Pharmacokinetics of selective PI3Kδ inhibitors after intratracheal instillation in mice

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AIMS & METHODS

The aim of this study was to characterize biological activity of a family of potent PI3Kδ-selective inhibitors and analyze their pharmacokinetics after intratracheal administration.

Compounds and cell lines: Raji cells were purchased from ATCC. Compounds were synthesized by Celon Pharma Medicinal Chemistry Department.

PI3K kinase activity assay: PI3Kα was purchased from Carna Biosciences and PI3Kβ, PI3Kγ, and PI3Kδ from Merck Millipore. The inhibition of examined kinases was evaluated by measuring their ability to converse ATP to ADP in the presence of increasing doses of compounds using ADP-Glo assay from Promega. IC_{50} values were calculated using GraphPad Prism version 5.03. The curves were fit using a nonlinear regression model with a log (inhibitor concentration) versus response formula.

Kinase selectivity panel: The selectivity among protein kinases was performed on the in-house panel of enzymes (Carna Biosciences) whose activity was measured at 1 µM concentration of compounds using ADP-Glo assay. No inhibition was observed for any kinase when compounds were tested up to 10 µM concentration.

Western blot analysis: For Western blot analysis cells were grown on 6-well plates and treated with the compounds for 1h. 15min before cell lysis Raji cells were stimulated with anti-IgM antibody [5 µg/ml] (Jackson Immunoresearch). The proteins were examined by Western blotting according to the protocols provided by the antibodies suppliers. The antibodies against pAkt (Ser473) and Akt1 were purchased from Cell Signaling Technology.

Metabolic stability assays: Mouse liver microsomes were purchased from Invitrogen. Donepezil and verapamil (Sigma Aldrich) were used as stable and unstable control, respectively. The protocol was optimized for testing only phase I metabolic enzymes.

Animals and experimental protocol: All animal experiments were approved by the Local Ethical Committee on Animal Testing at the Medical University of Lodz. Female BALB/C mice, 8-10 weeks of age were obtained from Nofer Institute of Occupational Medicine, Lodz, Poland. Compounds were dissolved in DMSO, diluted with 0,9%NaCl to final concentration of 0,2% DMSO and administered by a single intratracheal instillation in a volume of 2 µl per gram of body weight at the doses of 0,3 mg/kg and 1 mg/kg. Before intratracheal instillation animals were anesthetized with halothane. For pharmacokinetic analysis, plasma was sampled at 10min, 30min, and 1/2/4/7/12h. Bronchoalveolar lung fluid (BALF) was collected by lavaging lungs in situ with 2*1 ml volumes of 0,9% NaCl. After euthanasia lungs were harvested and homogenized in H_2O:DMSO (9:1) in a volume of 3ml per gram of tissue.

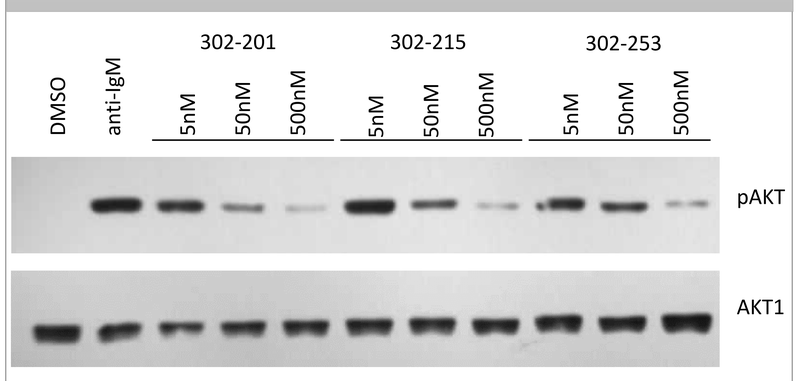
RESULTS

IN VITRO KINASE ACTIVITY

Cpd N.o.	IC_{50} PI3Kδ [µM]	IC_{50} PI3Kα [µM]	*Sel. PI3Kα [fold X]*	IC_{50} PI3Kβ [µM]	*Sel. PI3Kβ [fold X]*	IC_{50} PI3Kγ [µM]	*Sel. PI3Kγ [fold X]*
302-201	0.0066	12.47	*1890*	5.47	*830*	>60	*>9000*
302-215	0.0053	0.34	*64*	10.69	*2000*	11.03	*2000*
302-253	0.0007	1.29	*1800*	3.28	*4600*	27.07	*>38000*

