

Identification of Potent, Selective JAK2 Inhibitors Using a Fragment-Based Screening Approach

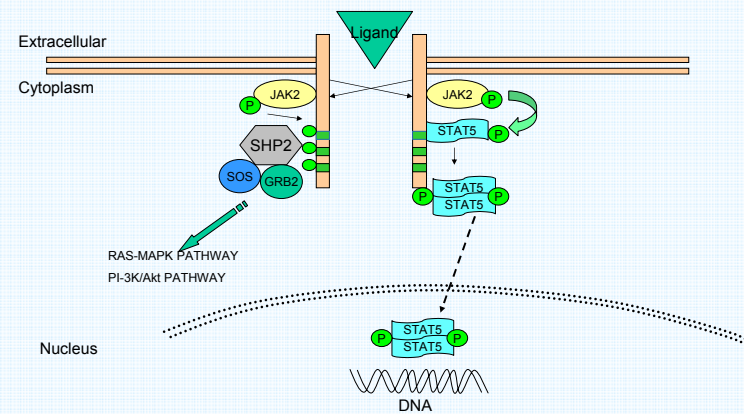
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INTRODUCTION

Janus Kinase 2 (JAK2) has become a key target in myeloproliferative diseases (MPD) since the discovery of the activating JAK2V617F mutation in a significant proportion of MPD patients. This mutation, found in the pseudokinase domain of JAK2, causes the kinase to become constitutively active, upregulating the phosphorylation of Signal Transducer and Activator of Transcription (STAT) 5 and signalling through this pathway. This results in cytokine-independent proliferation of cells expressing erythropoietin receptors.

Signalling through JAK2 pathway

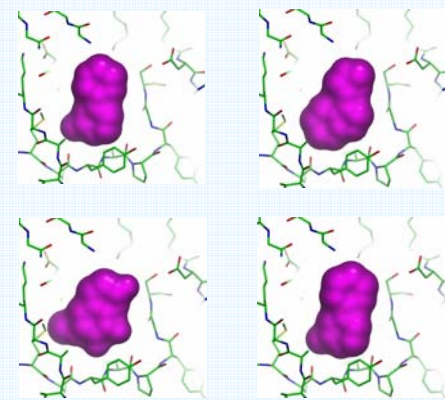


IDENTIFICATION AND OPTIMISATION OF FRAGMENT HITS

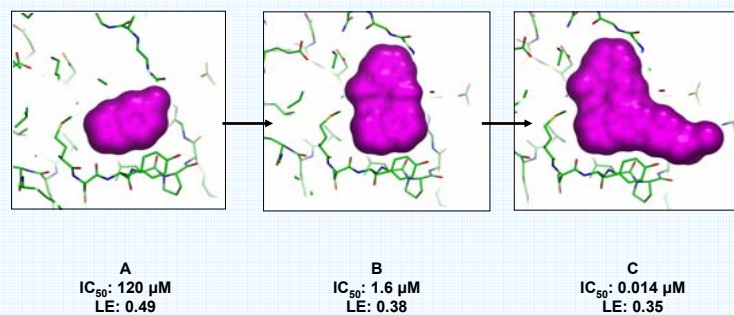
A novel soakable X-ray system was established for the kinase domain of JAK2. Astex's fragment-based screening approach, Pyramid™, was then used to screen our fragment libraries and identify multiple low molecular weight compounds that bound to this kinase domain. More than 10 distinct chemotypes were identified.

Selected weakly binding hits were then optimised into potent lead compounds using a structure-guided approach. Potent inhibitors were identified in a number of series.

Fragment hits bound to the JAK2 kinase domain



Optimisation of a fragment hit into a potent JAK2 inhibitor



INHIBITION OF JAK2 BY LEAD COMPOUNDS

Potent nanomolar inhibitors of the isolated JAK2 enzyme were identified. These inhibitors were tested in a number of JAK2-dependent cell-based systems. The effect of compounds on JAK2-driven proliferation was assessed in an engineered TEL-JAK2 Ba/F3 cell line and phosphorylation of STAT5, the direct downstream substrate of JAK2, was monitored by in-cell western in the V617F JAK2 activated human erythroleukemia (HEL) cell line. Compounds were identified with μM potency in both these systems.

Potency of lead compounds against isolated JAK2 enzyme and in JAK2-dependent cellular systems

Compound	Inhibition of JAK2 enzyme IC ₅₀ (nM)	Inhibition of TEL-JAK2 Ba/F3 proliferation IC ₅₀ (μM)	Inhibition of STAT5 phosphorylation in HEL cells IC ₅₀ (μM)
1	3.2	0.59	0.51
2	6.6	0.74	3
3	7.1	2.9	2
4	23	4.1	1.7
5	28	2.1	2.5
6	43	47% at 3 μM	NT

Cell proliferation was inhibited in a number of JAK2-activated cell lines including those carrying the V617F JAK2 mutation (HEL, SET-2) as well as the TEL-JAK2 engineered Ba/F3 line.

