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Structure activity optimization of 6*H*-pyrrolo[2,3-*e*][1,2,4]triazolo [4,3-*a*]pyrazines as Jak1 kinase inhibitors

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ABSTRACT

Previous work investigating tricyclic pyrrolopyrazines as kinase cores led to the discovery that 1-cyclohexyl-6*H*-pyrrolo[2,3-*e*][1,2,4]triazolo[4,3-*a*]pyrazine (**12**) had Jak inhibitory activity. Herein we describe our initial efforts to develop orally bioavailable analogs of **12** with improved selectivity of Jak1 over Jak2.

The Janus protein kinase family members (Jak1, Jak2, Jak3, and Tyk2) are cytoplasmic tyrosine kinases often associated with membrane cytokine receptors.[1] Cytokine binding to these receptors initiates their Jak dependent phosphorylation and in turn results in the formation of binding sites for Src homology 2 (SH2) containing proteins such as signal transduction activators of transcription (STAT) factors. The binding and subsequent activation of the STATs play a critical role in facilitating STAT nuclear translocation, ultimately resulting in cytokine production and cellular trafficking. This process aids in a number of biological processes including regulation of immune and inflammatory responses.[2,3]

Clinically, Jak kinase inhibition was shown to be highly effective in managing the signs and symptoms of rheumatoid arthritis (in many cases achieving remission) with the first generation broad Jak family inhibitors Tofacitinib **1**[4–6] and Baricitinib **2** (Fig. 1).[4,7] Despite encouraging efficacy, these agents have failed to reach their full potential due to dose-limiting tolerability issues.[8–10] For example, the incidence of severe anemia was reported to be a dose-limiting factor during Phase II development of Tofacitinib for RA.[9] It is reasonable to hypothesize that this adverse effect could arise due to inhibition of erythropoietin signaling mediated via Jak2.[11] Based on this observation we set out to explore the hypothesis that compounds with higher Jak1 selectivity over Jak2 could result in therapies that provide improved efficacy while limiting the side effects associated with the first generation Jak inhibitors, in particular, anemia. More recently, additional publications on Jak1 selective inhibitors have appeared in the literature.[12,13]

In order to determine the best approach to identify selective Jak1 inhibitors, we examined our Tyk2 crystal structures in complex with a number of ligands as reported previously.[14] A model of Tofacitinib **1** in Jak1 subsequently was created (Fig. 2). Through the examination of this model versus a published structure of Jak2[15] (2B7A), we hypothesized that induced fit of the glycine-rich loop was critical for Jak isoform selectivity. A comparison of the primary structure of Jak1 and Jak2 revealed that the end of the glycine-rich loop contains a His885 (Jak1)/Asn859 (Jak2) residue difference (seen in Fig. 2). We postulated that a histidine at this residue position (His885) either directly or indirectly (via solvent) interacts with the conserved catalytic base Asp1003 thereby creating a more closed glycine-rich loop conformation in Jak1. This led

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0960-894X/

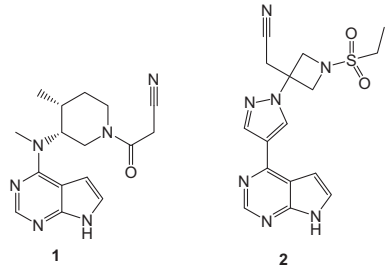


Figure 1. First generation Jak inhibitors: Tofacitinib **1** and Baricitinib **2**.

us to hypothesize that Jak1 inhibitors with improved selectivity over Jak2 could be obtained by optimization of the substituent projecting under the glycine-rich loop. This 'closed glycine-rich loop' structural hypothesis was later confirmed by the crystal structure of **1** in Jak1 (3EYG)[16] where a solvent-mediated interaction between His885 and Asp1003 was observed. Under this premise, we postulated that in addition to the cyanoacetamide moiety in Tofacitinib **1**, other capping groups could be considered to provide an improved van der Waals interaction with the glycine-rich loop of Jak1 thus improving the Jak1/2 selectivity ratio.

