In-vitro evaluation of a new potent, selective pan-Janus kinase (JAK) inhibitor VR588

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Rationale

Janus kinases (JAK) transduce multiple cytokine receptors reported to play an important role in asthma and COPD. VR588 is a selective and potent pan-JAK inhibitor suited to inhalation delivery and therefore may represent a new opportunity for the treatment of these conditions. These studies were designed to characterise the *in-vitro* kinase inhibition profile of VR588 and to determine its selectivity versus non-JAK kinases.

Methods

Comparison of kinase inhibitory activity was conducted in a non-cell (Z'Lyte™ Florescence assay) and in cell based assays using stimulation of human whole blood (IL-2 stimulated INFγ production and JAK1/JAK3 and pSTATa/b activation and IL-6 stimulated pSTAT3 to assess JAK1, JAK2 and Tyk2 activity).

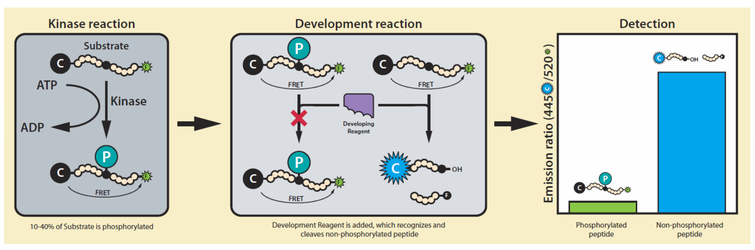


Diagram of Z'Lyte™ Florescence assay method

In addition, the selectivity of VR588 (1mM) against a panel of 93 human kinases was assessed using a competitive displacement assay. VR588 displaced DNA-tagged kinase from its immobilised active site ligand; binding to the kinases was determined by quantitative PCR (KinomeSCAN™).

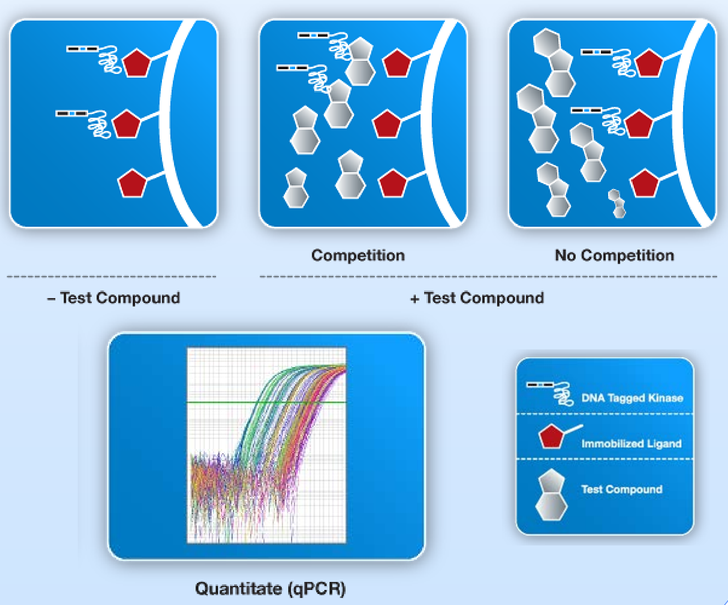


Diagram of KinomeSCAN™ method

Results I

VR588 potently inhibited JAK 1, 2, 3 and Tyk2 kinases in the non-cell based assay. Low nM potency was achieved against all JAK kinases.

Kinase inhibition IC_{50} (nM)

Kinase	VR588	Ruxolitinib (INCB-18424)	Fostamatinib (R-406)	Tofacitinib (CP-690550)
Jak3	2.1	59	75	4.7
Jak2	0.7	3.1	11.2	11.8
Jak1	4.2	5.2	136	9.1
Tyk2	6.0	1.2	11.2	19.5

Results II

In the same non-cell based assay VR588 showed poor inhibitory activity against non–JAK kinases including FLT3, PDGFB, JNK2 and Syk.