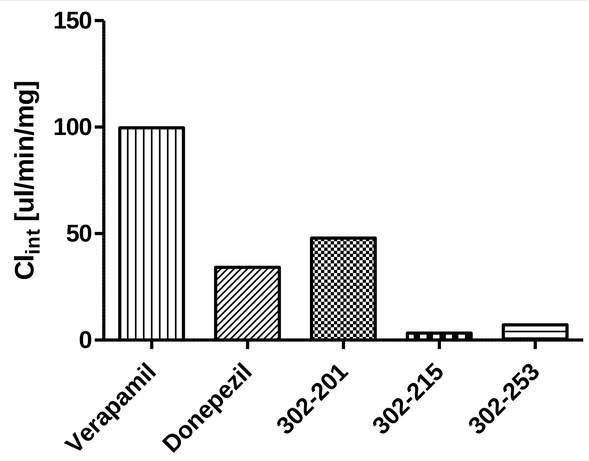
Obtained results indicated that all tested inhibitors blocked PI3Kδ kinase activity at low nanomolar or sub-nanomolar concentration. The compounds had no inhibitory activity on protein kinases from our in-house selectivity panel (data not shown). Although compounds' properties differ in terms of selectivity within PI3K family, selectivity ratios remain high.

LOSS OF AKT PHOSPHORYLATION IN RAJI CELLS



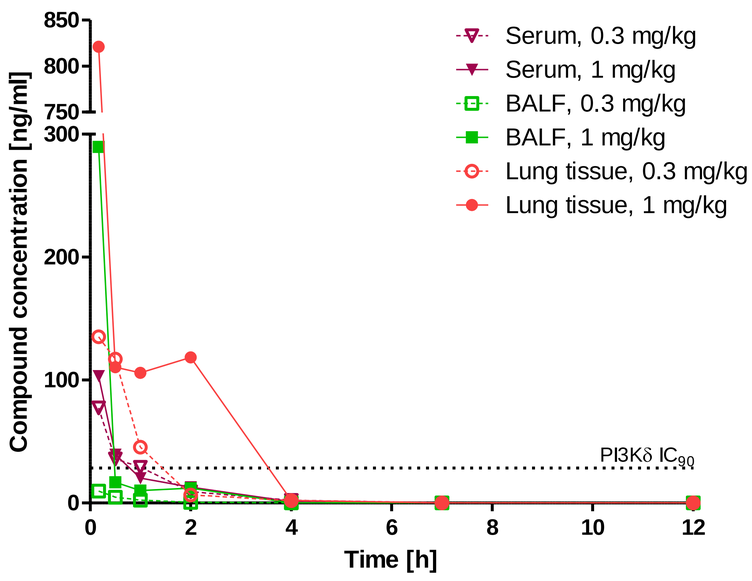
Western blot analysis has shown that inhibitors from tested series significantly inhibited phosphorylation of AKT in Raji cell line after anti-IgM stimulation. Both 302-201 and 302-215 achieved IC_{50} values close to 50 nM, with 302-253 having slightly lower effect in this concentration. All three compounds significantly lowered pAKT levels in the concentration of 500 nM.