To identify leads for Jak1 selective inhibitors, we utilized work previously described[17] to afford 6*H*-pyrrolo[2,3-*e*][1,2,4]triazolo[4,3-*a*]pyrazines. A general synthetic route (Scheme 1) was designed to provide access to analogs to explore the Gly-rich loop region of the protein. Commercially available aminopyrazine **3** was treated with NBS to give dibromide **4** which underwent a regioselective Sonogashira cross-coupling reaction with TMS-acetylene. The resulting alkyne **5** was reacted with sodium hydride in the presence of tosyl chloride and after aqueous acidic workup tosyl protected pyrrolopyrazine **6** was obtained. Buchwald–Hartwig amination with bis-Boc protected hydrazine yielded compound **7** which was deprotected with acid and neutralized to give the free hydrazine **8**. Hydrazides of general structure **9** were formed using peptide coupling reagents such as EDC with a variety of carboxylic acids, some of which contained protected amines. The cyclization of hydrazides of general structure **9** was accomplished with thionyl chloride in the presence of TEA and heat to give triazolopyrazines **10**. In the cases where the triazolopyrazines contained protected amines in X, these compounds were deprotected and then further functionalized as appropriate. For example, Boc deprotection of *tert*-butyl ((1*S*,3*R*)-3-(6-tosyl-6*H*-pyrrolo[2,3-*e*][1,2,4]triazolo[4,3-*a*]pyrazin-1-yl)cyclopentyl)carbamate (precursor to **11h**) with HCl followed by reaction with cyclopropylsulfonyl chloride under

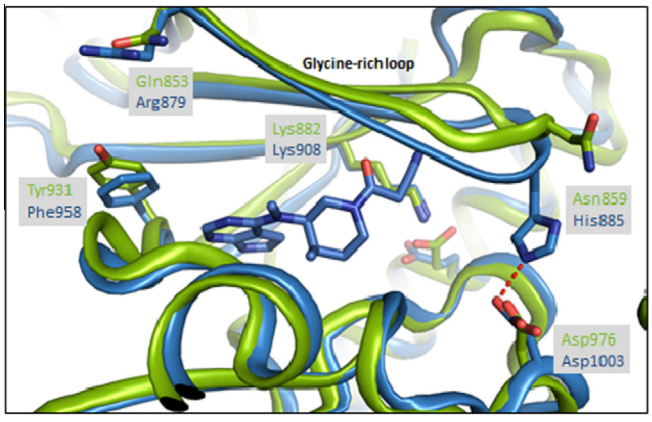
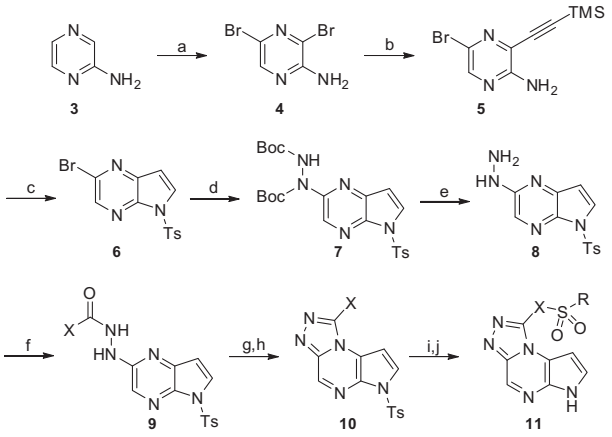


Figure 2. Overlay of Tofacitinib **1** modeled in Jak1 (blue) and Jak2 crystal structure 2B7A[13] (green).



