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Thiophene carboxamide inhibitors of JAK2 as potential treatments for myeloproliferative neoplasms

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ABSTRACT

A series of carboxamide-substituted thiophenes demonstrating inhibition of JAK2 is described. Development of this chemical series began with the bioisosteric replacement of a urea substituent by a pyridyl ring. Issues of chemical and metabolic stability were solved using the results of both in vitro and in vivo studies, ultimately delivering compounds such as **24** and **25** that performed well in an acute PK/PD model measuring p-STAT5 inhibition.

Over the past decade, the Janus kinase (JAK) family members (JAK1, JAK2, JAK3, and TYK2) have been the focus of a large amount of research due to their crucial cytokine-mediated signaling roles that affect both the immune system and hematopoiesis.[1] The JAKs are cytoplasmic kinases that associate themselves with cytokine receptors, and upon receiving a signal from specific cytokines they phosphorylate transcription factors called STAT (Signal Transducer and Activator of Transcription) proteins that in turn migrate to the nucleus and influence gene transcription. Aberrant JAK–STAT signaling has been linked to many myeloproliferative neoplasms (MPNs), which include diseases like polycythemia vera (PV), essential thrombocythemia (ET), and primary myelofibrosis (PMF). In 2005, it was found that JAK2 mutations were present in the majority (>95%) of PV patients, further boosting interest in developing JAK inhibitors for this disease.[2,3]

A major milestone in targeting the JAK family to treat MPNs was achieved through the FDA's approval of Jakafi® (ruxolitinib, INCB018424[4]) in 2011 for the treatment of intermediate- and high-risk myelofibrosis (primary and post-PV/ET). Jakafi® is a JAK1/JAK2 dual inhibitor, and it is still an open question whether a more selective JAK2 inhibitor might be a better treatment for PV patients since it would avoid interacting with the JAK1-mediated inflammatory cytokine signaling cascade. Medicinal chemistry programs at Merck and other companies/institutions[5] have sought to develop JAK2-selective inhibitors to answer this question.

When our JAK2 inhibitor program began, it was believed that JAK3, not JAK1, was the family member that posed the biggest risk in terms of detrimental off-target effects. Mutations in the JAK3 gene can cause the severe combined immunodeficiency (SCID) phenotype featuring nearly absent T cells and non-functional B cells.[6] Thus, our efforts began with a high throughput screening campaign that delivered multiple compounds with a kinase selectivity profile like **1**, i.e. selective for JAK2 over JAK3 (JAK3 served as our main selectivity counterscreen within the JAK family). Although **1** had several hit-like properties (e.g. ligand efficiency of 0.55),[7] further optimization towards an oral drug could have

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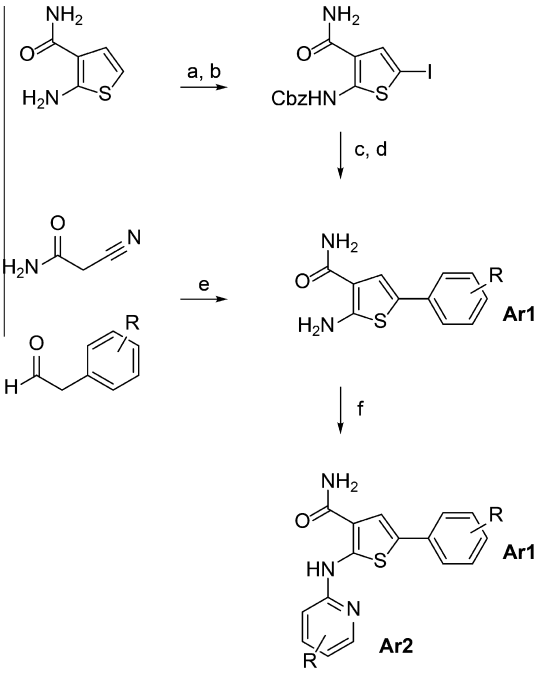
been hindered by the high number of hydrogen bond donors (HBD = 5) present in the initial hit. Viewed another way, **1** has a polar surface area (PSA) of 105 Å^2, which doesn't allow for much additional polarity to be added to the compound while staying in the range of polarity exhibited by most orally bioavailable drugs.[8] In order to further explore this chemical class, alternatives to the urea moiety were considered. Scientists at Pfizer had recently published on a class of isothiazole TrkA inhibitors in which they were able to replace a urea involved in kinase hinge binding with various heterocycles.[9] Performing this bioisosteric replacement led to the 2-pyridyl scaffold **2**, which was nearly equipotent and maintained the selectivity over JAK3 while greatly reducing the PSA to 69 Å^2 and dropping the HBD count to 3 (Fig. 1). Other aryl groups that did not feature a ring nitrogen adjacent to the linking amino group were less potent in the JAK2 assay (e.g. analogs **3**–**5**), and it was hypothesized that the sulfur-nitrogen interaction keeping the thiophene and pyridyl ring of **2** in the same plane is responsible for this preference;[10,11] it has been previously observed that a high degree of planarity is beneficial for binding to the JAK family enzymes,[12] and the narrow binding pocket seen in the JAK2 crystal structure provides a basis for this preference.[13]

