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Discovery of Potent and Highly Selective Thienopyridine Janus Kinase 2 Inhibitors

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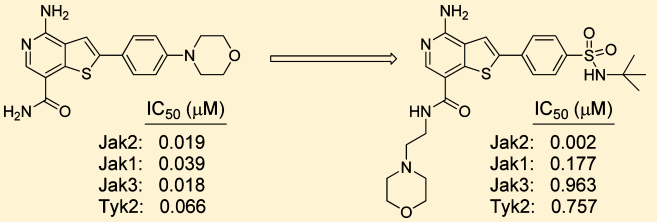
Supporting Information

ABSTRACT: Developing Janus kinase 2 (Jak2) inhibitors has become a significant focus for small molecule drug discovery programs in recent years due to the identification of a Jak2 gain-of-function mutation in the majority of patients with myeloproliferative disorders (MPD). Here, we describe the discovery of a thienopyridine series of Jak2 inhibitors that culminates with compounds showing 100- to >500-fold selectivity over the related Jak family kinases in enzyme assays. Selectivity for Jak2 was also observed in TEL-Jak cellular assays, as well as in cytokine-stimulated peripheral blood mononuclear cell (PBMC) and whole blood assays. X-ray cocrystal structures of **8** and **19** bound to the Jak2 kinase domain aided structure−activity relationship efforts and, along with a previously reported small molecule X-ray cocrystal structure of the Jak1 kinase domain, provided structural rationale for the observed high levels of Jak2 selectivity.



INTRODUCTION

The Janus kinases (Jak) are members of a family of intracellular tyrosine kinases that play important roles in cytokine receptor-mediated signal transduction via activation of downstream signal transducers and activators of transcription (STAT), phosphatidylinositol 3-kinase (PI3K), and mitogen-activated protein kinase (MAPK) pathways. There are four kinases in the Jak family [Jak1, Jak2, Jak3, and tyrosine kinase 2 (Tyk2)], and Jak2 has emerged in recent years as a potential therapeutic target. A significant proportion of patients with myeloproliferative disorders (MPD), which include polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (MF), have been shown to carry a mutation in the pseudokinase domain of Jak2 (V617F), which renders the kinase constitutively active and suggests that inhibition of Jak2 may be an effective approach for the treatment of MPD.[1] Several inhibitors of Jak family kinases showing efficacy in both preclinical models and in the clinic have been reported.[2] However, because a chronic dosing regimen may be anticipated for the treatment of PV and ET patients, an inhibitor that is selective for Jak2 over the broader kinome and the other Jak family kinases may be desirable to avoid unwanted immunosuppression and other off-target side effects. The discovery of Jak2 inhibitors having Jak family selectivity has posed a significant challenge due to high homology in the adenosine triphosphate (ATP) binding pocket among the Jak family kinases, although amino acid differences do exist,[3] and compounds with varying levels of Jak2 selectivity have been reported.[2c,e,4] Planning to exploit the subtle sequence differences in the active sites of the Jak family kinases via a combination of structure−activity relationships (SAR) and structure-based design, we initiated a program to develop highly selective Jak2 inhibitors. Here, we describe a series of potent thienopyridine Jak2 inhibitors that demonstrated high Jak family selectivity in enzyme and cell assays. X-ray cocrystal structures of small molecules bound to the Jak2 and Jak1 kinase domains helped guide SAR efforts and enabled the formation of hypotheses for the observed high selectivity.

CHEMISTRY

All thienopyridine final compounds were prepared from key intermediate **1** (Scheme 1).[5] The primary amide **3** was synthesized via hydrolysis of **1** with sulfuric acid at room temperature to provide iodide **2**, followed by Suzuki reaction with 4-morpholinophenylboronic acid. Treatment of **1** with concentrated HCl at 95 °C provided in good yield the carboxylic acid intermediate **4**, from which analogues bearing diverse amide and aryl group substitutions could be accessed. Secondary amides **8**−**13** and **21**−**26** were prepared via cross-coupling of **4** with either 4-morpholinophenylboronic acid

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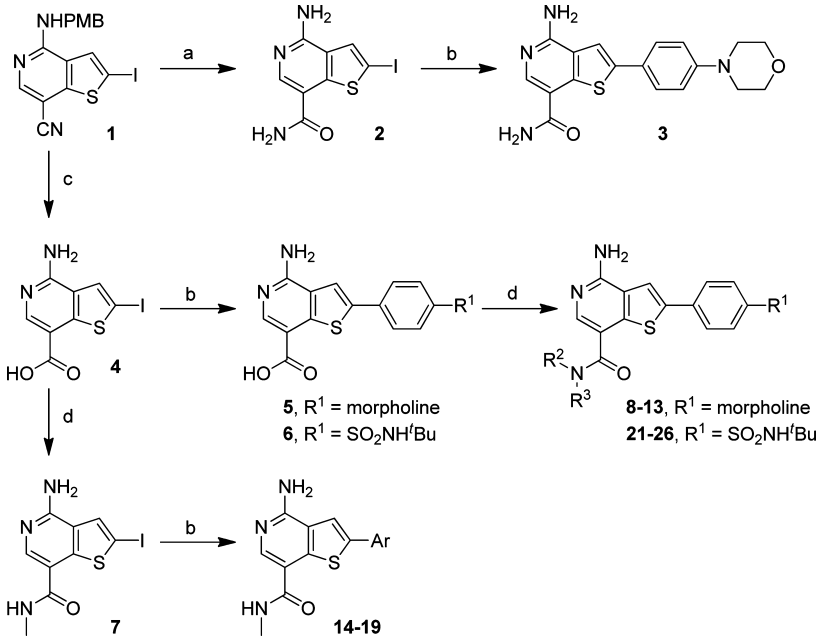
Scheme 1. Synthesis of Thienopyridines[a]



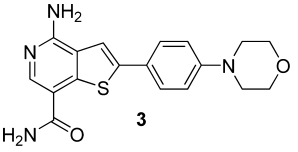
[a]Reagents and conditions: (a) H_2SO_4, 23 °C, 84%. (b) $Pd(PPh_3)_4$, aqueous Na_2CO_3, $ArB(OH)_2$, DMF, 100 °C, or $Pd(dppf)Cl_2$, aqueous Na_2CO_3, $ArB(OH)_2$, dioxane, 100 °C, 10–93%. (c) Concentrated HCl, 95 °C, 84%. (d) EDC-HCl, HOBt hydrate, Hunig's base, R^2R^3NH, DMF, 25–58%.

or 4-*tert*-butylsulfonamidophenylboronic acid to give intermediates **5** and **6**, respectively, followed by amide couplings with the appropriate amines using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide (EDC)/*N*-hydroxybenzotriazole (HOBt). Conversion of **4** to the methyl amide intermediate **7** followed by Suzuki reaction with the appropriate aryl boronic acid furnished compounds **14**–**19**.

RESULTS AND DISCUSSION

To identify suitable chemical matter from which selective Jak2 inhibitors could be developed, a high-throughput screen (HTS) of Amgen's compound collection was performed using a Jak2 enzymatic assay. Thienopyridine **3** was a representative primary amide hit (Table 1). Although it showed good potency in the Jak2 enzyme assay, **3** showed little selectivity over Jak1, Jak3, and Tyk2. Compound **3** was tested at 1 μM against a custom panel of 100 human kinases and had promising kinome selectivity, as only seven other kinases showed >90% inhibition (Table 1).[6]

Table 1. HTS Hit 3 in Jak Family and Other Kinase Assays



Jak family kinases[a,b]		other kinases[b]	
kinase	IC_{50} (μM)	kinase	inhibition (%)
Jak2	0.019	Jak2	96
Jak1	0.039	Tyk2, YSK4, TAK1, KIT, MEK5, GAK, IRAK4	>90
Jak3	0.018	8 kinases	70–85
Tyk2	0.066	84 kinases	<70

[a]Measured at the apparent ATP K_m.[7] [b]Each value represents the average of ≥2 independent experiments, where each experiment consisted of a single determination.

Because of its kinome selectivity and the modular nature of its synthesis, compound **3** was selected for additional studies. Analogues were prepared to determine if changes to either side of the molecule would impact Jak family selectivity. Efforts to establish SAR around the thienopyridine core began with preparation of secondary amides **8**–**13** (Table 2). The methyl-, ethyl-, phenyl-, and benzyl-amides **8**–**11** were only moderately less potent than **3** in a Jak2 enzyme assay, while the sterically bulky *tert*-butyl- and *N*,*N*-dimethyl amides **12** and **13** were poorly tolerated. Compounds **8**–**11** also showed significantly improved Jak family selectivity profiles, particularly over Jak3 and Tyk2. Methyl amide **8** was noteworthy, as it showed 43-fold selectivity against both Jak3 and Tyk2 and 7-fold selectivity against Jak1.

An X-ray cocrystal structure of thienopyridine **8** with the kinase domain of Jak2 was solved (Figure 1a). Compound **8** binds Jak2 in the ATP pocket and makes several key interactions with the protein. The pyridine nitrogen forms a hydrogen bond with the backbone NH of hinge residue Leu932, while the amide NH in **8** forms an additional hydrogen bond with the carbonyl of Leu932. The NH_2 of the aminopyridine moiety binds the protein through two hydrogen bonds, one with the backbone carbonyl of Glu930 along the hinge and the other through a water-mediated hydrogen bond to the backbone carbonyl of Gly993. Gly993 is the residue immediately preceding the conserved DFG motif and is present in Jak2, Jak1, and Tyk2 but is instead an alanine in Jak3, which results in a unique conformation at this site in Jak3 relative to the other three Jak family members.[7] This conformational difference does not readily explain the selectivity gains observed over Jak3, as the relatively unselective screening hit **3** should share the same hydrogen-bonding pattern as **8**. Jak1 and Tyk2 have sequences that are identical with Jak2 in the DFG region,

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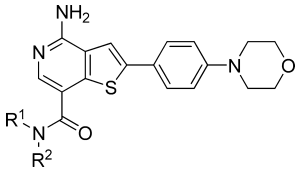
Table 2. Data in Jak Enzymatic Assays for 3 and 8−13



			enzyme: IC_{50} (μM) (selectivity vs Jak2, x-fold)[a]			
no.	R^1	R^2	Jak2	Jak1	Jak3	Tyk2
3	H	H	0.019	0.039 (2)	0.018 (1)	0.066 (3)
8	H	Me	0.031	0.211 (7)	1.34 (43)	1.33 (43)
9	H	Et	0.048	0.133 (3)	0.967 (20)	1.07 (22)
10	H	Ph	0.072	0.406 (6)	2.87 (40)	1.99 (28)
11	H	CH_2Ph	0.100	0.307 (3)	>125 (>250)	2.60 (26)
12	H	^{t}Bu	1.17	8.82 (8)	10.3 (9)	6.98 (6)
13	Me	Me	1.17	3.10 (3)	1.66 (1)	5.15 (4)

[a]Measured at the apparent ATP K_m.[7] Each IC_{50} value represents the average of ≥2 independent experiments, where each experiment consisted of a single determination.