Inhibition of JAK2-dependent proliferation

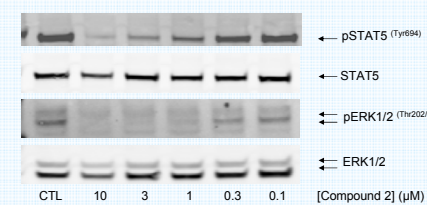
Compound	Inhibition of JAK2-driven Ba/F3 proliferation IC ₅₀ (μM)	Inhibition of HEL proliferation IC ₅₀ (μM)	Inhibition of SET-2 proliferation IC ₅₀ (μM)
2	0.74	1.2	0.84
3	2.9	1.9	3.1

HEL and SET-2 cell lines were obtained from DSMZ, Germany. TEL-JAK2 Ba/F3 cell line was engineered in-house.

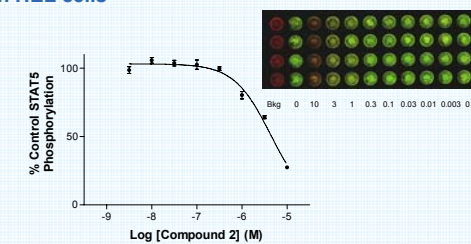
INHIBITION OF JAK2 SIGNALLING

Compounds were evaluated for their ability to inhibit JAK2 signalling. Compounds inhibited phosphorylation of the direct downstream substrate STAT5 and also phosphorylation of downstream signalling markers such as ERK in both V617F JAK2-activated cell lines (HEL) and cytokine-stimulated TF-1 cells with wild type JAK2. Inhibition was seen at concentrations consistent with the inhibition of proliferation.

Inhibition of V617F-activated JAK2 signalling pathways in HEL cells

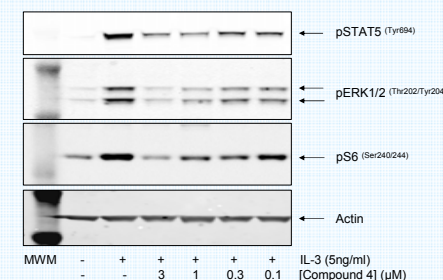


HEL cells treated with indicated concentrations of compound 2 for 1 hour



Levels of pSTAT5, measured by in cell western, in HEL cells treated with varying concentrations of compound 2.

Inhibition of IL-3 stimulated wild type JAK2 signalling pathways in TF-1 cells



TF-1 cells treated with the indicated concentrations of compound 4 for 2 hours and then stimulated with IL-3 to activate the JAK2 signalling pathway.

SELECTIVITY OF LEAD COMPOUNDS

The selectivity of our potent inhibitors for JAK2 over other kinases, including JAK-family kinases, was investigated. High levels of selectivity were seen for JAK2 over non-JAK family kinases with good selectivity over JAK1 and JAK3 and less selectivity for the other JAK-family member Tyk2.

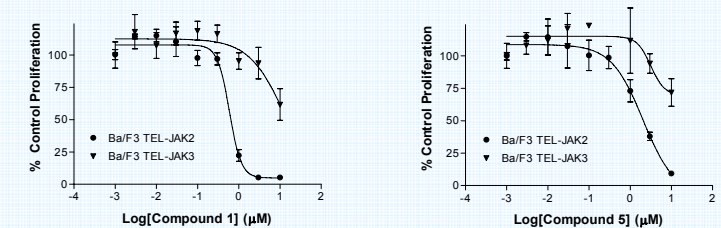
Selectivity of lead compounds for JAK2 over other kinases

Compound	JAK2 IC ₅₀ (nM)	JAK3 IC ₅₀ (nM)	JAK1 IC ₅₀ (nM)	Tyk2 IC ₅₀ (nM)	FGFR3 IC ₅₀ (nM)	Aurora B IC ₅₀ (nM)	CDK4 % inhibition
1	3.2	150 (47-fold)	380 (120-fold)	~30 (9-fold)	3900 (1200-fold)	2800 (880-fold)	9% at 100 μM (> 5000-fold)
5	28	1200 (43-fold)	1800 (64-fold)	220 (8-fold)	5100 (180-fold)	3500 (125-fold)	9% at 1000 μM (> 5000-fold)
6	43	1300 (30-fold)	6400 (150-fold)	530 (12-fold)	8100 (190-fold)	8200 (190-fold)	29% at 300 μM (> 5000-fold)
4	23	290 (13-fold)	300 (13-fold)	130 (6-fold)	4000 (170-fold)	840 (36-fold)	2% at 30 μM (> 1000-fold)

Selectivity for JAK2 over JAK3 was further investigated in cells using Ba/F3 cell lines activated by TEL-JAK2 and TEL-JAK3 respectively. The selectivity of compound 1, which showed 47-fold selectivity for JAK2 over JAK3 in isolated enzyme systems, translated into the cellular system with inhibition of proliferation in the JAK2 system being at least 17-times more potent than in the equivalent JAK3-driven system.

Selectivity for JAK2 over JAK3 in cells

Compound	JAK2 Ba/F3 Proliferation IC ₅₀ (μM)	JAK3 Ba/F3 Proliferation % Inhibition	Fold Selectivity
1	0.59	38% at 10 μM	> 17
5	2.1	28% at 10 μM	> 5
6	~3	38% at 10 μM	> 3
4	4.1	55% at 10 μM	2



CONCLUSIONS

- A fragment-based screen of JAK2 identified multiple low molecular weight hits, which were rapidly optimised into potent inhibitors
- Lead compounds were identified with sub-10 nM potency against JAK2, sub-μM potency in cellular systems and a mechanism of action consistent with JAK2 inhibition.
- Examples from the series show selectivity for JAK2 over JAK3 both against isolated enzymes and in a cellular system