To access 2-amino-3-carboxamide-2,5-substituted thiophenes such as **2**, two main synthetic strategies were used; each was suited to examining the SAR of a particular region of the molecule. To investigate the aryl group (**Ar2)** appended to the thiophene 2-amino substituent, access to the core with appropriate handles for substitution was achieved through two methods—a functionalization of 2-aminothiophene-3-carboxamide or a de novo thiophene synthesis via the Gewald reaction[14] (Scheme 1). The **Ar2** group in both cases was appended via Pd-catalyzed cross coupling. The choice of solvent for this reaction was very important as it greatly affected how much the amide nitrogen participated in this reaction; we found that 1,4-dioxane promoted reactivity at both the aniline and amide nitrogens, while switching to *tert*-butanol or *tert*-amyl alcohol (to avoid the freezing of *tert*-butanol at ambient temperatures) gave the desired aniline coupling product in high yields. Similar reaction condition-dependent variation in regiochemical preference in a similar ortho amide/aniline system had been observed previously by Buchwald and co-workers, although they varied the catalytic species as well as the solvent.[15] Compounds designed for exploration of the thiophene's 5 position (**Ar1**) were made by performing this selective heteroaniline arylation first and then subjecting the product to a halogenation/Suzuki cross coupling sequence (Scheme 2).

Although the 2-pyridyl ring was optimal in terms of potency, it was soon discovered that these compounds were not stable upon treatment with acid under fairly mild and standard conditions.



Scheme 1. Synthetic routes used to explore 2-amino substituents (**Ar2**). Reagents and conditions: (a) CbzCl, DIPEA, THF; (b) *N*-Iodosuccinimide, CH_2Cl_2; (c) R-B(OR)$_2$, $PdCl_2(PPh_3)_2$, Na_2CO_3, 1,2-dimethoxyethane/water, 100 °C; (d) Pd/C, H_2, ethanol, 80 °C; (e) S_8, morpholine, ethanol, 70 °C; (f) 2-halo pyridine, $Pd_2(dba)_3$, X-Phos, K_2CO_3, *t*-amyl alcohol, 100 °C.

Upon reverse-phase HPLC purification using trifluoroacetic acid in the solvents or attempting to form HCl salts with only a slight excess (3 equiv) of acid, a ring closure was observed in which the pyridyl nitrogen attacked the amide carbonyl, ultimately eliminating ammonia and forming a fused tricycle (Scheme 3). This issue was resolved by optimizing for both JAK2 potency and compound stability upon incubation in an aqueous pH 2 buffer solution (with DMSO present to solubilize the substrate); this simple assay was designed as a rough mimic of conditions found in human stomachs. Two independent solutions arose from this screening effort: either a substituent (methyl or larger) at the 6 position of the pyridyl ring (e.g. compounds **6** and **13**) or an electron withdrawing group at the 5 position (e.g. compounds **8** and **12**) gave molecules in this series the necessary stability for oral dosing (Table 1). Because the latter strategy was limited in the types of functional groups that could be employed, these groups typically possess a