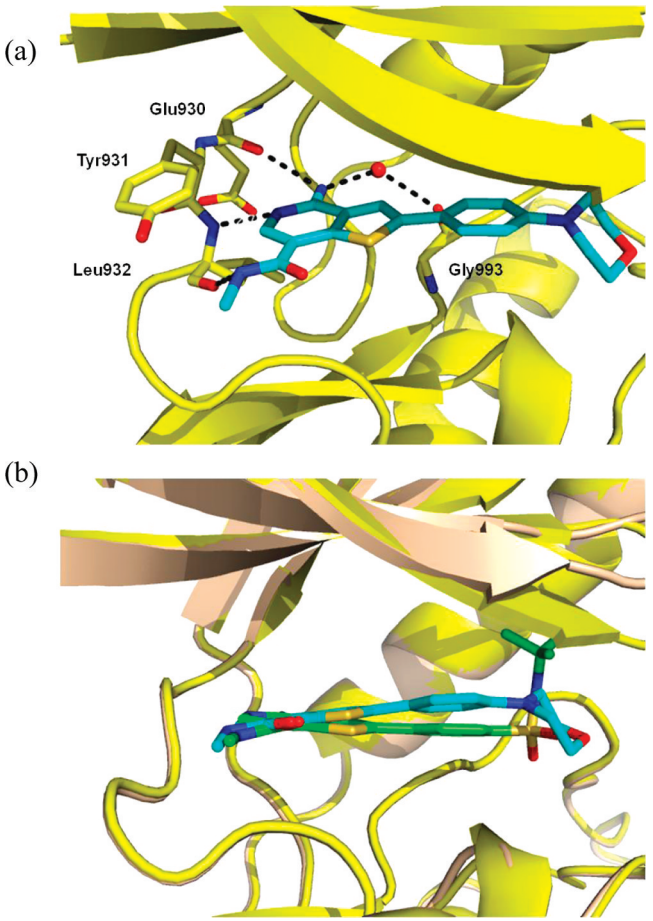


Figure 1. (a) X-ray cocrystal structure (PDB ID: 3TJC) of **8** bound to the ATP pocket of the Jak2 kinase domain at 2.4 Å resolution. (b) Overlay of the X-ray cocrystal structures of **8** (blue) at 2.4 Å resolution and **19** (green) (PDB ID: 3TJD) at 2.9 Å resolution bound to the ATP pocket of Jak2 (yellow for **8**/Jak2 and tan for **19**/Jak2).

so the selectivity of **8** for Jak2 over Jak1 and Tyk2 also cannot be rationalized by the observed hydrogen-bonding interactions.

Although a structural rationale for the improved selectivity profile of **8** for Jak2 was not readily apparent, SAR studies were conducted around the 4-morpholinophenyl moiety, which occupies a pocket beneath the conserved glycine-rich loop. Replacement of the morpholine group with a hydrogen atom provided compound **14**, which was slightly less potent against Jak2 and showed diminished selectivity over Jak3 and Tyk2 relative to **8** (Table 3). This suggested that 4-substitution of the phenyl ring may enhance both Jak2 potency and selectivity, and a set of compounds containing a range of 4-substituents was prepared (Table 3).

Compounds **3**, **8**, and **14** were also tested in a panel of high-throughput Jak family cellular assays consisting of Ba/F3 cell lines expressing translocated ETS leukemia (TEL) fusions of each Jak kinase domain, which confer constitutive kinase activity.[8] Whereas **8** was relatively potent (IC_{50} = 0.031 μM) in the Jak2 enzyme assay and showed promising Jak family selectivity, a significant enzyme to cell shift was observed (IC_{50} = 3.94 μM, 127-fold) in the TEL-Jak2 cell assay. Compounds **3** and **14** also showed large potency shifts in the TEL-Jak2 cell assay. Discrepancies between enzyme and cell assay data have been well-documented for several kinase targets,[9] and large enzyme to cell shifts have also been reported for other Jak kinase inhibitors.[4b−d] Numerous factors, including cell permeability and efflux, may play a role in enzyme to cell assay shifts. The data for a subset of thienopyridines show that the majority of the compounds have high average apparent permeability and are likely efflux substrates.[10] Despite the potency shifts observed between enzyme and cell assays, further development of thienopyridine SAR was expedited by the TEL-Jak panel, which provided a facile method to rapidly assess cellular potency and selectivity for all analogues. Importantly, these assays enabled us to study relationships between selectivity in enzyme assays and selectivity in cells for each of the individual Jak family kinases. The enzyme and TEL-Jak cell data for compound **8** illustrated a general trend that was present across the thienopyridine and several other series.[11] While enzyme to cell shifts existed for all of the Jak kinases, the magnitude of the shift for each kinase was different, with Jak1 showing a significantly smaller shift than Jak2. Thus **8**, which showed 7-fold selectivity for Jak2 over Jak1 in enzyme assays, showed no selectivity for Jak2 over Jak1 in cells. On the basis of these data, we hypothesized that achieving a higher threshold of selectivity over Jak1 in enzyme assays than that observed for **8** may be required to obtain significant selectivity over Jak1 in the TEL-Jak cell assay.

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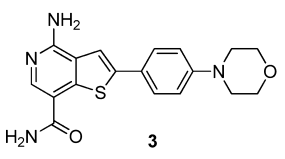
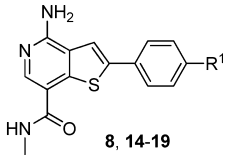
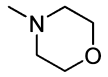
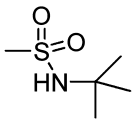
Table 3. Jak Family Enzyme and Cellular Data for 3, 8, and 14−19



3



8, 14-19

#	R^1	Enzyme: IC_{50}, μM [a] (*Selectivity vs. Jak2, x-fold*)				TEL-Jak Cell: IC_{50}, μM [b] (*Selectivity vs. Jak2, x-fold*)			
		Jak2	Jak1	Jak3	Tyk2	Jak2	Jak1	Jak3	Tyk2
3		0.019	0.039	0.018	0.066	>25	0.748 (<1)	>25	>25
8		0.031	0.211 (*7*)	1.34 (*43*)	1.33 (*43*)	3.94	1.81 (*<1*)	>25 (*>6*)	>25 (*>6*)
14	H	0.058	0.290 (*5*)	0.767 (*13*)	0.937 (*16*)	*71%* [c]	*52%* [c]	>25	>25
15	Me	0.027	0.377 (*14*)	0.545 (*20*)	0.888 (*33*)	12.6	>25 (>2)	>25 (>2)	>25 (>2)
16	^{t}Bu	0.010	0.039 (*4*)	0.188 (*19*)	0.202 (*21*)	*59%* [c]	*60%* [c]	>25	>25
17	OCF_3	0.012	0.070 (*6*)	0.365 (*30*)	0.208 (*17*)	2.89	6.85 (*2*)	>25 (*>9*)	>25 (*>9*)
18		0.004	0.036 (*9*)	0.118 (*30*)	0.145 (*36*)	1.37	1.16 (*1*)	>25 (*>18*)	>25 (*>18*)
19		0.001	0.030 (*30*)	0.307 (*307*)	0.266 (*266*)	1.01	1.09 (*1*)	>25 (*>25*)	>25 (*>25*)

[a]Measured at the apparent ATP K_m.[7] Each IC_{50} value represents the average of ≥2 independent experiments, where each experiment consisted of a single determination. [b]Each IC_{50} value represents the average of ≥2 independent experiments, where each experiment consisted of two replicates. [c]Percent inhibition at 25 μM. The IC_{50} could not be calculated because complete inhibition was not achieved in the concentration range tested.

SAR studies at the 4-position of the phenyl ring continued with replacement of the morpholine ring in **8** with a methyl group (**15**) or a *tert*-butyl group (**16**), which provided compounds having comparable Jak2 potency but with slightly diminished selectivity over Jak3 and Tyk2 (Table 3). Trifluoromethoxy substitution (**17**) was equipotent with *tert*-butyl substitution (**16**) in the Jak2 enzyme assay; yet, it was also active in the TEL-Jak2 assay with a similar selectivity profile to **8** in cell assays. While nitrile **18** and sulfonamide **19** achieved additional improvements in enzyme and cellular potency, the bulkier *tert*-butylsulfonamide (**19**) provided a significant breakthrough in Jak2 enzyme selectivity. Compound **19** was potent against Jak2 (IC_{50} = 0.001 μM) and was 30-fold selective over Jak1 and approximately 200-fold selective over both Jak3 and Tyk2 in enzyme assays. Although it showed improved selectivity over Jak1 enzyme, Jak1 cell selectivity in the TEL-Jak assays was not achieved. Compound **19** appeared comparable to **18** in terms of cellular potency and selectivity, but its relatively high selectivity for Jak2 in enzymatic assays warranted further investigation.