Scheme 1. Reagents and conditions: (a) NBS, Acetontrile/DMF, 0–5 °C, 3 h, 76%; (b) TMS-acetylene, $PdCl_2(PPh_3)_2$, CuI, TEA, THF, −5 to 0 °C, 1.5 h, 88%; (c) NaH, TsCl, DMF, 0 °C to rt, 1 h, 54%; (d) di-*tert*-butyl hydrazodicarboxylate, $Pd(OAc)_2$, Xantphos, K_2CO_3, *t*-AmOH/dioxane, 95 °C, 2 h, 91%; (e) H_3PO_4, THF/heptane, 70 °C, 2 h, then K_2CO_3, H_2O, rt, 1 h, 94%; (f) X-COOH, EDC-HCl, DCM, rt, 15 h, 43–85%; (g) thionyl chloride, TEA, dioxane, 80 °C, 1 h, 40–95%; (h) 4 M HCl in dioxane, dioxane, 60 °C, 4 h, 75–92%; (i) RSO_2Cl, TEA, DMF, rt, 75 min, 62–91%; (j) 1 M aq NaOH, dioxane, 60 °C, 2 h, 30–95%.

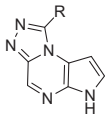
basic conditions and subsequent removal of the tosyl protecting group provided sulfonamide **11h**. In some cases in which hydrazides **9** contained a Boc-protected amine, partial deprotection occurred during cyclization. In such examples, the resulting mixture was subjected to anhydrous acidic conditions (such as trifluoroacetic acid) to effect complete amine deprotection then treated as above.

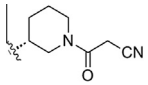
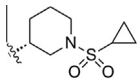
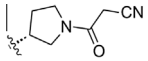
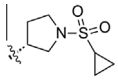
Compounds were profiled in Jak1, Jak2, Tyk2, Jak3 enzyme assays[18] and Jak1, Jak2 cellular assays.[19] We focused on assessing Jak1 over Jak2 selectivity by screening in IL-6-stimulated TF1 and EPO-stimulated UT-7 based cellular assays to benefit from full length Jak enzymes and physiologically relevant ATP concentrations. While there is some literature on IL-6 driven signaling also involving Jak2, our data indicate that IL-6 signaling can be separated from Jak2-dependent Epo signaling. This suggests that Jak2 is unlikely to be important for the IL-6-induced phospho-STAT3 signal we measured.

Lead tricyclic pyrrolopyrazine **12** showed reasonable potency against Jak1 (Table 1). Initial analogs with the piperidine amide moiety from **1** (albeit without the methyl group) as represented by compound **13** did not result in improvement of potency or selectivity compared to **12**. We hypothesized that this could be a result of the additional constraint posed by the tricyclic core causing the cyano group to project in a different orientation compared to **1**. Therefore we attempted to optimize both the size and trajectory of group appended to the core (piperidine to pyrrolidine) and the nature of the terminal group (amide to sulfonamide) to see if potency and/or selectivity could be increased. Neither the piperidine nor the pyrrolidine linking group attached to the tricyclic moiety (compounds **13**–**16**) produced potency levels comparable to the pyrrolopyrimidine hinge binders, such as Tofacitinib. In fact, these initial changes led to a loss of potency in both enzyme and cellular assays.

Given the undesired results from compounds **13**–**16**, additional modeling on 5- and 6-membered rings with other terminal groups indicated that alkylsulfonamide substituents via an exocyclic amine should provide an alternative and improved trajectory to interact with the Gly-rich loop. Thus we turned our attention to compounds with sulfonamide-substituted cyclohexyl and cyclopentyl groups (Table 2). Both the stereochemical configuration and length of the linking group significantly affected Jak1 in vitro

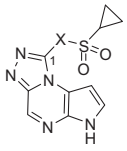
Table 1
In vitro Jak enzyme and cellular activity

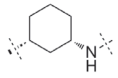
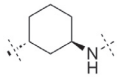
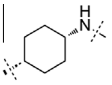
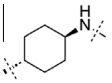
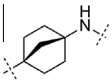
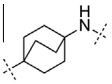
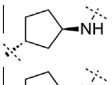
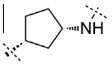