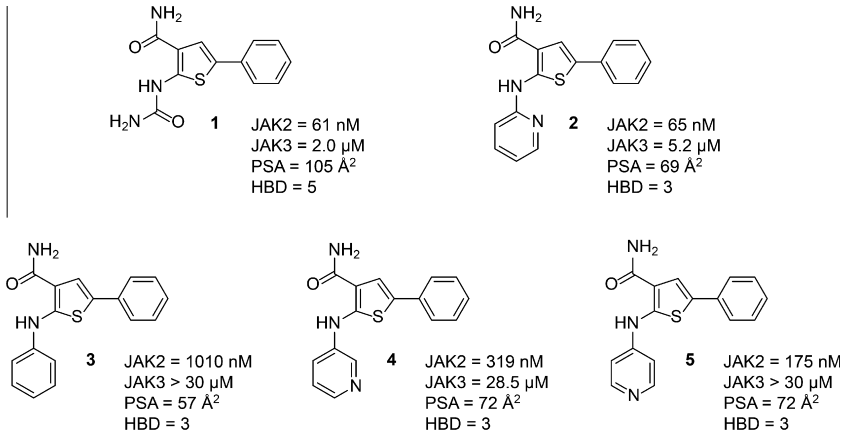
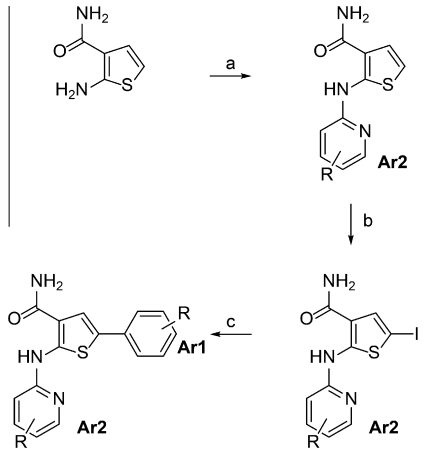
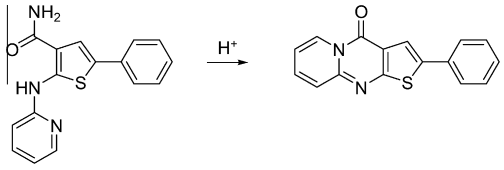


Figure 1. Attempted bioisosteric replacement of a urea with aromatic rings.



Scheme 2. Synthetic route used to explore phenyl substituents (**Ar1**). Reagents and conditions: (a) 2-halo pyridine, $Pd_2(dba)_3$, X-Phos, K_2CO_3, *t*-amyl alcohol, 100 °C; (b) *N*-Iodosuccinimide, CH_2Cl_2/DMF; (c) R-B(OR)$_2$, $PdCl_2(PPh_3)_2$, Na_2CO_3, 1,2-dimethoxyethane/water, 100 °C.



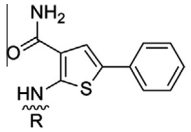
Scheme 3. Acidic conditions promote a self-condensation reaction.

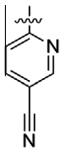
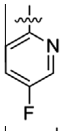
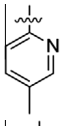
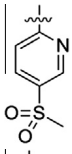
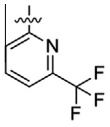
large amount of PSA, and the acceptable functionality was generally fairly planar, most of the analogs of the **Ar2** ring made subsequently relied on the former strategy, that is encumbering the 6 position to avoid the cyclization event. This allowed for the use of groups known to impart solubility (basic amines, ethereal side chains) to otherwise planar molecules (vide infra).

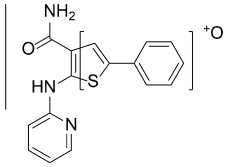
With the chemical stability issue under control, our attention turned to metabolic stability as it had been discovered that the parent compounds were also rapidly metabolized. A metabolite identification study performed with rat hepatocytes demonstrated the major metabolic event was an oxidative modification of the phenyl ring at the 5-position of the thiophene (Fig. 2). In response to these data, a library of thiophene carboxamides with substituted phenyl rings (as well as some heterocyclic replacements for the phenyl, compounds not shown) was generated and then these compounds were tested for microsomal stability, which should capture the phase I metabolism seen in the metabolite identification study (Table 2). Halogen substituents on the phenyl ring had a pronounced effect on both JAK2 potency and microsomal stability; unfortunately, the most potent substituents were also the least stable. A general trend that emerged was placing a fluorine or chlorine atom in the ortho position hurts stability while placing a substituent in the para position was beneficial.