An X-ray cocrystal structure of sulfonamide **19** in the kinase domain of Jak2 was solved, and an overlay with the Jak2/**8** structure is shown in Figure 1b. Compound **19** binds Jak2 in the same orientation as **8** in the ATP pocket and forms the same set of key hydrogen bonds with the protein. The heteroatoms of the sulfonamide do not form any additional hydrogen bonds with Jak2. Comparison of the structures of **8** and **19** suggests that the improved potency, Jak3 selectivity, and Tyk2 selectivity of **19** may be due to the orientation of the *tert*-butanesulfonamide moiety beneath the glycine-rich loop, which is shifted in the Jak2/**19** structure to accommodate the bulky *tert*-butyl group. This movement of the glycine-rich loop, coupled with the shift of the phenylsulfonamide portion of **19** downward in the active site versus that of **8**, may be better tolerated in Jak2 and Jak1 than in Jak3 and Tyk2.

In an effort to better understand and improve selectivity over Jak1, a study of the Jak1 active site was undertaken. Data from two published[3] Jak1 X-ray cocrystal structures showed that the ATP pocket of Jak1 is smaller than that of Jak2. The average distance between the two Jak1 structures, measured from Pro960 of the hinge region to Asp1021 of the DFG motif, is 14.9 ± 0.1 Å, while the same distance in the Jak2/**19** structure is 15.4 ± 0.1 Å. An overlay of the published cocrystal structure of the pan-Jak inhibitor CP-690550[2a] (**20**) with the Jak1 kinase domain and the Jak2/**19** structure (Figure 2) illustrates the subtle difference in active site size between Jak1 and Jak2. These data suggested that increased steric bulk along the hinge region might be better tolerated in Jak2 than Jak1 and could

dx.doi.org/10.1021/jm200911r|J. Med. Chem. 2011, 54, 8440−8450

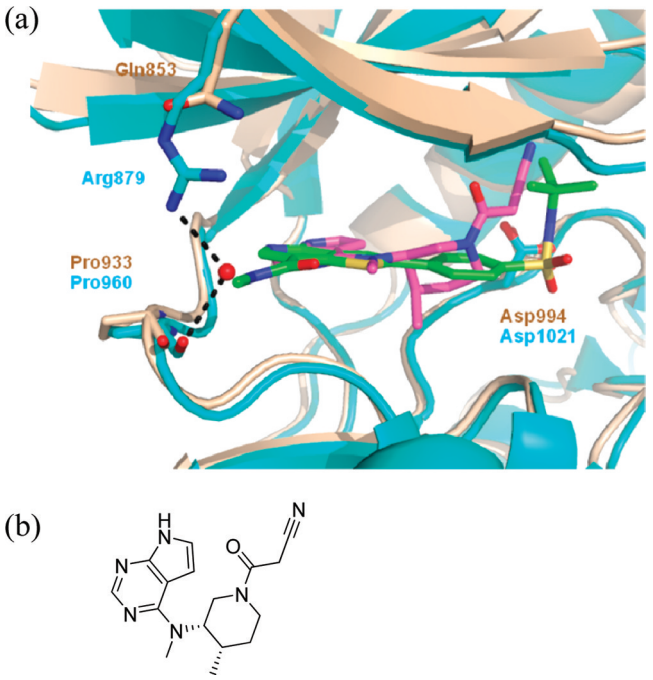


Figure 2. (a) Overlay of the X-ray cocrystal structures of **19** (PDB ID: 3TJD) bound to Jak2 (tan) and **20** (PDB ID: 3EYG) bound to Jak1 (blue). (b) Structure of **20**.

therefore further improve selectivity over Jak1. This hypothesis is consistent with the observed increase in Jak1 selectivity upon replacement of the primary amide in **3** with the methyl amide in **8**. Further increasing bulk in this region also had the potential to exploit an active site residue difference between Jak1 (Arg879) and Jak2 (Gln853) (Figure 2a). Arg879 of Jak1 engages in a water-mediated hydrogen bond with the hinge Pro960. The pyrrolopyrimidine moiety of **20** participates in this hydrogen-bonding network in Jak1, which may contribute to its Jak1 potency.[3] It was hypothesized that incorporation of a tertiary amine into the thienopyridine scaffold, which would be protonated at physiological pH, could disrupt this hydrogen bond and introduce a charge−charge clash with Arg879 of Jak1 that would not occur in Jak2, thereby providing increased selectivity.

To test these structure-based hypotheses for improving Jak1 selectivity, a series of 4-sulfonamides with a variety of amide moieties was prepared (Table 4). Data from the enzyme and TEL-Jak cell assays for **20** are included for comparison. As previously reported, **20** shows good potency against all of the Jak family members in enzyme assays,[2a] with cell shifts that are comparable to those of the thienopyridines and compounds representing other series.[11] Alcohol **21** was less selective over Jak1 than **19** in enzyme assays, but capping the alcohol with a methyl group to give methoxy amide **22** provided increased Jak1 selectivity relative to **19** with concomitant improvements in Jak3 and Tyk2 selectivity. Furthermore, its 44-fold selectivity for Jak2 over Jak1 in enzyme assays appeared to be sufficient for translation into Jak1 cell selectivity, as **22** was 7-fold more potent in the TEL-Jak2 cell assay than in the TEL-Jak1 assay. Introduction of a tertiary amine moiety provided additional improvement in Jak1 selectivity. Compounds **23**−**25**, which incorporate both larger amide substituents and tertiary amines along the hinge region of the ATP pocket, approached and exceeded 100-fold selectivity for Jak2 over Jak1 and also maintained the >400-fold selectivity over Jak3 and Tyk2 observed for **22**. Consistent with the charge−charge clash hypothesis, morpholine **25**, which is the least basic among **23**−**25**, was the most potent of the three compounds against Jak1, and piperidine **24**, which is the most basic among **23**−**25**, was the most selective for Jak2 over Jak1. Consistent with their improved Jak1 selectivity versus compound **22** in enzyme assays, compounds **23** and **24** showed >10-fold selectivity for Jak2 over Jak1 in the TEL-Jak assays, and **25** also showed promising levels of cellular selectivity.[12] Compound **26**, which contains an additional methylene between the core and the morpholine, showed diminished potency and selectivity versus **25**. Although several compounds have been reported to achieve selectivity for Jak2 over Jak3 in TEL-Jak assays,[4] **22**−**25** represent a compound class that is one of few reported to achieve selectivity for Jak2 over all of the Jak kinases in TEL-Jak cell assays.[2d]

Compound **25** was profiled against the same 100 human kinases as HTS hit **3** and showed improved selectivity versus the broader kinome. Against Jak2 at 1 μM, **25** showed 100% inhibition, and GAK (98%) and YSK4 (91%) were the only other kinases tested that showed >90% inhibition. Five additional kinases were inhibited at 65−80%,[13] and 92 kinases returned values of <50% inhibition.

Selected thienopyridines were next tested in primary cell assays in which cytokine stimulation results in activation of full-length endogenous Jak kinases. These assays served to establish functional activity and selectivity in a more physiologically relevant context than the TEL-Jak cell assays. Purified peripheral blood mononuclear cells (PBMC) and human whole blood (WB) were stimulated with either thrombopoietin (TPO)[14] or interleukin-2 (IL-2)[15] to activate the Jak2 and Jak1/Jak3 pathways, respectively. Because inhibition of Jak1 and Jak3 is required for blockade of signaling through γC-containing cytokines, which leads to downstream inhibition of STAT5 phosphorylation,[16] only compounds that are selective for Jak2 over both Jak1 and Jak3 should be selective in these assays. As expected, **3**, which showed no selectivity for Jak2 over the other Jak kinases, also did not exhibit any selectivity for the Jak2-driven pathway in PBMC and was slightly more potent in the IL-2-stimulated assay (Table 5).

A similar phenomenon has been reported in the literature for the pan-Jak kinase inhibitor **20**, which is equipotent against Jak1, Jak2, and Jak3 in enzyme assays but is more potent in cellular assays driven by a combination of Jak1 and Jak3 activities than those driven by Jak2 alone.[2a,16] Compound **20** was approximately 5-fold more potent in our IL-2-stimulated assays than our TPO-stimulated assays (Table 5), which is in general agreement with reported values that show good selectivity for inhibition of Jak1/Jak3- versus Jak2-mediated cytokine signaling in WB.[17] Experiments reported in the literature with **20** and a Jak3 selective inhibitor also showed that Jak1 plays a dominant role over Jak3 in signal transduction through γC-containing cytokines,[18] which suggests that high selectivity over Jak1 in particular may be necessary to observe significant Jak2 selectivity in physiologically relevant assays. Likewise, the Jak2/Jak1 inhibitor CYT387, which is 9-fold selective for Jak2 over Jak3 in enzyme assays, shows only marginal selectivity (<2-fold) for a Jak2-driven cellular assay over a combined Jak1/Jak3-driven cellular assay.[4c] This suggests that its lack of cellular selectivity is driven by its Jak1 potency. Although it was obtained from a variety of

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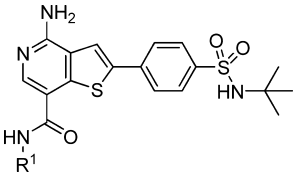
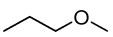
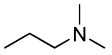
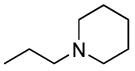
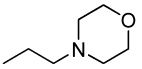
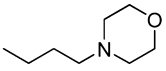
Table 4. Jak Family Enzyme and Cellular Data for 20−26



		Enzyme: IC_{50}, μM (*Selectivity vs. Jak2, x-fold*)[a]				TEL-Jak Cell: IC_{50}, μM (*Selectivity vs. Jak2, x-fold*)[b]			
#	R^1	Jak2	Jak1	Jak3	Tyk2	Jak2	Jak1	Jak3	Tyk2
20		0.004	0.003	0.0006	0.052	0.265	0.026	0.054	1.20
21		0.008	0.078 (*10*)	0.434 (*54*)	0.932 (*117*)	*64%*[c]	*65%*[c]	>25	>25
22		0.003	0.129 (*43*)	1.28 (*427*)	1.48(*493*)	0.959	6.61 (*7*)	>25 (>*26*)	>25 (>*26*)
23		0.008	0.708 (*89*)	3.33 (*416*)	4.10 (*513*)	2.40	>25 (>10)	>25 (>*10*)	>25 (>*10*)
24		0.005	0.654 (*131*)	2.38 (*476*)	2.67 (*534*)	2.13	>25 (>*12*)	>25 (>*12*)	>25 (>*12*)
25		0.002	0.177 (*89*)	0.963 (*482*)	0.757 (*379*)	1.62	*61%*[c]	>25 (>*15*)	>25 (>*15*)
26		0.005	0.206 (*41*)	0.645 (*129*)	1.55 (*310*)	4.72	12.6 (3)	>25 (>*5*)	>25 (>*5*)

[a]Measured at the apparent ATP K_m.[7] Each IC_{50} value represents the average of ≥2 independent experiments, where each experiment consisted of a single determination. [b]Each IC_{50} value represents the average of ≥2 independent experiments, where each experiment consisted of two replicates. See the Experimental Section for details. [c]Percent inhibition at 25 μM. The IC_{50} could not be calculated because complete inhibition was not achieved in the concentration range tested.