Compound	R	Jak1 HTRF (μM)	Jak2 HTRF (μM)	Jak3 HTRF (μM)	Tyk2 HTRF (μM)	Jak1 cell (μM)	Jak2 cell (μM)
Tofacitinib **1**	na	0.003	0.004	0.003	0.14	0.045[a]	1.12
Baricitinib **2**	na	0.006	0.006	0.18	0.036	0.017[a]	0.31
12		0.03	0.09	3.23	0.53	0.29[a]	11.8
13		0.01	0.08	1.02	0.29	0.36	6.42
14		0.23	0.59	5.65	2.6	na	>20
15		0.08	na	10.8	3.95	>1.0	>20
16		0.49	na	20.4	8.1	na	na

[a] Assays performed in 384 well format.[19]

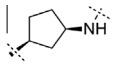
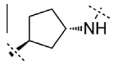
Table 2
In vitro Jak enzyme and cellular activity



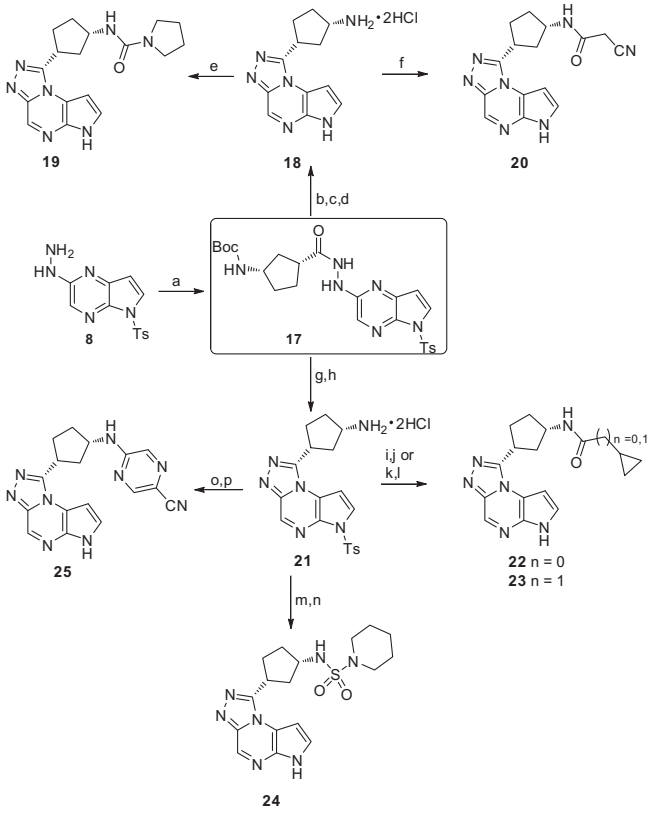
Compound	X	Jak1 HTRF (μM)	Jak1 cell (μM)	Jak2 cell (μM)	Jak2/Jak1 cellular selectivity
11a	 racemic mixture of *cis*-isomers	0.02	0.27	2.4	9×
11b	 racemic mixture of *trans*-isomers	0.37	1.9	>20	>10×
11c		0.21	>1.0	>20	–
11d		0.15	0.94	>20	>20×
11e		0.05	>1.0[a]	14.6	–
11f		0.01	0.02	4.5	225×
11g		0.06	>1.0[a]	8.2	–
11h		0.02	0.11	1.9	17×

(*continued on next page*)

Table 2 (*continued*)

Compound	X	Jak1 HTRF (μM)	Jak1 cell (μM)	Jak2 cell (μM)	Jak2/Jak1 cellular selectivity
11i		0.70	na	>20	–
11j		0.70	na	na	–

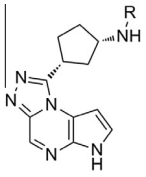
[a] Assays performed in 384 well format.[19]

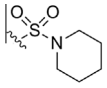
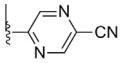


