Novel, highly potent and selective JAK3 inhibitor CPL-409-057 disrupting IL-4/IL-13 signaling in asthma therapy

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AIMS

- Development of a potent and selective JAK3 inhibitor
- Good *in vitro* activity with no drug-induced cytotoxicity
- Targeting specific epithelial cytokine response
- Pharmacokinetic assessment of the compound

METHODS

The activity of CPL-409-057 was measured with ADP-Glo™ Kinase Assay (Promega). Recombinant kinases were purchased from Carna Biosciences or ProQinase. Kd's were calculated by DiscoverX. Cell lines: TF-1, L-540 were purchased from DSMZ. SET-2, HEK293, HepG2 and Primary Bronchial Tracheal Epithelial Cells were purchased from ATCC. Viability assays were performed after a 72 h treatment with the compound, using ATPlite™ Luminescence Assay (Perkin Elmer). Phosphorylation of STATs was assessed for cells that have been treated with the compound for 1 h. Western Blot analysis was performed with primary antibody from Cell Signaling Technology. Eotaxin release was measured by ELISA (R&D Systems) after 72 h of cell co-incubation with CPL-409-057 and IL-4 or IL-13. Mouse liver microsomes for stability experiments were purchased from Invitrogen. The protocol was optimized for testing only phase I metabolic enzymes. For pharmacokinetic study, C57BL male mice were treated with 25 mg/kg *p.o.* and 10 mg/kg *i.v.* in 10%DMSO/40%PEG. Animal experiments were approved by the Local Ethical Committee on Animal Testing at the Medical University of Bialystok.

RESULTS

KINASE ACTIVITY AND SELECTIVITY

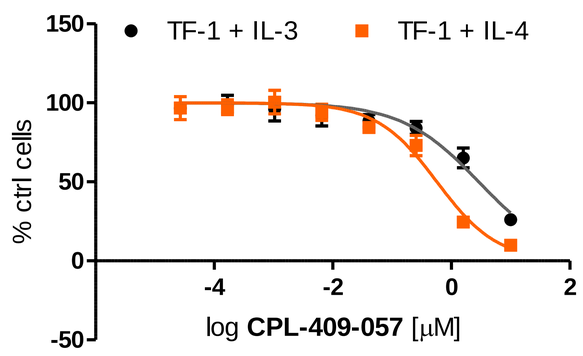
KINASE	Kd [nM]
JAK1	1.12
JAK2	0.12
JAK3	**0.056**
TYK2	3.97

KINASE	IC50 [nM]
JAK1	2.34
JAK2	0.93
JAK3	**0.02**
TYK2	44.97

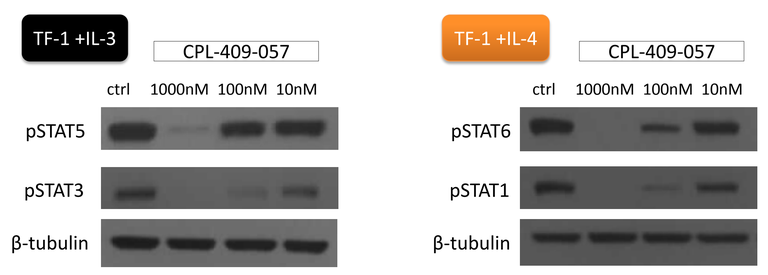
KINASE	IC50 [nM]
AURA	2210
BTK	858,1
EGFR	>15000
FGFR2	3193
FLT3	158.5
IGF-1R	3750
KDR	2875
PDGFR	48.69

IN VITRO ACTIVITY

IL-3→JAK2→STAT5
IL-4→JAK1/3→STAT1/6



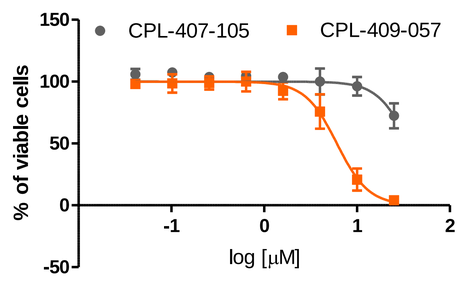
	TF-1 + IL-3	TF-1 + IL-4
IC50	2.901µM	0.5726µM



IN VITRO CELLULAR SELECTIVITY

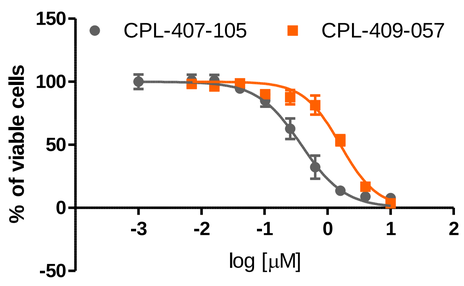
CPL-407-105 was used as a selective JAK2 inhibitor[1]

L-540 (JAK3)



	CPL-407-105	CPL-409-057
IC50	36.34 µM	6.072 µM

SET-2 (JAK2)