Table 5. Activity of Selected Thienopyridines and 20 in Cytokine-Stimulated PBMC and WB Assays

	PBMC (IC_{50}, μM)[a]			WB (IC_{50}, μM)[a]				
compd	TPO	IL-2	fold selectivity	TPO	IL-2	fold selectivity	human Fu (%)[b]	TPO-stimulated WB IC_{50}/PBMC IC_{50}
3	0.761	0.548	<1	>25	>25		ND[d]	>33
19	0.048	0.625	13	1.48	>25	>17	1.2	37.5
20	0.155	0.033	<1	0.682	0.143	<1	24[c]	4.4
22	0.135	4.45	33	3.95	>25.0	>6	2.3	29.3
23	0.515	>25.0	>49	7.83	>25.0	>3	6.4	15.2
25	0.148	7.30	49	2.19	>25.0	>11	10.1	14.8

[a]Each IC_{50} value represents the average of ≥2 independent experiments, where each experiment consisted of two replicates. Measures inhibition of phosphorylation of STAT5 using intracellular phosflow staining and analysis. [b]Fraction unbound. Binding to human plasma proteins was determined by equilibrium dialysis. [c]Reported value, determined using by equilibrium dialysis. See ref 2a. [d]Not determined.

different cell assay formats, data for these previously reported inhibitors and compound **3** taken together illustrate the difficulty in identifying Jak2 selective inhibitors using enzyme assays alone, and it highlights the importance of establishing relationships between apparent Jak2 selectivity in enzyme assays with that in downstream cellular assays.

Interestingly, compound **19**, which was highly selective for Jak2 over Jak3 in enzyme and TEL-Jak assays but showed discordant Jak1 enzyme (30-fold selectivity for Jak2) and Tel-Jak1 (equipotent with Jak2) data, was 13-fold selective for the Jak2 driven pathway in the PBMC assay. Compound **19** is an outlier in that its selectivity over Jak1 and Jak3 in TEL-Jak assays was not predictive of selectivity in the PBMC assays. As expected, compounds that showed good cellular selectivity in the TEL-Jak2 assay over all of the Jak kinases, such as **22**, **23**, and **25**, were more selective than **19** for TPO versus IL-2 stimulation in the PBMC assays. Compounds **23** and **25** are particularly noteworthy as they were ≥49-fold more potent in the TPO-stimulated assay than in the IL-2 stimulated assay, thus demonstrating high selectivity for the Jak2-driven pathway.

In WB assays, **19**, **22**, **23**, and **25** returned micromolar IC_{50} values with TPO stimulation. Although multiple factors could contribute to shifts between the IC_{50} values in the TPO-stimulated PBMC and the TPO-stimulated WB assays, the values obtained in the WB assay for **19**, **20**, **22**, **23**, and **25** (Table 5) were all less than or equal to PBMC assay IC_{50} values after normalization for human plasma free fraction (TPO

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PBMC IC_{50}/Fu). Notably, despite its potency shift into WB, compound **19** still showed good selectivity for the TPO- over the IL-2 stimulated pathway (>17-fold). Because **22**, **23**, and **25** showed higher selectivity than **19** in PBMC assays, it was reasonable to anticipate that they would also show >17-fold selectivity in WB. However, accurate determination of IC_{50} values in the IL-2-stimulated assays was not possible in the concentration ranges tested.

CONCLUSION

Here, we have described the identification of a highly selective thienopyridine series of Jak2 inhibitors. SAR developed around the phenyl and amide moieties combined with structure-based design led to the discovery of compounds possessing 100-fold selectivity for Jak2 over Jak1, >400-fold selectivity over Jak3, and >500-fold selectivity over Tyk2 in enzyme assays. Use of a high-throughput panel of TEL-Jak cellular assays enabled us to determine that very high selectivity for Jak2 in enzyme assays was required to observe selectivity in both the engineered TEL-Jak and the more biologically relevant cytokine-stimulated PBMC and WB assays. Despite large potency shifts between enzyme and cellular assays for the compounds in this series, two of the most Jak2 selective inhibitors in enzyme and TEL-Jak cell assays (**23** and **25**) approached and exceeded 50-fold selectivity for the TPO-stimulated over the IL-2-stimulated pathway in PBMC assays. These thienopyridines represent two of only a small number of Jak2 inhibitors reported to show significant selectivity in cells. In addition to serving as valuable tools for understanding Jak family selectivity, the thienopyridines provided novel insight about the active site of Jak2 kinase. Additional chemical series designed to take advantage of information learned from this work will be reported in future publications.

EXPERIMENTAL SECTION

Chemistry. Unless otherwise noted, all materials were obtained from commercial suppliers and used without further purification. Anhydrous solvents were obtained from Aldrich and used directly. Strong cation exchange chromatography was performed using Biotage Isolute SCX-2 columns. Silica gel chromatography was performed using medium-pressure liquid chromatography (MPLC) on a CombiFlash Companion (Teledyne Isco) with RediSep normal-phase silica gel (35−60 μm) columns and UV detection at 254 nm. Preparative reversed-phase high-pressure liquid chromatography (HPLC) was performed on a Gilson (215 liquid handler), YMC-Pack Pro C18, 150 mm × 30 mm i.d. column, eluting with a binary solvent system A and B using a gradient elusion [A: H_2O with 0.1% trifluoroacetic acid (TFA); B: CH_3CN with 0.1% TFA] with UV detection at 254 nm. All final compounds were purified to ≥95% purity as determined by Agilent 1100 Series HPLC with UV detection at 254 nm (method A: Zorbax SB-C8, 4.6 mm × 150 mm, 15 min; 1.5 mL/min flow rate; 0−100% 0.1% TFA in CH_3CN/0.1% TFA in H_2O; method B: Phenomenex Synergi, 2 mm × 50 mm, 3 min, 1.0 mL/min flow rate, 5−95% 0.1% formic acid in CH_3CN/0.1% formic acid in H_2O). Low-resolution mass spectral (MS) data were determined on an Agilent 1100 Series LCMS with UV detection at 254 nm and a low resonance electrospray mode (ESI). High-resolution mass spectra (HRMS) were obtained on an Agilent 1100 HPLC/MS time-of-flight mass spectrometer. Nuclear magnetic resonance (NMR) spectra were acquired with either a Bruker AVANCE-400 (at 400.13 MHz) or a Bruker AVANCE III-500 (at 500.34 MHz) spectrometer operating at 298 K. Chemical shifts are reported in ppm relative to the residual proton signal in the solvent [dimethylsulfoxide (DMSO)-d_6 at 2.50 ppm]. Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, br = broad, and m = multiplet), coupling constants (in Hz), and number of protons.

*4-Amino-2-iodothieno[3,2-c]pyridine-7-carboxamide (**2**).* A vial was charged with **1** (300 mg, 0.712 mmol) and neat sulfuric acid (1.42 mL), and the dark red mixture was stirred at room temperature (RT) for 1 h. Ice was added, and the resulting precipitate was filtered, washed with water, and dried. The solids were dissolved in DMSO/MeOH and passed through an SCX column, which was washed with MeOH. The product was eluted with 2.0 M NH_3/MeOH, and the filtrate was concentrated to provide **2** (190 mg, 84%) as a tan solid. MS: m/z 319.6 ($[M + H]^+$). ^1H NMR (400 MHz, DMSO-d_6): δ 8.44 (s, 1H), 7.94 (s, 2H), 7.29 (br s, 1H), 7.22 (s, 2H).

*4-Amino-2-(4-morpholinophenyl)thieno[3,2-c]pyridine-7-carboxamide (**3**).* To a solution of **2** (80.0 mg, 0.251 mmol) in dimethylformamide (DMF) (3.0 mL) and Na_2CO_3 (2.0 M, 0.5 mL) was added 4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)phenyl)-morpholine (83.4 mg, 0.288 mmol). The solution was degassed, and $Pd(PPh_3)_4$ (11.6 mg, 10.0 μmol) was added. The reaction was heated at 100 °C for 1 h and was cooled to RT. The mixture was filtered and washed with dichloromethane (DCM) and water to give **3** (60.0 mg, 68%) as a light green solid. MS: m/z 355.1 ($[M + H]^+$). ^1H NMR (400 MHz, DMSO-d_6): δ 8.44 (s, 1H), 7.86 (s, 1H), 7.57 (d, J = 8.8 Hz, 2H), 7.01−7.10 (m, 4H), 3.71−3.79 (m, 4H), 3.15−3.22 (m, 4H). HRMS for $C_{18}H_{18}N_4O_2S$ $[M + H]^+$: calcd, 355.1223; found, 355.1224.

*4-Amino-2-iodothieno[3,2-c]pyridine-7-carboxylic Acid (**4**).* A pressure bottle was charged with **1** (5.00 g, 11.87 mmol), concentrated HCl (23.74 mL, 11.87 mmol), and DMSO (11.9 mL). The bottle was sealed, and the reaction was heated at 95 °C overnight. After they were cooled to RT, the solids were filtered, washed with water, and air-dried to provide **4** as the hydrochloride salt (3.57 g, 84%) as a tan solid. MS: m/z 320.6 ($[M + H]^+$). ^1H NMR (400 MHz, DMSO-d_6): δ 8.90 (br s, 2H), 8.35 (s, 1H), 8.32 (s, 1H).