This microsomal screening effort was done in parallel with in vivo rat PK screening in order to build an in vitro-in vivo correlation. A breakthrough came when the rat PK properties of **14** and **23** were compared: addition of the benzylic morpholine, originally done to increase solubility as discussed above, gave a 4 fold increase in exposure (Table 3). Since the microsomal data showed the ortho fluoro was one of the worst substituents on the phenyl ring for metabolic stability, it was hypothesized that switching to some of the preferred substituents would afford compounds with even more favorable pharmacokinetic properties. SAR exploration had shown that a tertiary alcohol at the para position of **Ar1** was

Table 1
The effect of pyridyl substituents on potency and chemical stability



Compound	R	JAK2 IC_{50} (nM)	$t_{1/2}$ @ PH 2
2		65	<1 h
6		275	>18 h
7		398	<1 h
8		146	>18 h
9		161	1 h
10		227	<1 h
11		178	<1 h
12		16	>18 h
13		891	>18 h

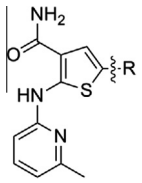


1% of **2** remaining after 25 min incubation with rat hepatocytes

Figure 2. Metabolic ID experiments on prototypical compound **2**.

tolerated in terms of potency, and as seen in Table 2, para substitution was relatively beneficial for microsomal stability. Moreover, the tertiary alcohol's higher polarity relative to substituents like trifluoromethyl helped in general to decrease the log*D* to a more

Table 2
The effect of **Ar1** phenyl substituents on potency and microsomal stability (rat and human)



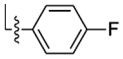
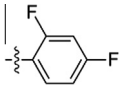
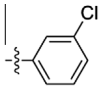
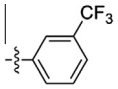
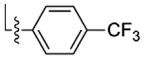
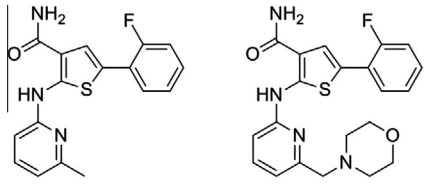
Compound	R	JAK2 IC_{50} (nM)	RKM $t_{1/2}$ (min)	HLM $t_{1/2}$ (min)
6		270	8	24
14		36	2	19
15		155	13	32
16		140	29	39
17		178	23	24
18		80	5	17
19		93	3	11
20		312	19	33
21		690	29	36
22		380	53	61

Table 3
Addition of a benzylic morpholine improves pharmacokinetic properties



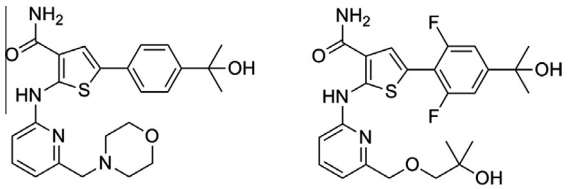
14 **23**

JAK2 (IC_{50})	36 nM	34 nM
Rat Cl_p	181 mL/min/kg	38 mL/min/kg
Rat AUC (Norm)	0.28 μM h kg/mg	1.05 μM h kg/mg
Rat V_D	11.8 L/kg	4.7 L/kg
Rat $t_{1/2}$	0.9 h	3.5 h
PSA	68 $Å^2$	85 $Å^2$
H-bond donors	3	3

drug-like level,[16] leading to compound **24** that had greatly improved pharmacokinetic properties. Further optimization in other respects, chiefly on-target potency and hERG inhibition, was also needed; **23** exhibited strong hERG channel inhibition in vitro[17] (IC_{50} = 161 nM), and the superior analog **24**'s IC_{50} value of 1410 nM was still deemed too low relative to the JAK2 potency. To increase this window, efforts to both lower the on-target and raise the off-target potency were pursued. Through the addition of fluorines to **Ar1** (JAK2 potency increase) and replacing the morpholine with an ethereal chain (decreasing hERG inhibition),[18] an acceptable difference (>1000×) in these values was achieved with compound **25** (Table 4).

To exemplify how these thiophene-based JAK2 inhibitors might bind to the enzyme, compound **25** was docked into a JAK2 crystal structure (Fig. 3).[19] As discussed previously, the sulfur of the thiophene and nitrogen of the pyridine were constrained to be coplanar during docking, providing a fairly flat surface for binding near the hinge. Both the primary amide N–H and O as well as the biaryl nitrogen's N–H are making critical interactions with the hinge backbone residues Glu 930 and Leu 932. The tertiary hydroxyl appears to be able to hydrogen bond with Asn 981 or alternatively to Asp 994, while the gem-dimethyl group can pack under the glycine-rich loop. The ethereal side chain is predicted to project out toward solvent, although its exact location is unknown; the docking experiments revealed many reasonable alternatives, and the interaction with Gln 853 shown in Figure 3 is just one possibil-