Scheme 2. Reagents and conditions: (a) (1*R*,3*S*)-3-((*tert*-butoxycarbonyl)amino)-cyclopentanecarboxylic acid, EDC·HCl, DCM, rt, 2 h, 97%; (b) $SOCl_2$, TEA, dioxane, 80 °C, 1.5 h; (c) 2 M aq Na_2CO_3, dioxane, 80 °C, 20 h, 85% over 2 steps; (d) 4 M HCl in dioxane, dioxane, 60 °C, 2 h, 95%; (e) pyrrolidine-1-carbonyl chloride, TEA, THF, 60 °C, 16 h, 40%;(f) 2-cyanoacetic acid, EDC, HOBt, DIEA, perfluorophenyl 2-cyanoacetate, rt, 5 h, 21%; (g) $SOCl_2$, TEA, dioxane, 80 °C, 1.5 h, 85%; (h) 4 M HCl in dioxane, dioxane, 60 °C, 2 h, 93%; (i) cyclopropanecarboxylic acid, EDC·HCl, DIEA, DCM, rt, 20 h, 89%; (j) 2 M aq Na_2CO_3, dioxane/ethanol (1:1), 60 °C, 16 h, 55%; (k) cyclopropylacetic acid, EDC·HCl, DIEA, DCM, rt, 20 h, 79%; (l) 2 M aq Na_2CO_3, dioxane/ethanol (1:1), 60 °C, 16 h, 61%; (m) piperidine-1-sulfonyl chloride, DIEA, DMF, rt, 8 h, 72%; (n) 1 M aq NaOH, dioxane, 60 °C, 1 h, 91%; (o) 5-chloropyrazine-2-carbonitrile, DIEA, *n*-propanol, μW, 150 °C, 30 min, 80%; (p) 1 M aq NaOH, dioxane, 60 °C, 80 min, 64%.

potency. In the cyclohexyl series, *cis*-3-sulfonamide substituted **11a** possessed superior Jak1 potency compared to the *trans* analog **11b** and both 4-substituted analogs (**11c** and **11d**). Interestingly, although bridged bicycle **11e** was less potent than parent **11a**, the bulkier bicycle **11f** demonstrated excellent enzymatic and cellular potency as well as greatly improved selectivity versus Jak2. In the cyclopentyl series, the absolute *R*-stereochemistry at the point of attachment of the group (X) to C-1 of the tricycle proved to be critical as compounds **11g** and **11h** showed significantly better potency in the Jak1 enzymatic assay compared to compounds **11i** and **11j**. Absolute stereochemistry at the sulfonamide attachment point was also important as **11h** showed 3–9× improved Jak1

Table 3
In vitro Jak enzyme and cellular activity



Compound	R	Jak1 HTRF (μM)	Jak1 cell (μM)	Jak2 cell (μM)
11h		0.02	0.11	1.9
18	H	1.9	na	na
19		0.70	na	>20
20		0.09	>1.0	>20
22		0.44	na	>20
23		1.2	na	>20
24		0.05	>1.0[a]	1.8
25		0.04	0.5[a]	>20

[a] Assays performed in 384 well format.[19]

potency in both enzymatic and cellular assays compared to **11g**. We were encouraged by the fact that the initial set of analogs demonstrated good in vitro permeability and solubility. The increased chemical reactivity of the cyclopentyl amine versus the hindered [2,2,2]-bicyclooctyl amine allowed for exploration of more diverse chemical space, and thus **11h** was selected over **11f** as a starting point for further exploration.