*4-Amino-2-(4-morpholinophenyl)thieno[3,2-c]pyridine-7-carboxylic Acid (**5**).* A vial was charged with $Pd(dppf)Cl_2$ (7.70 mg, 10.52 μmol), 4-(morpholino)phenylboronic acid (65.3 mg, 0.316 mmol), **4** (75 mg, 0.210 mmol), aqueous Na_2CO_3 (2.0 M, 316 μL, 0.631 mmol), and dioxane (841 μL). The vial was sealed, and the mixture was heated at 100 °C for 2 h. After it was cooled to RT, the mixture was passed through an SCX column, which was washed with MeOH. The product was eluted with 2.0 M NH_3/MeOH, and the filtrate was concentrated to provide **5** (52 mg, 70%) as a tan solid. MS: m/z 356.0 ($[M + H]^+$). ^1H NMR (400 MHz, DMSO-d_6): δ 8.40 (s, 1H), 7.90 (s, 1H), 7.58 (d, J = 8, 2H), 7.24 (br s, 2H), 7.05 (d, J = 8, 2H), 3.76 (m, 4H), 3.19 (m, 4H).

*4-Amino-2-(4-(N-tert-butylsulfamoyl)phenyl)thieno[3,2-c]-pyridine-7-carboxylic Acid (**6**).* Following the procedure described for **5**, 4-(*N*-*tert*-butylsulfamoyl)phenylboronic acid and **4** provided the title compound as the hydrochloride salt (660 mg, 93%) as a brown solid. MS: m/z 405.8 ($[M + H]^+$). ^1H NMR (400 MHz, DMSO-d_6): δ 8.46 (s, 1H), 8.28 (s, 1H), 7.91 (m, 4H), 7.59 (m, 3H), 1.13 (s, 9H).

*4-Amino-2-iodo-N-methylthieno[3,2-c]pyridine-7-carboxamide (**7**).* A round-bottom flask was charged with **4** (2.65 g, 7.43 mmol), Hunig's base (2.86 mL, 16.35 mmol), HOBt hydrate (1.707 g, 11.15 mmol), EDC-HCl (2.137 g, 11.15 mmol), DMF (29.7 mL), and methylamine (2.0 M in THF, 5.57 mL, 11.15 mmol). The reaction mixture was stirred at RT for 15 h. After completion, the mixture was concentrated. The crude material was purified by silica gel chromatography, 10−100% 90/10/1 DCM/MeOH/NH_4OH in DCM to provide **7** (1.4 g, 56.5%) as a tan solid. MS: m/z 333.8 ($[M + H]^+$). ^1H NMR (400 MHz, DMSO-d_6): δ 8.37 (m, 2H), 7.92 (s, 1H), 7.09 (br s, 2H), 2.77 (d, J = 4, 3H).

*4-Amino-N-methyl-2-(4-morpholinophenyl)thieno[3,2-c]-pyridine-7-carboxamide (**8**).* Following the procedure described for **7**, methylamine and **5** provided the title compound (62 mg, 40%). MS: m/z 369.3 ($[M + H]^+$). ^1H NMR (400 MHz, DMSO-d_6): δ 8.24 (s, 1H), 8.05 (s, 1H), 7.60 (d, J = 8, 2H), 7.05 (d, J = 8, 2H), 3.77−3.73 (m, 4H), 3.18−3.14 (m, 4H), 2.81 (s, 3H). HRMS for $C_{19}H_{20}N_4O_2S$ $[M + H]^+$: calcd, 369.1380; found, 369.1377.

*4-Amino-N-ethyl-2-(4-morpholinophenyl)thieno[3,2-c]pyridine-7-carboxamide (**9**).* Following the procedure described for **7**, ethylamine and **5** provided the title compound (83 mg, 52%). MS: m/z 383.2 ($[M + H]^+$). ^1H NMR (400 MHz, DMSO-d_6): δ 8.40

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(t, J = 4. 1H), 8.37 (s, 1H), 7.82 (s, 1H) 7.58 (d, J = 8, 2H), 7.03 (d, J = 8, 2H), 3.75−3.72 (m, 4H), 3.29 (dq, J = 4, J = 8, 2H), 3.17−3.15 (m, 4H), 1.12 (t, J = 8, 3H). HRMS for $C_{20}H_{22}N_4O_2S$ $[M + H]^+$: calcd, 383.1536; found, 383.1536.

*4-Amino-2-(4-morpholinophenyl)-N-phenylthieno[3,2-c]-pyridine-7-carboxamide (**10**).* Following the procedure described for **7**, aniline and **5** provided the title compound (47 mg, 26%). MS: m/z 431.0 ($[M + H]^+$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.10 (s, 1H), 7.91 (s, 1H), 7.79 (d, J = 8, 2H), 7.59 (d, J = 12, 2H), 7.35 (t, J = 8, 2H), 7.24 (s, 2H), 7.11−7.05 (m, 3H), 7.09−7.04 (m, 4H), 3.77−3.76 (m, 4H), 3.20−3.16 (m, 4H). HRMS for $C_{24}H_{22}N_4O_2S$ $[M + H]^+$: calcd, 431.1536; found, 431.1540.

*4-Amino-N-benzyl-2-(4-morpholinophenyl)thieno[3,2-c]pyridine-7-carboxamide (**11**).* Following the procedure described for **7**, benzylamine and **5** provided the title compound (25 mg, 13%). MS: m/z 445.0 ($[M + H]^+$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.97 (t, J = 4, 1H), 8.51 (s, 1H), 7.87 (s, 1H), 7.56 (d, J = 8, 2H), 7.34−7.31 (m, 4H), 7.26−7.24 (m, 1H), 7.09−7.04 (m, 4H), 4.95 (d, J = 4, 2H), 3.76−3.74 (m, 4H), 3.20−3.17 (m, 4H). HRMS for $C_{18}H_{18}N_4O_2S$ $[M + H]^+$: calcd, 445.1693; found, 445.1692.

*4-Amino-N-tert-butyl-2-(4-morpholinophenyl)thieno[3,2-c]-pyridine-7-carboxamide (**12**).* Following the procedure described for **7**, *tert*-butylamine and **5** provided the title compound (40 mg, 23%). MS: m/z 411.0 ($[M + H]^+$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.45 (s, 1H), 8.37 (s, 1H), 7.85 (s, 1H) 7.65 (s, 1H), 7.57 (d, J = 8, 2H), 7.04 (d, J = 8, 2H), 7.06 (s, 2H), 3.76−3.74 (m, 4H), 3.18−3.17 (m, 4H), 1.40 (s, 9H). HRMS for $C_{22}H_{26}N_4O_2S$ $[M + H]^+$: calcd, 411.1849; found, 411.1847.

*4-Amino-N,N-dimethyl-2-(4-morpholinophenyl)thieno[3,2-c]-pyridine-7-carboxamide (**13**).* Following the procedure described for **7**, dimethylamine and **5** provided the title compound (35 mg, 22%). MS: m/z 383.1 ($[M + H]^+$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.89 (s, 1H), 7.82 (s, 1H), 7.55 (d, J = 8, 2H), 7.01 (d, J = 8, 2H), 3.73−3.71 (m, 4H), 3.16−3.13 (m, 4H), 3.03 (s, 6H). HRMS for $C_{20}H_{22}N_4O_2S$ $[M + H]^+$: calcd, 383.1536; found, 383.1532.

*4-Amino-N-methyl-2-phenylthieno[3,2-c]pyridine-7-carboxamide (**14**).* Following the procedure described for **5**, phenylboronic acid and **7** provided the title compound (35 mg, 41%). MS: m/z 284.0 ($[M + H]^+$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.44 (s, 1H), 8.36 (q, J = 4, 1H), 8.05 (s, 1H), 7.72−7.66 (m, 2H), 7.53−7.44 (m, 2H), 7.40−7.36 (m, 1H), 7.12 (br s, 2H), 2.81 (d, J=4, 3H). HRMS for $C_{15}H_{13}N_3OS$ $[M + H]^+$: calcd, 284.0852; found, 284.0852.

*4-Amino-N-methyl-2-p-tolylthieno[3,2-c]pyridine-7-carboxamide (**15**).* Following the procedure described for **5**, 4-methylphenylboronic acid and **7** provided the title compound (33 mg, 46%). MS: m/z 297.8 ($[M + H]^+$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.42 (s, 1H), 8.35 (q, J = 4, 1H), 7.99 (s, 1H), 7.59 (d, J=8, 2H), 7.30 (d, J = 8, 2H), 7.08 (br s, 2H), 2.81 (d, J = 4, 3H), 2.34 (s, 3H). HRMS for $C_{16}H_{15}N_3OS$ $[M + H]^+$: calcd, 284.0852; found, 284.0852. HRMS for $C_{18}H_{18}N_4O_2S$ $[M + H]^+$: calcd, 298.1009; found, 298.1007.

*4-Amino-2-(4-tert-butylphenyl)-N-methylthieno[3,2-c]pyridine-7-carboxamide (**16**).* Following the procedure described for **5**, 4-*tert*-butylphenylboronic acid and **7** provided the title compound (8 mg, 8%). MS: m/z 340.0 ($[M + H]^+$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.42 (s, 1H), 8.37 (q, J = 4, 1H) 7.99 (s, 1H), 7.63 (d, J = 8, 2H), 7.51 (d, J = 8, 2H), 7.09 (br s, 2H), 2.81 (d, J = 4, 3H), 1.32 (s, 9H).

