Highly active and selective JAK2 inhibitor CPL-407-220 as a new drug candidate for myeloproliferative disorders

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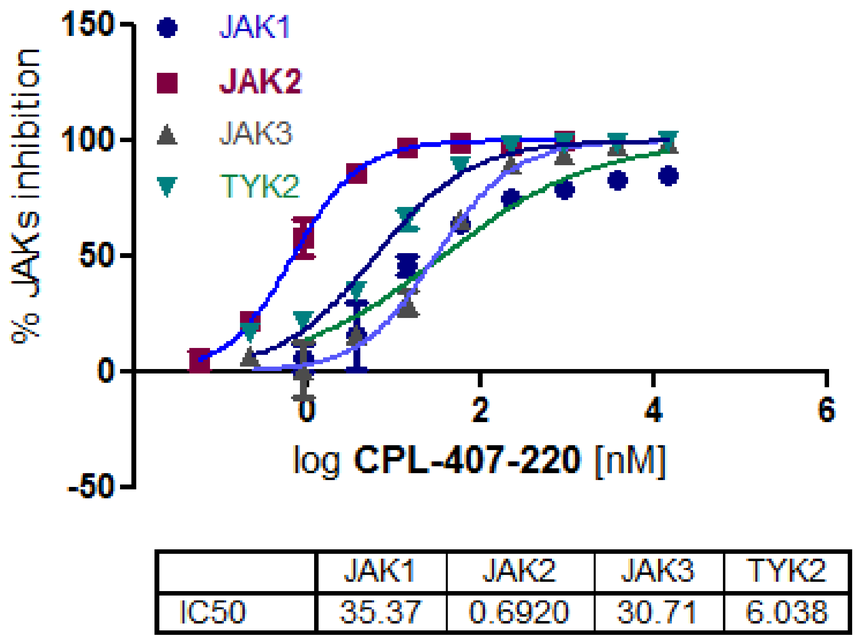
INTRODUCTION

Myeloproliferative neoplasms (MPNs) include a diverse group of cell disorders such as chronic myeloid leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). The deregulated signaling of the JAK/STAT pathway plays an important role in the pathogenesis of MPNs. JAK2 is important for hematopoietic growth factors signalling and *JAK2*V617F mutation occurs in the majority of patients with PV, ET, and PMF.

METHODS and RESULTS

Activity of CPL-407-220 was measured with ADP-Glo™ Kinase Assay (Promega). Recombinant kinase were purchase from Carna Biosciences Inc.
Our compound shows great potency against JAK2 kinase with IC_{50}=0.69 nM and has a good selectivity over other JAK family kinases: JAK1, JAK3 and TYK2 with IC_{50} of 35.37nM, 30.71nM and 6.04nM, respectively.

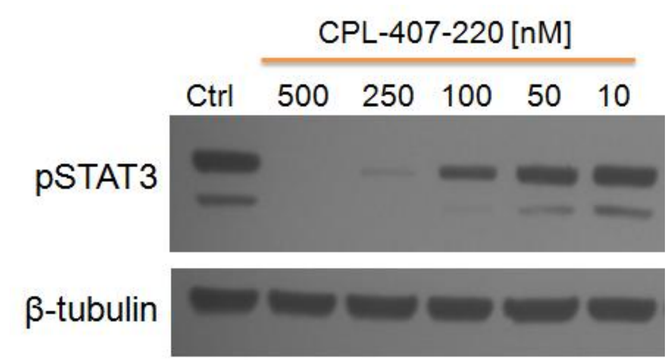


	JAK1	JAK2	JAK3	TYK2
IC50	35.37	0.6920	30.71	6.038

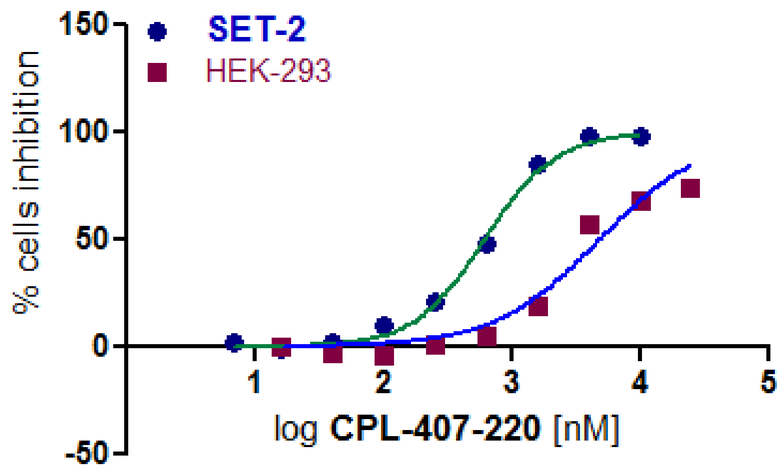
Selectivity of CPL-407-220 was investigated on a small panel of kinases. IC50 for recombinant kinases were measured with ADP-Glo™ Kinase Assay. Obtained IC_{50} values for AurA, FLT3, FGFR2, KDR, PDGFR2 and EGFR shows selectivity of approximately 28-, 51-, 19-, 773- and more than 1000-fold, respectively.

CPL-407-220	IC50 [nM] / JAK2-fold
AurA	19.11 / 28
FLT3	34.88 / 51
FGFR2	12.88 / 19
KDR	31.82 / 46
PDFGR2	498.70 / 773
EGFR	>1000

CPL-407-220 inhibitory effect was studied with JAK2-dependent HEL92.1.7(*JAK2V617F*), cell line purchased from ATCC. Cells were treated with tested compound for 1 hour and Western Blot analysis was performed. Our compound inhibits phosphorylation of STAT3 with IC_{50}=59.06nM.



The inhibition of cancer cells viability was examinated with SET-2 cells (*JAK2617F*), and the cytotoxicity with Human Embryonic Kidney 293 cells, both lines were purchased from ATCC. The viability assays were performed after 72 hours of compound treatment by ATPlite™ Luminescence Assay (Perkin Elmer).
CPL-407-220 shows good inhibition of JAK2-dependent SET-2 cells (IC_{50}=644.8nM) and moderate cytotoxicity against HEK293 cells (IC_{50}=4.8uM).



	SET-2	HEK-293
IC50	614.2	4833

CONCLUSIONS

- A novel highly potent JAK2-selective inhibitor CPL-407-220 was developed
- CPL-407-220 significantly reduces viability of JAK2-dependent cell line and shows moderated HEK293 cytotoxicity
- Our compound is promising for further development in therapy of myeloproliferative disorders

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