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Pyrrolo[1,2-*f*]triazines as JAK2 inhibitors: Achieving potency and selectivity for JAK2 over JAK3

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ABSTRACT

SAR studies of pyrrolo[1,2-*f*]triazines as JAK2 inhibitors is presented. Achieving JAK2 inhibition selectively over JAK3 is discussed.

JAK2 is a member of the Janus kinase (JAK) family of non-receptor tyrosine kinases comprising four family members (JAK1, JAK2, JAK3 and TYK2). Each JAK is composed of two carboxy terminal kinase domains, JH1 and JH2, with the JH2 domain representing a pseudokinase domain that negatively regulates enzymatic activity.[1] The amino terminal half of the protein contains a FERM domain (short for band 4.1 ezrin, radixin and moesin) that associates with cytokine receptors. JAKs are essential components to cytokine receptor signaling; they are activated upon ligand binding and subsequently phosphorylate a STAT (signal transducers and activators of transcription) which is translocated to the nucleus to initiate gene expression.[2] Imbalances in the JAK-STAT pathway have been implicated in various inflammatory diseases and cancers.[3]

The recent discovery of activating mutations in the tyrosine kinase gene, JAK2, and constitutive activation of the JAK2-STAT pathway in a large number of Philadelphia chromosome negative myeloproliferative disease patients has ignited considerable interest in these diseases, and has highlighted JAK2 as a therapeutic intervention point for drug discovery efforts.[4] Significant medical need exists as the current standard of care is only palliative, and does not change the course of these diseases.

High sequence homology, however, with other JAK family members has posed a major challenge to the design of selective JAK2 inhibitors.[5] Given that other JAK family members are involved in the regulation of immune function, it is important to maintain selectivity for JAK2 over these family members in order to mitigate the risks associated with undesired immunosuppression.[6] Several JAK2 inhibitors with varying selectivity profiles are currently being evaluated in preclinical settings as well as in clinical trials.[7] Herein we report initial 'hit to lead' optimization efforts that led to the identification of the potent and selective JAK2 inhibitor, pyrrolotriazine **29**.

A subset of an internal compound collection was evaluated in vitro in JAK2 and JAK3 kinase assays.[8] Pyrrolotriazine **1**, exhibiting weak JAK2 and JAK3 inhibitory activity, was identified as a hit. The modular nature of this chemotype made it an attractive starting point to explore SAR. Furthermore, preliminary molecular modeling studies of the chemotype in an X-ray structure of JAK2[9] suggested that the C4 substituent binds to the hinge region of the JAK2 catalytic domain, while the C2 substituent binds near the ribose pocket. Since a number of the JAK2 and JAK3 residues in this region are not conserved and the P-loop conformations appear to differ between the two, at least when compared to a

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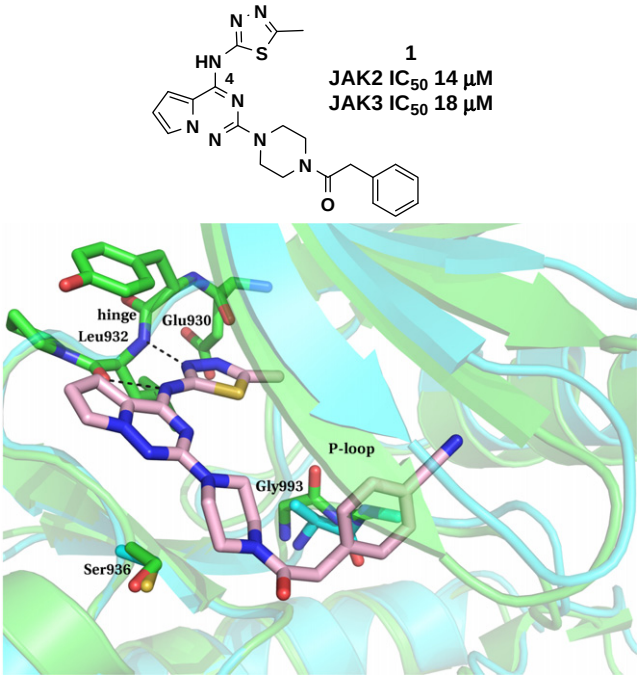
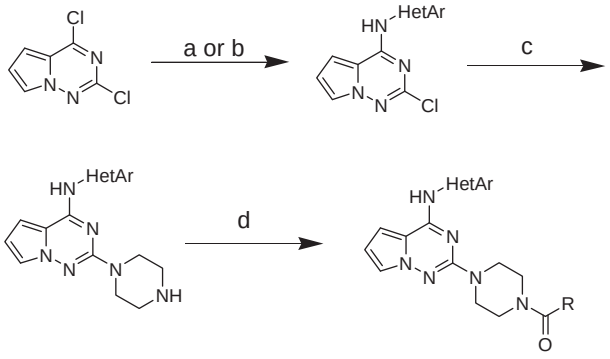


Figure 1. A binding model of thiadiazole **1** (carbons in magenta) in JAK2 (carbons and ribbon in green) superposed with an X-ray structure of JAK3 (carbons and ribbon in cyan). Two key residues that differ between the two kinases are highlighted, namely JAK2 Ser936 (Cys in JAK3) and Gly993 (Ala in JAK3). Hydrogen bonds to the hinge are indicated by dotted lines.