*4-Amino-N-methyl-2-(4-(trifluoromethoxy)phenyl)thieno[3,2-c]-pyridine-7-carboxamide (**17**).* Following the procedure described for **5**, 4-(trifluoromethoxy)phenylboronic acid and **7** provided the title compound (31 mg, 35%). MS: m/z 367.8 ($[M + H]^+$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.45 (s, 1H), 8.37 (q, J = 4, 1H) 8.09 (s, 1H), 7.81 (d, J = 8, 2H), 7.50 (d, J = 8, 2H), 7.16 (br s, 2H), 2.81 (d, J = 4, 3H). HRMS for $C_{16}H_{18}N_3O_2SF_3$ $[M + H]^+$: calcd, 368.0675; found, 368.0673.

*4-Amino-2-(4-(2-cyanopropan-2-yl)phenyl)-N-methylthieno[3,2-c]pyridine-7-carboxamide (**18**).* Following the procedure described for **5**, 4-(2-cyanopropan-2-yl)phenylboronic acid and **7** provided the title compound (28 mg, 33%). MS: m/z 350.8 ($[M + H]^+$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.44 (s, 1H), 8.37 (q, J = 4, 1H) 8.08 (s, 1H), 7.75 (d, J = 8, 2H), 7.64 (d, J = 8, 2H), 7.14 (br s, 2H), 2.81 (d, J = 4, 3H), 1.73 (s, 6H). HRMS for $C_{19}H_{18}N_4OS$ $[M + H]^+$: calcd, 351.1274; found, 351.1275.

*4-Amino-2-(4-(N-tert-butylsulfamoyl)phenyl)-N-methylthieno-[3,2-c]pyridine-7-carboxamide (**19**).* Following the procedure described for **5**, 4-(*N*-*tert*-butylsulfamoyl)phenylboronic acid and **7** provided the title compound (50 mg, 50%). MS: m/z 418.8 ($[M + H]^+$). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.46 (s, 1H), 8.39 (q, J = 4, 1H) 8.20 (s, 1H), 7.91 (d, J = 8, 2H), 7.86 (d, J = 8, 2H), 7.57, (s, 1H), 7.19 (br s, 2H), 2.82 (d, J = 4, 3H), 1.13 (s, 9H). HRMS for $C_{19}H_{22}N_4O_3S_2$ $[M + H]^+$: calcd, 419.1206; found, 429.1209.

*4-Amino-2-(4-(N-tert-butylsulfamoyl)phenyl)-N-(2-hydroxyethyl)-thieno[3,2-c]pyridine-7-carboxamide (**21**).* Following the procedure described for **7**, 2-aminoethanol and **6** provided the title compound (10 mg, 11%). MS: m/z 448.7 ($[M + H]^+$). ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 8.53 (s, 1 H), 8.40 (t, J = 5.60, 1 H), 8.21 (s, 2 H), 7.90−7.93 (m, 2 H), 7.85−7.89 (m, 2 H), 7.57 (s, 1 H), 7.21 (s, 2 H), 4.72 (t, J = 5.59, 1 H), 3.54 (q, J = 6.19, 2 H), 3.36 (q, J = 6.02, 2 H), 1.13 (s, 9 H).

*4-Amino-2-(4-(N-tert-butylsulfamoyl)phenyl)-N-(2-methoxyethyl)thieno[3,2-c]pyridine-7-carboxamide (**22**).* Following the procedure described for **7**, 2-methoxyethanamine and **6** provided the title compound (29 mg, 32%). MS: m/z 462.8 ($[M + H]^+$). ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 8.53 (s, 1 H), 8.48 (t, J = 5.34 Hz, 1 H), 8.22 (s, 1 H), 7.85−7.95 (m, 4 H), 7.57 (s, 1 H), 7.22 (br s, 2 H), 3.42−3.51 (m, 4 H), 3.28 (s, 3 H), 1.13 (s, 9 H). HRMS for $C_{21}H_{26}N_4O_4S_2$ $[M + H]^+$: calcd, 464.1496; found, 464.1497.

*4-Amino-2-(4-(N-tert-butylsulfamoyl)phenyl)-N-(2-(dimethylamino)ethyl)thieno[3,2-c]pyridine-7-carboxamide (**23**).* Following the procedure described for **7**, N^1,N^1-dimethylethane-1,2-diamine and **6** provided the title compound (26 mg, 28%). MS: m/z 475.8 ($[M + H]^+$). ^{1}H NMR (500 MHz, DMSO-d_6): δ ppm 8.50 (s, 1 H), 8.36 (t, J = 5.60 Hz, 1 H), 8.21 (s, 1 H), 7.90−7.94 (m, 2 H), 7.84−7.89 (m, 2 H), 7.57 (s, 1 H), 7.21 (br s, 2 H), 3.39 (q, J = 6.61 Hz, 2 H), 2.43 (t, J = 6.99 Hz, 2 H), 2.20 (s, 6 H), 1.13 (s, 9 H). HRMS for $C_{22}H_{29}N_5O_3S_2$ $[M + H]^+$: calcd, 476.1785; found, 476.1788.

*4-Amino-2-(4-(N-tert-butylsulfamoyl)phenyl)-N-(2-(piperidin-1-yl)ethyl)thieno[3,2-c]pyridine-7-carboxamide (**24**).* Following the procedure described for **7**, 2-(piperidin-1-yl)ethanamine and **6** provided the title compound (40 mg, 39%). MS: m/z 515.8 ($[M + H]^+$). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 8.49 (s, 1 H), 8.42 (m, 1 H), 8.22 (s, 1 H), 7.90−7.95 (m, 2 H), 7.82−7.90 (m, 2 H), 7.58 (s, 1 H), 7.24 (br s, 2 H), 3.44 (m, 2 H), 2.67−2.33 (m, 6H), 1.54 (m, 4 H), 1.41 (m, 2 H), 1.13 (s, 9 H). HRMS for $C_{25}H_{33}N_5O_3S_2$ $[M + H]^+$: calcd, 516.2098; found, 516.2103.

*4-Amino-2-(4-(N-tert-butylsulfamoyl)phenyl)-N-(2-morpholinoethyl)thieno[3,2-c]pyridine-7-carboxamide (**25**).* Following the procedure described for **7**, 2-morpholinoethanamine and **6** provided the title compound (42 mg, 41%). MS: m/z 517.8 ($[M + H]^+$). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 8.49 (s, 1 H), 8.38 (t, J = 5.77 Hz, 1 H), 8.21 (s, 1 H), 7.89−7.95 (m, 2 H), 7.82−7.89 (m, 2 H), 7.57 (s, 1 H), 7.22 (br s, 2 H), 3.53−3.61 (m, 4 H), 3.37−3.47 (m, 2 H), 2.54−2.52 (m, 2H), 2.38−2.46 (m, 4 H), 1.13 (s, 9 H). HRMS for $C_{24}H_{31}N_5O_4S_2$ $[M + H]^+$: calcd, 518.1890; found, 518.1896.

*4-Amino-2-(4-(N-tert-butylsulfamoyl)phenyl)-N-(3-morpholinopropyl)thieno[3,2-c]pyridine-7-carboxamide (**26**).* Following the procedure described for **7**, 3-morpholinopropan-1-amine and **6** provided the title compound (32 mg, 30%). MS: m/z 531.8 ($[M + H]^+$). ^{1}H NMR (400 MHz, DMSO-d_6): δ ppm 8.50 (s, 1 H), 8.45 (t, J = 5.48 Hz, 1 H), 8.21 (s, 1 H), 7.89−7.94 (m, 2 H), 7.83−7.90 (m, 2 H), 7.57 (s, 1 H), 7.22 (br s, 2 H), 3.58 (m, 4 H), 3.61−3.51 (m, 2 H), 3.34−3.31 (m, 2H), 2.31−2.40 (m, 6 H), 1.71 (m, 2 H), 1.13 (s, 9 H). HRMS for $C_{25}H_{33}N_5O_3S_2$ $[M + H]^+$: calcd, 532.2047; found, 532.2049.

TR-FRET Jak Enzyme Assays. The Jak Homology 1 (JH1, catalytic kinase) domain of each human Jak (Jak1, Jak2, Jak3, and Tyk2) was expressed in Sf-9 cells as an N-terminal GST fusion. The kinase activity of the purified recombinant Jaks was assessed using a Lance TR-FRET assay, which measured the phosphorylation of a Tyk2-based peptide substrate (Biotin-LC-EQEDEPEGDYFEWLE, Biopeptide, San Diego, CA). Kinase assays were performed in black 384-well

assay plates (Costar) using a reaction buffer that contained 5 mM $MgCl_2$, 50 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.5, 4 mM dithiothreitol (DTT), and 0.05% bovine serum albumin (BSA). The enzyme reactions (40 μL) contained 0.5 μM peptide, apparent K_m concentrations of ATP (19 μM for Jak1, 10 μM for Jak2, 1.8 μM for Jak3, and 15 μM for Tyk2), and either 625 pM Jak1, 4 pM Jak2, 4 pM Jak3, or 75 pM Tyk2. Reactions were incubated for 90 min at RT with compound [in a 22 pt (1:2) titration series with final concentrations ranging from 62.5 μM to 29.8 pM]. Reactions were then stopped with 40 μL of a detection mix containing 400 pM Lance Eu-PT66 Antibody (Perkin-Elmer), 6.6 μg/mL streptavidin allophycocyanin (SA-APC) (Perkin-Elmer), 40 mM ethylenediaminetetraacetic acid (EDTA), 100 mM HEPES, pH 7.5, 100 mM NaCl, 0.1% BSA, and 0.5% Tween 20. Plates were incubated for 30 min at RT before reading the TR-FRET signal on the RUBYstar (BMG Labtek, Cary, NC) instrument using an excitation setting of 320 nm and an emission collection at both 615 and 665 nm. Phosphorylation of the peptide resulted in an increased emission at 665 nm; the ratio of signal generated at 665 nm over 615 nm was used in calculations. Automation was accomplished with a Mulitdrop 384 bulk liquid dispenser equipped with a 160-plate Titan stacker (Thermo Electron Corporation, Waltham, MA) for all additions (enzyme, substrate, and detection mix).