Kinase inhibition IC_{50} (nM)

Kinase	VR588	Ruxolitinib (INCB-18424)	Fostamatinib (R-406)	Tofacitinib (CP-690550)
FLT3	247	-	-	-
PDGFB	2350	-	-	-
JNK2	4000	-	-	-
Syk	8900	>3000	66	>10000

Results III

This potent and broad JAK inhibition was confirmed in the cell based assays with inhibition of IL-2 stimulated INFγ, IL-2 stimulated pSTATa/b & IL-6 stimulated pSTAT3.

IC_{50} values (nM)

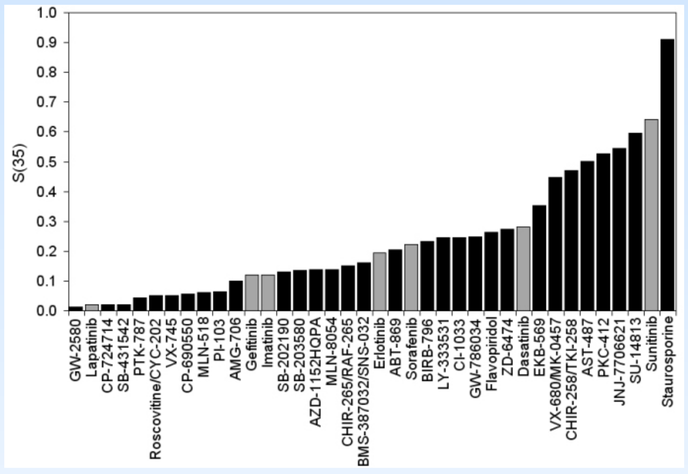
	VR588	Ruxolitinib (INCB-18424)	Tofacitinib (CP-690550)
I-2-IFNγ production (Jak1-Jak3)	209	394	188
IL-2-pSTATa/b (Jak1-Jak3)	29	34	28
IL-6-pSTAT3 (Jak1-Jak2-Jak3)	62	54	32

Results IV

Selectivity assay against a panel of human kinases revealed no relevant off- target effects. Of 93 non-mutant human kinases tested only 10 non-JAK kinases demonstrated binding of <35% of control.

Results IV

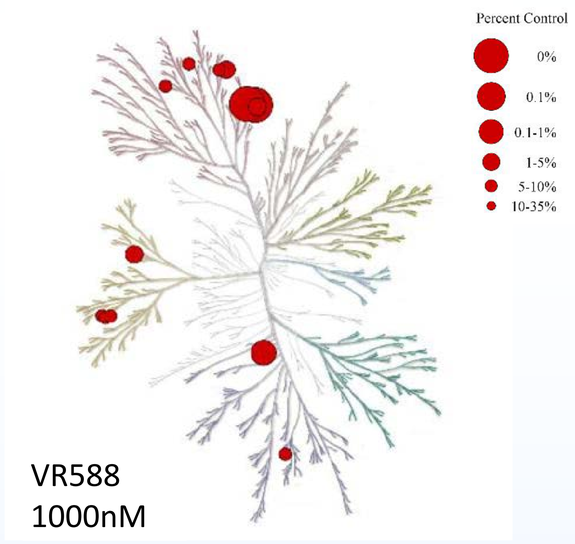
The range of these demonstrated between 14 to 128 fold lower affinity than binding to Jak2.



KinomeSCAN validation assay showing binding of 38 kinase inhibitors versus a panel of 287 human protein kinases and 3 lipid kinases. S(35) = (number of non-mutant kinases with %Ctrl<35%)/290 kinases. Compounds approved for human use (as of August 2007) are highlighted in grey. The selectivity score S(35) for VR588 against 93 kinases was 0.144.

Results V

TREE*spot*™ visualisation of kinase binding, where larger circles indicate higher affinity binding.



Conclusion

VR588 represents a potent and balanced inhibitor of the JAKs and the lack of off-target effects mitigates the potential for unwanted kinase activity. These results suggest VR588 may have utility as a pharmacological treatment of asthma and COPD and have prompted the characterisation of absorption, distribution, metabolism, and excretion (ADME) and *in-vivo* efficacy via the inhaled route.

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