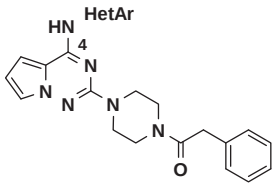
Scheme 1. Preparation of 2,4-disubstituted pyrrolo[1,2-*f*]triazines. Reagents and conditions: (a) $HetArNH_2$, Et_3N, *i*PrOH, 45 °C, 16 h; (b) $HetArNH_2$, NaH, DMF; (c) piperazine, NMP, 125 °C, 2 h; (d) RCOOH, HATU, DIPEA, DMF, rt, 18 h.

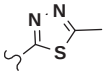
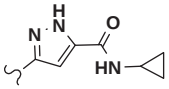
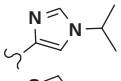
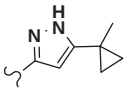
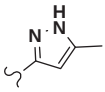
published JAK3 structure,[10] compound **1** offered reasonable potential for achieving selectivity (Fig. 1).

At first, we explored various heteroaromatic amines as C4 substituents. The results are summarized in Table 1. The poor potency of lead thiadiazole **1** can be attributed to repulsive interaction between the lone pair of electrons on N4 of thiadiazole with carbonyl of Glu-930. When thiadiazole is replaced with an unsubstituted thiazole (compound **5**), this repulsive interaction is replaced with a weak attractive interaction between the CH at 4 position of thiazole and Glu-930. Further variations at C4 revealed that 5-methylpyrazole **7** was more potent than 5-cyclopropylmethylpyrazole **6**, possibly due to small size of the binding pocket. 5-Methyl pyrazole **7** exhibited a good balance between JAK2 potency, selectivity versus JAK3 and cellular potency (proliferation of SET-2 cell line is dependent on JAK2 activity[11]) and hence, was chosen for investigating SAR at the C2-position. No clear trend in selectivity versus JAK1 was observed with in the compounds disclosed herein.[12]

The compounds were synthesized via sequential SNAr reactions of dichloropyrrolotriazine as outlined in Scheme 1. The first SNAr

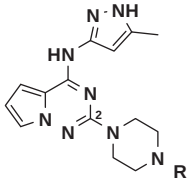
Table 1
JAK2 inhibition and selectivity data for various heteroaromatic anilines at C4

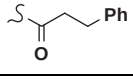


Compd	HetAr=	JAK2 IC_{50} (μM)	JAK3/ JAK2	JAK1/ JAK2	SET-2 (IC_{50}, μM)
1		14	1.3	nd	0.69
2		2.7	19	0.75	1.4
3		0.19	41	11	nd
4		0.11	15	1.3	0.67
5		0.059	37	2.6	1.2
6		0.022	9.0	7.7	0.98
7		0.0028	19	4.5	0.37

nd = not determined.

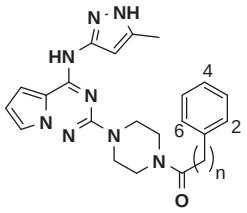
Table 2
JAK2 inhibition and selectivity data for various piperazine substituents at C2



Compd	R=	JAK2 IC_{50} (μM)	JAK3/JAK2	JAK1/JAK2	SET-2 (IC_{50}, μM)
8		0.031	15	16	nd
9		0.022	5	nd	nd
10		0.0093	11	5.0	nd
11		0.0022	19	13	0.062
7		0.0028	19	4.5	0.37
12		0.0020	11	1.7	0.076

nd = not determined.

Table 3
JAK2 inhibition and selectivity data for various piperazine substituents at C2



Compd	*n*	Phenyl-substituent			JAK2 IC_{50} (μM)	JAK3/ JAK2	JAK1/ JAK2	SET-2 IC_{50} (μM)
		2	4	6				
13	0	Me			0.0030	33	12	0.16
14	0		Me		0.0045	3.9	1.1	0.059
15	0	CN			0.0060	27	1.7	0.14
16	0		CN		0.0048	1.3	0.7	0.0062
17	0	OMe			0.0035	24	4.5	0.090
18	0	Cl			0.0073	22	3.0	0.071
19	0	Br			0.0021	47	9.1	0.18
20	0	SO_2Me			0.0011	94	3.9	0.19
21	1	Me			0.0053	9.6	1.7	0.12
22	1		Me		0.0011	20	2.8	0.31
23	1	F			0.0046	17	3.8	0.17
24	1		F		0.0009	25	6.5	0.054
25	1		OMe		0.0012	30	7.9	0.63
26	1		Cl		0.0006	16	nd	0.59
27	1	F	F		0.0024	22	2.1	0.054
28	1	F	Cl	F	0.0010	67	3.8	0.080
29	1	F	OMe	F	0.0018	69	3.4	0.11

nd = not determined.

