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 ProPase. Kf'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 ATP-Rbite™ 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 Białystok.

RESULTS

IN VITRO ACTIVITY

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (μM)</th>
<th>% of viable cells</th>
<th>S.D. [%]</th>
</tr>
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<tbody>
<tr>
<td>TF-1 + IL-3</td>
<td>2.901 μM</td>
<td>50</td>
<td>±3.4</td>
</tr>
<tr>
<td>TF-1 + IL-4</td>
<td>0.5726 μM</td>
<td>100</td>
<td>±0.2</td>
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IN VITRO CELLULAR SELECTIVITY

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CONCLUSIONS
- We discovered a novel JAK3 inhibitor, potent and selective to both JAK family and small panel of diverse kinases.
- 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 Th2-driven diseases, e.g. asthma.