AlphaScreen pSTAT5 Detection Assay. TEL-Jak Ba/F3 cells were washed 1× with assay buffer (HBSS, 0.1% BSA, and 5 mM HEPES, pH 7.0) and then resupended to a density of 5×10^6 cells/mL (7.2×10^6 cells/mL for TEL-Jak2 Ba/F3). Three microliters of cell suspension was added to a 384-well low volume white-walled polystyrene Proxiplate (Perkin-Elmer, Downers Grove, IL), which contained 1 μL of compound [in a 22 pt (1:2) titration series with final concentrations ranging from 25.0 μM to 11.9 pM] in 2% DMSO (98% assay buffer) per well. The low control contained a final concentration of 10 μM pan Jak standard inhibitor (at least 10× the cellular IC_{50} of each Jak). Cells were incubated with compound (final DMSO concentration of 0.5%) at RT for 60 min before proceeding with pSTAT5 detection. Measurement of pSTAT5 was accomplished with the SureFire pSTAT5 Assay kit (Perkin-Elmer). Cells were lysed with 1 μL of 5 × lysis buffer and incubated at RT for 10 min. Next, 4.3 μL of a solution containing reaction buffer, activation buffer, and acceptor beads prepared as per manufacturer's protocol was added. Plates were incubated overnight in the dark at RT before adding 1.8 μL of a mixture containing dilution buffer and donor beads also prepared as per manufacturer's protocol. After a final incubation of 1 h at RT, TEL-Jak plates were read on the EnVision (Perkin-Elmer) using the AlphaScreen setting. Automation of cell, lysis, and detection reagent additions was performed with a FlexDrop (Perkin-Elmer) bulk liquid dispenser.

Cytokine-Stimulated pSTAT5 PBMC Assays. Peripheral blood samples were obtained from healthy donors, and PBMC were isolated via density gradient separation using Ficoll-Paque PREMIUM (GE Healthcare, Uppsala, Sweden). PBMC (300000−400000 per well) in 36 μL of Dulbecco's phosphate-buffered saline (DPBS) were dispensed into a 96-well (deep well, v-bottom) polypropylene plate (Costar, Corning, NY) containing 4 μL of 10 × compound [in a 10 pt (1:3) titration series with final concentrations ranging from 25.0 μM to 1.27 nM] in 10% DMSO (90% DPBS) per well. Cells were pretreated with compound for 30 min at 37 °C followed by stimulation with EC_{90} concentrations of either TPO at 10 ng/mL or IL-2 at 10 ng/mL for a further 15 min at 37 °C (R&D Systems, Minneapolis, MN). The low control was defined by vehicle in buffer only (1% DMSO in DPBS). PBMCs were fixed with 800 μL of Lyse Fix buffer (BD, Franklin Lakes, NJ) for 15 min at 37 °C. Next, cells were pelleted, resuspended, and permeabilized with 600 μL of 100% MeOH (JT Baker, Phillipsburg, NJ) and stored at −40 °C in MeOH for at least 1 h before staining.

For intracellular pSTAT5 staining, permeabilized cells were centrifuged and resuspended in 200 μL of blocking buffer [1% mouse serum (Sigma-Aldrich) in Stain buffer (BD)] and incubated for 20 min at RT. After the blocking buffer was removed by centrifugation followed by aspiration, cells were stained with Alexa Fluor 647 conjugated mouse antiphospho-STAT5 (Y694) (BD Biosciences, San Jose, CA) according to the manufacturer's suggestion and incubated at 37 °C for 30 min. Cells were then washed and resuspended in 150 μL of wash buffer (0.5% BSA in DPBS). Events were acquired on the FACS Calibur flow cytometer (BD Biosciences) equipped with a Cytek Development AMS 96-well plate loader using side and forward scatter properties. Greater than 10000 events were captured for each sample.

Cytokine-Stimulated WB Assays. WB assays were performed similar to PBMC assays with the following exceptions: WB, about 3−4 × 10^5 cells were preincubated with compounds; final rHuIL-2 concentrated used for WB stimulation was 60 ng/mL; only half of the cells were subjected to Ab staining after fixation and permeabilization.

Data Analysis. AlphaScreen and TR-FRET data were analyzed with the mFit nonlinear regression algorithm and the Screener (Genedata AG, Basel, Switzerland) data analysis software. Phosflow IC_{50} determination was performed using a Levenberg−Marquardt nonlinear regression algorithm and with Activity base Global fit software (IDBusiness Solution Limited, Emeryville, CA).

Cell Permeability. Data were obtained from MDR1-LLC-PK1 cells, pig kidney cells expressing human MDR1, at 5 μM in the presence of 0.1% BSA.[19]

Plasma Protein Binding. Data were obtained by equilibrium dialysis.[20]

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Permeability data for key compounds; enzyme to cell shift data for the thienopyridines, compound **20**, and a larger set of Jak kinase inhibitors; and statistical data for key compounds in the enzyme, TEL-Jak cell, PBMC, and WB assays. This material is available free of charge via the Internet at http://pubs.acs.org.

Accession Codes

PDB ID for the **8**-Jak2 complex: 3TJC; PDB ID for the **19**-Jak2 complex: 3TJD.

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■ ABBREVIATIONS USED

ATP, adenosine triphosphate; BSA, bovine serum albumin; DPBS, Dulbecco's phosphate-buffered saline; DCM, dichloromethane; DMF, dimethylformamide; DMSO, dimethylsulfoxide; DTT, dithiothreitol; EDC, 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide; EDTA, ethylenediaminetetraacetic acid; ESI, electrospray ionization; ET, essential thrombocythemia; HEPES, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid; HOBt, *N*-hydroxybenzotriazole; HPLC, high-pressure liquid chromatography; HRMS, high-resolution mass spectrometry; HTS, high-throughput screen; IL-2, interleukin-2; Jak, Janus kinase; MAPK, mitogen-activated protein kinase; MF, primary myelofibrosis; MPD, myeloproliferative disorder; MPLC, medium-pressure liquid chromatography; NMR, nuclear magnetic resonance; PBMC, peripheral blood mononuclear cells; PI3K, phosphatidylinositol 3-kinase; PV, polycythemia vera; RT, room temperature; SA-APC, streptavidin allophycocyanin; SAR, structure−activity relationship; STAT, signal transducers

and activators of transcription; TEL, translocated ETS leukemia; TFA, trifluoroacetic acid; Tyk2, tyrosine kinase 2; TPO, thrombopoietin; WB, whole blood

REFERENCES

(1) (a) Baxter, E. J.; Scott, L. M.; Campbell, P. J.; East, C.; Fouroclas, N.; Swanton, S.; Vassiliou, G. S.; Bench, A. J.; Boyd, E. M.; Curtin, N.; Scott, M. A.; Erber, W. N.; Green, A. R. Acquired Mutation of the Tyrosine Kinase Jak2 in Human Myeloproliferative Disorders. *Lancet* **2005**, *365*, 1054. (b) Kralovics, R.; Passamonti, F.; Buser, A. S.; Teo, S.-S.; Tiedt, R.; Passweg, J. R.; Tichelli, A.; Cazzola, M.; Skoda, R. C. A Gain-of-Function Mutation of *Jak2* in Myeloproliferative Disorders. *N. Engl. J. Med.* **2005**, *352*, 1779−1790. (c) James, C.; Ugo, V.; Le Couédic, J.-P.; Staerk, J.; Delhommeau, F.; Lacout, C.; Garçon, L.; Raslova, H.; Berger, R.; Bennaceur-Griscelli, A.; Villeval, J. L.; Constantinescu, S. N.; Casadevall, N.; Vainchenker, W. A Unique clonal *JAK2* Mutation Leading to Constitutive Signalling Causes Polycythaemia Vera. *Nature* **2005**, *434*, 1144−1148. (d) Levine, R. L.; Wadleigh, M.; Cools, J.; Ebert, B. L.; Wernig, G.; Huntly, B. J. P.; Boggon, T. J.; Wlodarska, I.; Clark, J. J.; Moore, S.; Adelsperger, J.; Koo, S.; Lee, J. C.; Gabriel, S.; Mercher, T.; D'Andrea, A.; Fröhling, S.; Döhner, K.; Marynen, P.; Vandenberghe, P.; Mesa, R. A.; Tefferi, A.; Griffin, J. D.; Eck, M. J.; Sellers, W. R.; Meyerson, M.; Golub, T. R.; Lee, S. J.; Gilliland, D. G. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thromobcythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell* **2005**, *7*, 387−397.