displacement reaction at C4 with electron rich heteroaryl amines proceeded at room temperature with triethylamine as base. However, electron deficient heteroaryl amines required a stronger base such as sodium hydride to effect complete reaction at C4. The second SNAr reaction at C2 required heating the reaction mixture in NMP up to 125 °C.[13]

Investigation of N-substitution of the piperazine revealed improvements in JAK2 potency upon acylation of nitrogen (Table 2). Further, larger acyl groups containing an aromatic ring were preferred as exemplified by compounds **11**, **7** and **12**. Amongst the acyl substituents, benzoyl compound **11** and phenacetyl compound **7** exhibited the best selectivity versus JAK3. Based upon these results and the fact that certain residues differ near the C2 binding pocket of JAK2 and JAK3, compounds **11** and **7** were chosen for further fine tuning of the selectivity against JAK3. This SAR is summarized in Table 3.

Amongst the benzoyl compounds ($n = 0$), substitution at the 4-position of the phenyl ring improved SET-2 potency at the expense of JAK3 selectivity (e.g., **14** and **16**). However, substitution at the 2-position of the phenyl ring consistently provided greater selectivity (compared to substitution at 4-position) versus JAK3 (see **13**, **15**, **17**–**20**) with retention of cellular potency. Further substitutions such as 2,4- on the phenyl ring did not lead to any appreciable improvements in selectivity (data not shown). In the case of phenacetyl compounds ($n = 1$), electron withdrawing substituents at the *ortho* and *para* positions afforded no significant improvements in JAK3 selectivity (e.g., **23**–**27**). However 2,4,6-trisubstitution on the phenyl ring led to the identification of potent and JAK3 selective (>60-fold) compounds (e.g., **28–29**) with excellent antiproliferative activity in the SET-2 cell line. The SAR could be explained by analysis of an X-ray structure of compound **29** bound to the JAK2 kinase domain (Fig. 2, PDB id code 3Q32) which was in agreement with the docking-based hypothesis described earlier. The increased potency of the C4 pyrazole ring analogs was rationalized by the observation that the pyrazole basic nitrogen accepts a hydrogen bond from the amide NH of hinge residue

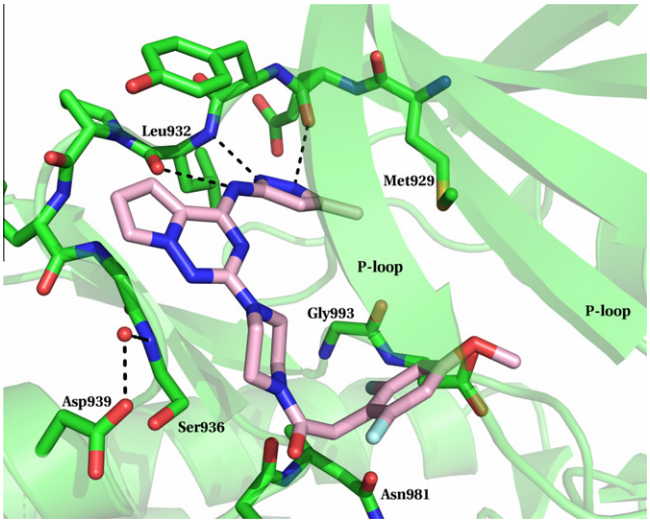


Figure 2. X-ray crystal structure of pyrrolotriazine **29** (carbons in magenta) bound to JAK2 (carbons and ribbon in green). Hydrogen bonds to the hinge are indicated by dotted lines.

Leu932, and the pyrazole NH donates a hydrogen bond to the hinge carbonyl of Glu930. The *ortho* substituent on phenyl ring binds near Gly in JAK2 versus Ala in JAK3, which might explain the modulation in selectivity observed with *ortho* substituted compounds. In addition, the benzyl group is tucked under the P-loop which may also be a source of selectivity between JAK family members because of subtle packing differences mentioned by authors of an analysis of JAK3 and TYK2 X-ray structures in comparison with JAK1 and JAK2 structures.[14]

In summary, hits to lead optimization established SAR at the C-2 and C-4 positions of the initial pyrrolotriazine hit, and led to the discovery of analogs (**20**, **28** and **29**) with significantly improved in vitro biochemical and cellular potency and JAK3 selectivity. No significant pattern in selectivity versus JAK1 was observed across the set of compounds disclosed in this manuscript. Unfortunately, most of the compounds suffered from poor metabolic stability (data not shown). Efforts towards achieving improved metabolic stability will be forthcoming.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.01.022.

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