METABOLIC STABILITY



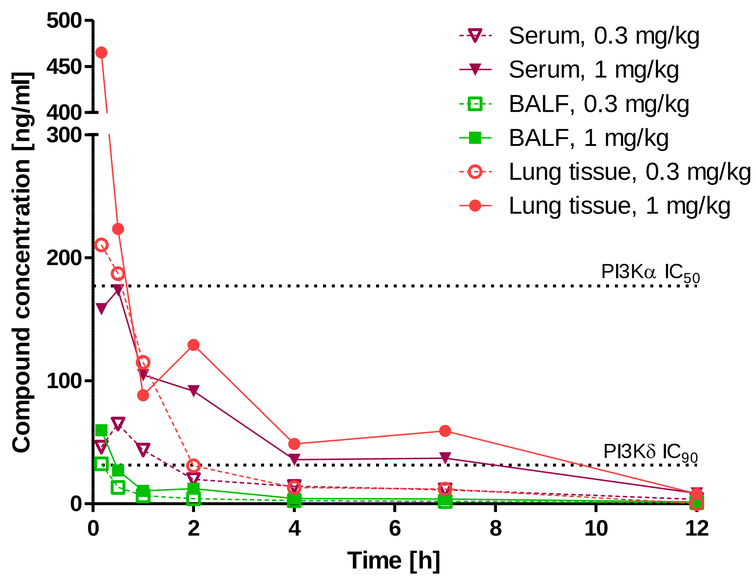
Metabolic stability assays using mouse liver microsomes showed that 302-201 had the highest clearance from tested compounds. That would be preferred when the expected route of administration was by inhalation. 302-215 and 302-253 were much more stable, having clearence values ten times lower than donepezil (3.3 and 7.3 vs 34.2 µl/min/mg, respectively).

302-201



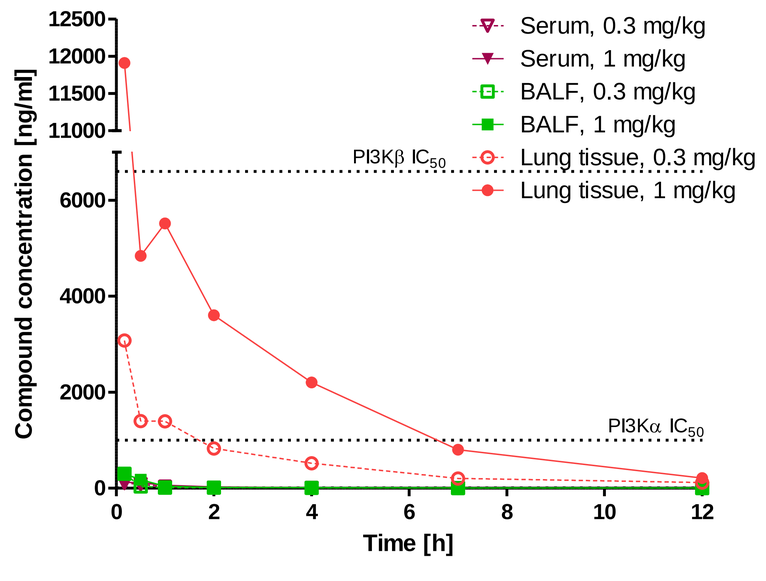
302-201 absorbed quickly, showing very rapid loss of the analyte in BALF. It achieved low concentration in serum, similar for both doses used. In lung tissue the compound showed rapid drop of concentration 30 min after administration of the higher dose, followed by a sustained concentration up to 2 h.

302-215



302-215 showed higher levels of the compound in serum, with T_{max}=0.5h. The lower dose allowed for 90% inhibition of PI3Kδ activity up to 2 h, whereas the higher dose allowed for the same effect for at least 7 h in lung tissue. PI3Kα activity could be interfered by the compound in lungs and in serum.

302-253



302-253 showed much greater concentration and retention in lung tissue compared to other compounds: IC_{90} for PI3Kδ (~7ng/ml) is exceeded up to the last sampling point (12 h) for both doses tested. Serum levels are low, pointing low systemic exposure after this route of administration.

CONCLUSIONS

- The concentration needed for 90% of inhibition of PI3Kδ was achieved in lungs for at least 2h for 302-201, 7h for 302-215 and 12h for 302-253, after administration of 1mg/kg dose.
- High selectivity of 302-201 translates for low risk of off-target activities: even in higher dose no significant inhibition of other kinases was detected.
- High retention of 302-253 in lung tissue enables possibilities of low-dose, once-a-day administration.
- Novel PI3Kδ inhibitors demonstrate very good absorption after intratracheal instillation, completely blocking PI3Kδ signalling in very low doses. Drug administration by inhalation would be beneficial for low systemic exposure of PI3Kδ inhibitors.

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