(2) (a) Flanagan, M. E.; Blumenkopf, T. A.; Brissette, W. H.; Brown, M. F.; Casavant, J. M.; Shang-Poa, C.; Doty, J. L.; Elliott, E. A.; Fisher, M. B.; Hines, M.; Kent, C.; Kudlacz, E. M.; Lillie, B. M.; Magnuson, K. S.; McCurdy, S. P.; Munchhof, M. J.; Perry, B. D.; Sawyer, P. S.; Strelevitz, T. J.; Subramanyam, C.; Sun, J.; Whipple, D. A.; Changelian, P. S. Discovery of CP-690,550: A Potent and Selective Janus Kinase (JAK) Inhibitor for the Treatment of Autoimmune Diseases and Organ Transplant Injection. *J. Med. Chem.* **2010**, *53*, 8468−8484. (b) Quintás-Cardama, A.; Vaddi, K.; Liu, P.; Manshouri, T.; Li, J.; Scherle, P. A.; Caulder, E.; Wen, X.; Li, Y.; Waeltz, P.; Rupar, M.; Burn, T.; Lo, Y.; Kelley, J.; Covington, M.; Shepard, S.; Rodgers, J. D.; Haley, P.; Kantarjian, H.; Fridman, J. S.; Verstovsek, S. Preclinical Characterization of the Selective JAK1/2 Inhibitor INCB018424: Therapeutic Implications for the Treatment of Myeloproliferative Neoplasms. *Blood* **2010**, *115*, 3109−3117. (c) Wernig, G.; Kharas, M. G.; Okabe, R.; Moore, S. A.; Leeman, D. S.; Cullen, D. E.; Gozo, M.; McDowell, E. P.; Levine, R. L.; Doukas, J.; Mak, C. C.; Noronha, G.; Martin, M.; Ko, Y. D.; Lee, B. H.; Soll, R. M.; Tefferi, A; Hood, J. D.; Gilliland, D. G. Efficacy of TG101348, a Selective JAK2 Inhibitor, in Treatment of a Murine Model of Jak2V617F-Induced Polycythemia Vera. *Cancer Cell* **2008**, *13*, 311−320. (d) Hedvat, M.; Huszar, D.; Herrmann, A.; Gozgit, J. M.; Schroeder, A.; Sheehy, A.; Buettner, R.; Proia, D.; Kowolik, C. M.; Xin, H.; Armstrong, B.; Bebernitz, G.; Weng, S.; Wang, L.; Ye, M.; McEachern, K.; Chen, H.; Morosini, D.; Bell, K.; Alimzhanov, M.; Ioannidis, S.; McCoon, P.; Cao, Z. A.; Yu, H.; Jove, R.; Zinda, M. The JAK2 Inhibitor AZD1480 Potently Blocks Stat3 Signaling and Oncogenesis in Solid Tumors. *Cancer Cell* **2009**, *16*, 487−497. (e) Ioannidis, S.; Lamb, M. L.; Wang, T.; Almeida, L.; Block, M. H.; Davies, A. M.; Peng, B.; Su, M.; Zhang, H.-J.; Hoffman, E.; Rivard, C.; Green, I.; Howard, T.; Pollard, H.; Read, J.; Alimzhanov, M.; Bebernitz, G.; Bell, K.; Ye, M.; Huszar, D.; Zinda, M. Discovery of 5-Chloro-N^2-[(1*S*)-1-(5-fluoropyrimidin-2-yl)ethyl]-N^4-(5-methyl-1*H*-pyrazol-3-yl)-pyrimidine-2,4-diamine (AZD 1480) as a Novel Inhibitor of the Jak/Stat Pathway. *J. Med. Chem.* **2011**, *54*, 262−276.

(3) Williams, N. K.; Bamert, R. S.; Patel, O.; Wang, C.; Walden, P. M.; Wilks, A. F.; Fantino, E.; Rossjohn, J.; Lucet, I. S. Dissecting Specificity in the Janus Kinases: The Structures of JAK-Specific Inhibitors Complexed to the JAK1 and JAK2 Protein Tyrosine Kinase Domains. *J. Mol. Biol.* **2009**, *387*, 219−232.

(4) (a) Ioannidis, S.; Lamb, M. L.; Almeida, L.; Guan, H.; Peng, B.; Bebernitz, G.; Bell, K.; Alimzhanov, M.; Zinda, M. Replacement of pyrazol-3-yl amine hinge binder with thiazol-2-yl amine: Discovery of potent and selective Jak2 inhibitors. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1669−1673. (b) Ioannidis, S.; Lamb, M. L.; Davies, A. M.; Almeida, L.; Su, M.; Bebernitz, G.; Ye, M.; Bell, K.; Alimzhanov, M.; Zinda, M. Discovery of pyrazol-3-ylamino pyrazines as novel JAK2 inhibitors. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6524−6528. (c) Burns, C. J.; Bourke, D. G.; Andrau, L.; Bu, X.; Charman, S. A.; Donohue, A. C.; Fantino, E.; Farrugia, M.; Feutrill, J. T.; Joffe, M.; Kling, M. R.; Kurek, M.; Nero, T. L.; Nguyen, T.; Palmer, J. T.; Phillips, I.; Shackleford, D. M.; Sikanyika, H.; Styles, M.; Su, S.; Treutlein, H.; Zeng, J.; Wilks, A. F. Phenylaminopyrimidines as inhibitors of Janus kinases (JAKs). *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5887−5892. (d) Wang, T.; Duffy, J. P.; Wang, J.; Halas, S.; Salituro, F. G.; Pierce, A. C.; Zuccola, H. J.; Black, J. R.; Hogan, J. K.; Jepson, S.; Shlyakter, D.; Mahajan, S.; Gu, Y.; Hoock, T.; Wood, M.; Brinley, F. F.; Frantz, J. D.; Dauffenbach, L. M.; Germann, U. A.; Fan, B.; Namchuk, M.; Bennani, Y. L.; Ledeboer, M. W. Janus Kinase 2 Inhibitors. Synthesis and Characterization of a Novel Polycyclic Azaindole. *J. Med. Chem.* **2009**, *52*, 7938−7941. (e) Pissot-Soldermann, C.; Gerspacher, M.; Furet, P.; Gaul, C.; Holzer, P.; McCarthy, C.; Radimerski, T.; Regnier, C. H.; Baffert, F.; Drueckes, P.; Tavares, G. A.; Vangrevelinghe, E.; Blasco, F.; Ottaviani, G.; Ossola, F.; Scesa, J.; Reetz, J. Discovery and SAR of potent, orally available 2,8-diaryl-quinoxalines as a new class of JAK2 inhibitors. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2609−2613.

(5) Burkitt, S. A.; Cardozo, M. G.; Cushing, T. D.; DeGraffenreid, M. R.; Farthing, C. N.; Hao, X.; Jaen, J. C.; Jiao, X. Y.; Kopecky, D. J.; Labelle, M.; Lively, S. E.; McMinn, D. L.; Rasmussen, S. P.; Shin, Y.; Smith, A.; Smith, M.-L. Preparation of Thieno[3,2-c]pyridines and Related Compounds as Antiinflamatory Agents. U.S. Pat. Appl. Publ. US 2004/0097485 A1, 2004.

(6) Jak1 and Jak3 were not included in the 100 kinase custom panel, which used a DiscoveRx KINOMEscan screen.

(7) (a) Chrencik, J. E.; Patny, A.; Leung, I. K.; Korniski, B.; Emmons, T. L.; Hall, T.; Weinberg, R. A.; Gormley, J. A.; Williams, J. M.; Day, J. E.; Hirsch, J. L.; Kiefer, J. R.; Leone, J. W.; Fischer, D.; Sommers, C. D.; Huang, H.-C.; Jacobsen, E. J.; Tenbrink, R. E.; Tomasselli, A. G.; Benson, T. E. Structural and Thermodynamic Characterization of the TYK2 and JAK3 Kinase Domains in Complex with CP-690550 and CMP-6. *J. Mol. Biol.* **2010**, *400*, 413−433. (b) Boggon, T. J.; Li, Y.; Manley, P. M.; Eck, M. J. Crystal structure of the Jak3 kinase domain in complex with a staurosporine analog. *Blood* **2005**, *106*, 996−1002.

(8) Warmuth, M.; Kim, S.; Gu, X. J.; Xia, G.; Adrián, F. Ba/F3 cells and their use in kinase drug discovery. *Curr. Opin. Oncol.* **2007**, *19*, 55−60.

(9) Knight, Z. A.; Shokat, K. M. Features of Selective Kinase Inhibitors. *Chem. Biol.* **2005**, 621−637.

(10) Permeability data for compounds **8**, **19**, **22**, **23**, and **25** are included in the Supporting Information.

(11) Enzyme to cell assay shift correlations for multiple series are included in the Supporting Information.

(12) Fold selectivity for Jak2 over Jak1 in the TEL-Jak assays could not be determined because, although it showed >50% inhibition at 25 μM, no IC_{50} curve could be determined for **25**.

(13) DYRK1B (34%), EGFR (27%), MEK5 (26%), ROCK2 (20%), and Tyk2 (27%). Jak1 and Jak3 were not included in the 100 kinase custom panel.

(14) Tortolani, P. J.; Johnston, J. A.; Bacon, C. M.; McVicar, D. W.; Shimosaka, A.; Linnekin, D.; Longo, D. L.; O'Shea, J. J. Thrombopoietin Induces Tyrosine Phosphorylation and Activation of the Janus Kinase, JAK2. *Blood* **1995**, *85*, 3444−3451.

(15) Gaffen, S. L. Signaling Domans of the Interleukin 2 Receptor. *Cytokine* **2001**, *14*, 63−77.

(16) Thoma, G.; Nuninger, F.; Falchetto, R.; Hermes, E.; Tavares, G. A.; Vangrevelinghe, E.; Zerwes, H.-G. Identification of a Potent Janus Kinase 3 Inhibitor with High Selectivity within the Janus Kinase Family. *J. Med. Chem.* **2011**, *54*, 284−288.

(17) Meyer, D. M.; Jesson, M. I.; Li, X.; Elrick, M. M.; Funckes-Shippy, C. L.; Warner, J. D.; Gross, C. J.; Dowty, M. E.; Ramaiah, S. K.; Hirsch, J. L.; Saabye, M. J.; Barks, J. L.; Kishore, N.; Morris, D. L.

Anti-inflammatory activity and neutrophil reductions mediated by the JAK1/JAK3 inhibitor, CP-690,550, in rat adjuvant-induced arthritis. *J. Inflammation* **2010**, *7*, 41.

(18) Haan, C.; Rolvering, C.; Raulf, F.; Kapp, M.; Drückes, P.; Thoma, G.; Behrmann, I.; Zerwes, H.-G. Jak1 Has a Dominant Role over Jak3 in Signal Transduction through γc-Containing Cytokine Receptors. *Chem. Biol.* **2011**, *18*, 314−323.

(19) For full experimental details, see Huang, L.; Berry, L.; Ganga, S.; Janosky, B.; Chen, A.; Roberts, J.; Colletti, A. E.; Lin, M.-H. J. Relationship between Passive Permeability, Efflux, and Predictability of Clearance from In Vitro Metabolic Intrinsic Clearance. *Drug Metab. Dispos.* **2010**, *38*, 223−231.

(20) For full experimental details, see Huang, L.; Be, X.; Berry, L.; Moore, E.; Janosky, B.; Wells, M.; Pan, W. J.; Zhao, Z.; Lin, M.-H. J. In Vitro and In Vivo Pharmackokinetic Characterizations of AMG 900, an Orally Bioavailable Small Molecule Inhibitor of Aurora Kinases. *Xenobiotica* **2011**, *41*, 400−408.