Table 4
Comparison of two thiophene-based JAK2 inhibitors with acceptable pharmacokinetic parameters



24 **25**

JAK2 (IC_{50})	20 nM	7.5 nM
JAK3 (IC_{50})	1350 nM	763 nM
JAK2 pSTAT5 cell (IC_{50})	520 nM	90 nM
HPLC log*D* pH 7.4	2.2	2.6
Rat Cl_p	1.3 mL/min/kg	4.5 mL/min/kg
Rat AUC (Norm)	28 μM h kg/mg	7.6 μM h kg/mg
Rat V_D	1.1 L/kg	3.3 L/kg
Rat $t_{1/2}$	10.3 h	5.3 h
Rat %*F*	72%	100%
PSA	105 Å^2	117 Å^2
HBD	4	5
hERG inhibition (IC_{50})	1.4 μM	12.0 μM

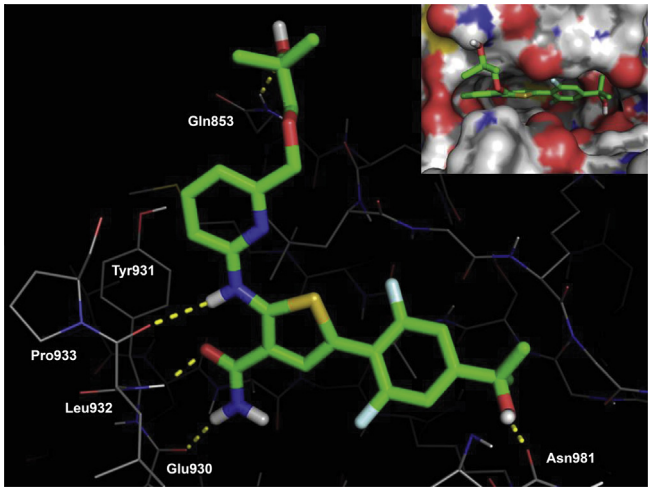


Figure 3. Docking model of **25** with the JAK2 enzyme.

ity. This flexibility correlates with the permissive SAR observed in this region of the molecule, which was useful for the fine-tuning of physical properties in this series.

Based on the favorable potency and pharmacokinetic profiles of compounds **24** and **25**, including reasonable activity in our cell-based JAK2 assay that monitors for inhibition of STAT5 phosphorylation,[20] these two molecules were further profiled. An acute PK/PD mouse model,[21] in which the inhibition of STAT5 phosphorylation serves as a read-out, was used as a filter for compounds before they were progressed into either a chronic wild-type mouse model of PCV or a more intensive *JAK2V617F* genetic knock-in mouse model.[22] The acute model involves treatment of mice with a high dose of recombinant erythropoietin (Aranesp®) to induce many of the clinical features seen in polycythemia vera such as elevated hematocrit, splenomegaly, and elevated levels of phosphorylated STAT5 (pSTAT5). The results of interrogating the acute model with compounds **24** and **25** are shown in Figure 4. Both compounds demonstrated in vivo activity; however, **25** (IC_{50} = 2.0 μM) was

