylcellulose.

Figure S3 : Initial range finding rat AIA study with **25** at doses of 5, 15, and 50 mpk.



Immunized rats were enrolled into groups and dosed with either: 50, 15, or 5 mg/kg of **25** or vehicle PO, QD. (a) Compared to vehicle, treatment with **25** significantly reduced disease severity measured by plethysmograph in the 50 mg/kg (Day 1-7), 15 mg/kg (Day 2-7), and 5 mg/kg (Day 3-7) dose groups. (b) On Day 7 post treatment, hind paw measurements show a significant reduction of disease for all treatments compared to vehicle control.

Table S6. Cellular activity in human whole blood (HWB) for compounds **19**, **25**, **35**, **36**.

Compound	19	25	35	36
IFNα pSTAT3 IC50 (uM)	0.155	0.189	0.216	0.160
IFNγ pSTAT1 IC50 (uM)	0.433	0.163	ND	0.192
CD3+ IL6 pSTAT1 IC50 (uM)	ND	0.343	0.573	0.439
CD14+ IL6 pSTAT1 IC50 (uM)	ND	0.163	0.271	0.312
IL10 pSTAT3 IC50 (uM)	4.627	2.511	ND	2.025
CD8+ IL15 pSTAT5 IC50 (uM)	ND	1.298	2.265	ND
IL-21 pSTAT3 IC50 (uM)	ND	0.511	ND	0.356
IL27 pSTAT3 IC50 (uM)	ND	0.271	ND	0.242
IL12 pSTAT4 IC50 (uM)	3.915	13.673	ND	>20.000
IL23 pSTAT3 IC50 (uM)	17.764	>16.452	ND	ND
CD34+ pSTAT5 IC50 (uM)	4.089	7.178	>6.934	6.811