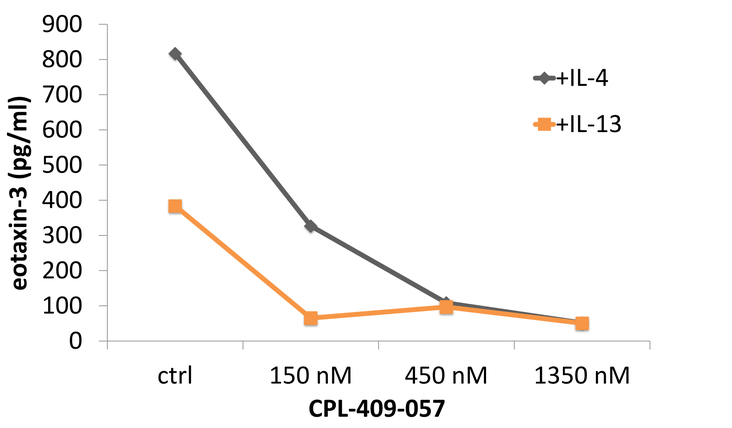
	CPL-407-105	CPL-409-057
IC50	0.3833 µM	1.599 µM

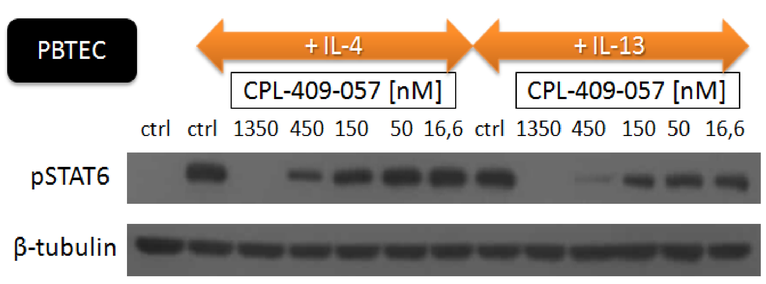
CYTOTOXICITY ON JAK-INDEPENDENT CELLS

HEK293	% of inhibition	S.D. [%]
10 µM	23.25	6.89
1.6 µM	-2.43	10.42
0.25 µM	-3.19	8.18

HepG2	% of inhibition	S.D. [%]
10 µM	57.48	2.01
1.6 µM	16.38	5.13
0.25 µM	1.78	3.55

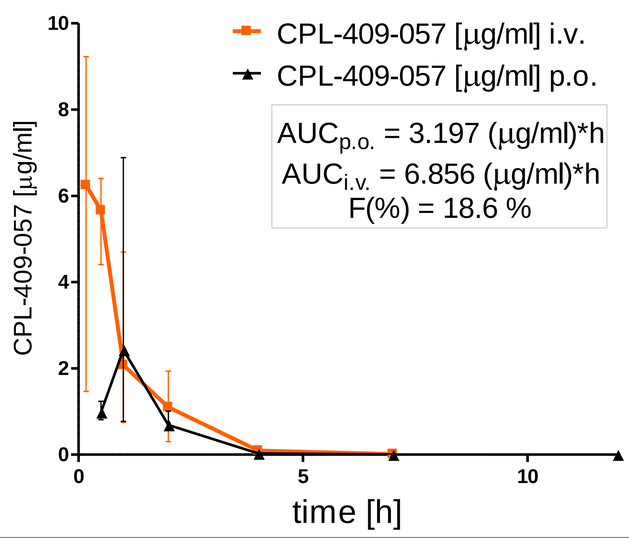
EOTAXIN RELEASE FROM PBTEC





METABOLIC STABILITY AND PHARMACOKINETICS

Compound	Cl_{int} [µl/min/µg protein]
Stable control (donepezil)	50
Unstable control (verapamil)	137
CPL-409-057	**20,1**



CONCLUSIONS

- We discovered a novel JAK3 inhibitor, potent and selective to both JAK family and small panel of diverse kinases.
- CPL-409-057 inhibition of IL4/JAK1,3/STAT6 signaling prevails over IL3/JAK2/STAT5 inhibition in TF-1 erythroleukemia cells.
- Compared to CPL-407-105 (JAK2 inhibitor), CPL-409-057 shows better activity against IL3-dependent Hodgkin lymphoma L-540 cells and better selectivity to JAK2-dependent thrombocythemia SET-2 cells.
- CPL-409-057 shows low cytotoxicity to embryonic HEK293 cells and moderate to hepatocellular carcinoma Hep G2 cells.
- Our compound strongly inhibits IL4/JAK1,3/STAT6 and IL13/JAK1,2/STAT6 signaling in primary bronchial-tracheal epithelial cells, and markedly decreases the secretion of eosinophil-attracting eotaxin-3 (CCL26).
- Low bioavailability of the compound points to other than oral route of its administration, including inhalatory route.
- CPL-409-057 emerged as an attractive candidate for further development in a therapy of Th_2-driven diseases, e.g. asthma.

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1. Gunerka P. et al. Therapeutic potential of CPL-407-105: a novel, potent JAK/STAT pathway inhibitor for the treatment of asthma. ERS 2014 Poster #P1509.