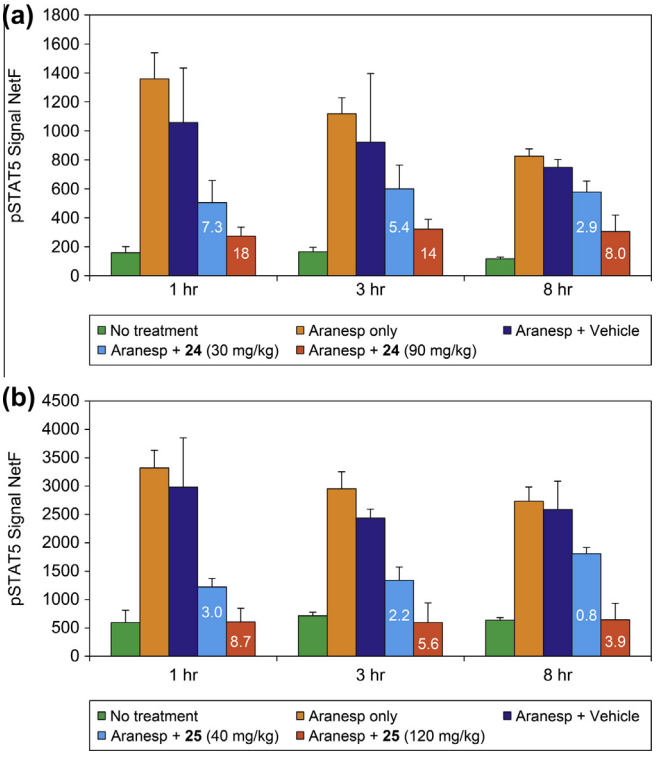


Figure 4. Results for compounds (a) **24** and (b) **25** tested in an acute PK/PD model. Numbers in red on the bar graph represent plasma concentrations at a given time point (μM).

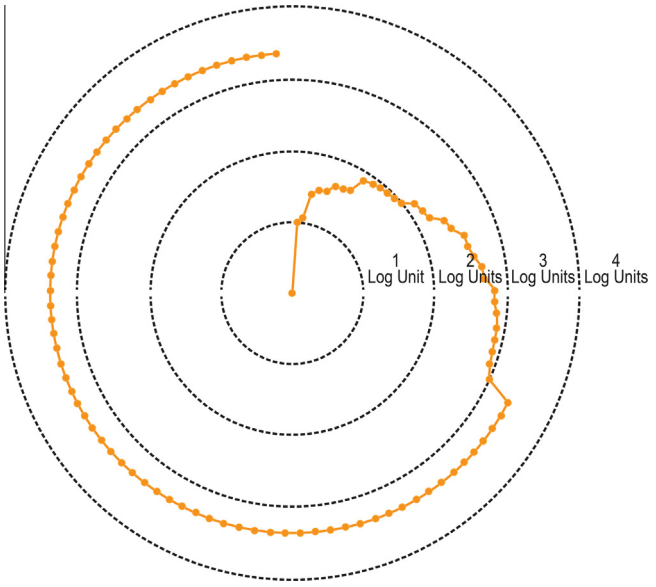


Figure 5. Radar plot indicating the in vitro kinase selectivity of compound **25**. The dotted circles represent inhibitor concentrations at 10-fold intervals above the JAK2 IC_{50}.

more potent than **24** (IC_{50} = 5.4 μM) and showed a longer lasting effect. The discrepancy between the difference in their cell potencies (~6-fold) and in vivo potencies (~3-fold) may be related to differences in the free drug concentrations; although the mouse plasma protein binding for these compounds was not measured, the higher log*D* of compound **25** and lack of a basic amine implies that it has a lower free fraction in vivo. In addition to sufficient

in vivo potency, **25** also proved to be selective for JAK2 over other kinases, including the JAK and Src family kinases; against an in vitro panel of 100 kinases, it showed 100% selectivity at 10-fold the JAK2 IC_{50} concentration, 97% selectivity at 30-fold, and 86% selectivity at 100-fold (Fig. 5).[23] Thus **25** was further progressed as a compound of interest for the program (results not shown).

In conclusion, we have described the discovery of novel and selective trisubstituted thiophene inhibitors of the JAK2 enzyme for the treatment of polycythemia vera. The urea of HTS hit **1** was rapidly replaced with a pyridyl substituent in an effort to reduce the PSA/HBD count of this series. This ring system was then held constant as physical and metabolic stability was optimized using an array of in vitro and in vivo assays. This effort eventually delivered compounds such as **25** that had excellent pharmacokinetic properties and showed PK/PD modulation of the JAK2 pathway in vivo.

References and notes

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20. Detection of STAT phosphorylation was performed using AlphaScreen™ SureFire™ p-STAT5 assay (Perkin–Elmer and TGR Biosciences) using both biotinylated anti-phospho-STAT5 antibody, which is captured by Streptavidin-coated donor beads, and anti-total STAT5 antibody, which is captured by Protein A conjugated acceptor beads. IC_{50} values are reported as the averages of at least two independent determinations; standard deviations are within ±25–50% of IC_{50} values. For further details see: WO2008156726.
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23. Kinases with a <30-fold potency difference relative to JAK2 in the kinase panel: JAK1, SRC, and TYK2; additional kinases with a <100-fold potency difference relative to JAK2 in the kinase panel: ABL1, CSF1R, DAPK1, FLT3, HCK, JAK3, KDR, LCK, NTRK1, NTRK3, and NAUK1.