We next sought to determine the optimal terminal substituent in the context of the cyclopentyl group. A versatile synthetic route allowed exploration of a wide range of terminal groups as outlined in Scheme 2. Hydrazide **17** was accessed through the coupling of hydrazine **8** and (1*R*,3*S*)-3-((*tert*-butoxycarbonyl)amino)cyclopentane-carboxylic acid with EDC and was readily cyclized to the desired 6*H*-pyrrolo[2,3-*e*][1,2,4]triazolo[4,3-*a*]pyrazine with thionyl chloride and TEA. Basic hydrolysis of the tosyl group followed by acidic cleavage of the Boc-protected amine afforded amine **18** as a dihydrochloride salt. Reaction of **18** with pyrrolidine-1-carbonyl chloride and TEA yielded urea **19**. Cyclopentylamine salt **18** was also subjected to amidation using 2-cyanoacetic acid with coupling agents EDC and HOBt; when this initial coupling failed, addition of perfluorophenyl-2-cyanoacetate to the reaction mixture resulted in conversion to **20**. Alternatively, hydrazide **17** can

be cyclized with thionyl chloride and TEA, followed by Boc deprotection under acidic conditions to give amine **21**, also as a dihydrochloride salt. Coupling of **21** with either cyclopropanecarboxylic acid or cyclopropylacetic acid and EDC followed by basic tosyl group hydrolysis led to amides **22** and **23**. Reaction of **21** with piperidine-1-sulfonyl chloride and Hunig's base followed by tosyl group removal yielded sulfonyl urea **24**. Finally, chlorine displacement of 5-chloropyrazine-2-carbonitrile with **21** and subsequent deprotection afforded aminoheterocycle **25**.

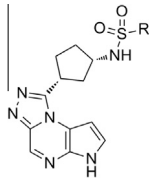
The primary amine, urea, amide, sulfonyl urea, and aminoheterocycle analogs (**18**–**20** and **22**–**25**, Table 3) all led to decreased potency in enzyme and/or cellular assays, confirming that the sulfonamide linker (as in **11h**) was optimal for Jak1 potency. Based on this data, we refocused our attention on optimization of the sulfonamide capping group in order to improve Jak1 potency and Jak1 versus Jak2 cellular selectivity.

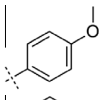
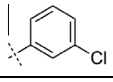
Multiple sulfonamide capping groups were evaluated (Table 4). Although both alkyl- (**26**–**28**) and cycloalkyl- (**29**–**31**) sulfonamides were tolerated, the cycloalkyl-analogs **11h**, **29**, and **30** appeared to be optimal from a combined potency and selectivity standpoint. **11h** was selected for further profiling based on the highest microsomal stability. Aryl sulfonamides **32**–**34** were generally potent but had significantly lower microsomal stability and were not advanced.

Compounds of interest were advanced into rat PK studies (Table 5). Compound **11a** had poor oral absorption as indicated by the low Cmax and AUC. Compound **11f** demonstrated moderate Cmax and total AUC compared to **11a**; however non-linear PK was observed upon dose escalation. In contrast, **11h** and **29** demonstrated acceptable oral exposure and IV half-life although plasma clearance was relatively high, warranting further optimization. In addition, **11h** and **29** demonstrated greater than 100-fold selectivity in an in-house kinome panel of 80 kinases broadly covering the kinome tree. Next, we evaluated these compounds in an acute in vivo model of pro-inflammatory cytokine production as a readout of Jak1 inhibition. Encouragingly, both compounds demonstrated significant levels of inhibition of Con A induced IFNγ production in rat.[21]

In summary, we have described initial optimization of 6*H*-pyrrolo[2,3-*e*][1,2,4]triazolo[4,3-*a*]pyrazines as Jak1 inhibitors. Compounds demonstrating a promising combination of Jak1 potency, Jak1 versus Jak2 cellular selectivity and desirable PK properties were identified. Further efforts to generate optimized Jak1 inhibitors from this series with improved in vivo efficacy will be described in due course.

Table 4
In vitro Jak enzyme and cellular activity



Compound	R	Jak1 cell (μM)	Jak2 cell (μM)	Jak2/Jak1 cellular selectivity	Microsomal stability, % remaining, human/rat[20]
11h		0.11	1.9	17×	100/98
26		0.69[a]	23.7	34×	na
27		0.6[a]	21.1	101×	—/100
28		0.92[a]	>20	>20×	na
29		0.08	18.2	227×	85/80
30		0.2	20.5	100×	90/68
31		0.67[a]	>50	>75×	na
32		0.69[a]	23.4	34×	42/31
33		0.69	18.2	26×	35/61
34		0.12[a]	6.51	54×	8/6

[a] Assays performed in 384 well format.[19]

Table 5
PK in male Sprague–Dawley rats

Compound	IV CLp[a] (L/h/kg)	PO $AUC_{0\text{-}inf}$[b] (μg·h/mL)	PO C_{max}[b] (μg/mL)	PO F(%)	Rat Con A[19] (%) @ 10 mg/kg
12	na	0.28	0.17	na	—
11a	na	0.05	0.01	na	0%
11f	3.1	0.41	0.06	13	31%
11h	2.9	1.1	0.23	29	55%
29	2.5	0.86	0.31	32	40%

[a] IV dose and vehicle—5 mg/kg in 10% EtOH, 65%PEG-400 qs. D5 W.
[b] PO dose and vehicle—10 mg/kg in 0.02% Tween 80 in 0.5% HPMC.

Disclosure

M.F., K.E.F., A.A., M.A.A., H.D., J.J.E, D.M.G., J.S.G., E.G., D.H., B.L., M.M., G.S., K.S., E.T., S.V.E., J.V., L.W., K.W and N.W. are employees at AbbVie.

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18. In vitro Jak1 kinase activity assay used Jakl kinase domain (aa 845–1142), biotin-TYR2 peptide (Biotin-(Ahx)-AEEEYFFLFA-amide; 2 μM) and 1 μM ATP in 50 mM MOPSO pH 6.5, 10 mM $MgC1_2$, 2 mM $MnC1_2$ (2 mM), DTT (2.5 mM), BSA (0.01% w/v), 0.1 mM Na_3VO_4 and ATP (0.001 mM). After 60 min, the enzymatic reaction was quenched by addition of EDTA (to final concentration 100 mM) and developed by addition of PT66-K (europium labeled antiphosphotyrosine antibody cat #61T66KLB Cisbio, Bedford, MA) and 3.12 μg/mL phycolink streptavidin-allophycocyanin acceptor (cat #PJ25S, Prozyme, San Leandro, CA). Following incubation for 60 min, 96-well plates were read via a Rubystar detector (BMG) using a 337 nm laser for excitation and emission wavelength of 665 nm. In vitro Jak2, Tyk2 and Jak3 enzymatic assays were performed in a similar fashion.
19. IL-6 dependent pSTAT3 in TF-1 and Epo-dependent pSTAT5 assays in UT-7 cells were conducted in 96 well plates unless indicated by Λ in which case they were conducted in 384 well plates as described in the Supplemental methods in Goedken, E. R.; Devanarayan, V.; Harris, C. M.; Dowding, L. A.; Jakway, J. P.; Voss, J. W.; Wishart, N.; Jordan, D. C.; Talanian, R. V. *J. Biomol. Screen.* **2012**, *17*, 857.
20. Microsomal stability assay: 25 min incubation at 37 °C, 0.25 mg microsomal protein, 1 mM NADPH and 1 μM compound. Measured % remaining at 25 min. For human a pool of male/female microsomes was used; for rat a pool of male SD rats microsomes was used.
21. Concanavalin A (con A) induced IFN inhibition assay: male lewis rats were given Con A at 10 mg/kg (0.002 mL/g body weight) iv at $t = 0$. Con A was formulated in Dulbecco's Phosphate Buffered Saline 1X. Compounds were dosed at $t = -30$ min. All compounds were formulated in 0.5% HPMC/0.02% tween80 (polyoxyethylene-sorbitan Mono-oleate) in MQ water (95%). Rats were cardiac bled at $t = 4$ h. IFN-gamma and IL-2 were measured by ELISA.

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