Discovery of Novel Small Molecule Inhibitors of Multiple Influenza A Strains

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Abstract

Efficacy of drugs currently used in anti-Influenza A therapy is decreasing because of emerging viral resistance. Prosetta has discovered novel antiviral compounds, which inhibit the propagation of influenza A virus, by employing a unique moderate throughput screen (MTS) based on the interaction of viral proteins with cellular host-factors. MTS of a small molecule chemical library identified seventeen distinct chemical series whose activity was validated against Influenza A H7N7, (fowl plague virus, (Bratislava) in MDCK cells. In this live virus cell culture assay, six chemical series showed EC50<20 µM, which resulted in the selection of PAV-8667 as the Pre-lead series. The synthesis of a small diversity set surrounding this series indicated a robust SAR existed and PAV-8667 is currently undergoing optimization for potency, ADMET, and safety profiles. Currently, the series has produced multiple compounds displaying EC50<1 µM. One compound from this series, PAV-616 shows EC50=10 nM, EC99=100 nM with a CC50 of 2.5-10 µM. Early pharmacokinetic studies in mice have shown promise, as PAV-616 shows a mean residence time >24h, Cmax >84 nM and 31% bioavailability after oral application at 3.6 mg/kg. Compounds in the series have been tested on Influenza A H1N1 (Puerto Rico 8) strain and showed activity comparable to that against FPV. Further optimization of potency, ADMET and safety profiles are underway as this series will soon enter animal efficacy studies.

Fluorescent 384-Well Plate Assay for a Viral Capsid Assembly Drug Screen

A cell-free system capable of assembling putative influenza nucleocapsids was developed and adapted to a 384-well moderate through-put ELISA format for screening small molecules modulators of virus assembly.

C : Capsid Protein Complex

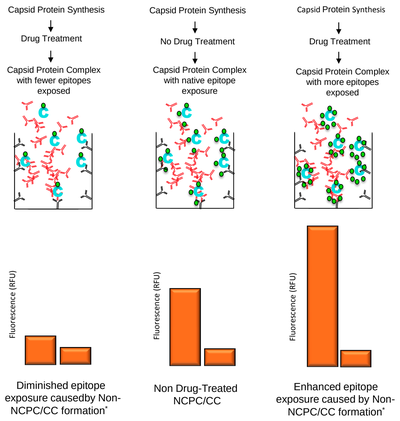
: Epitope

: Biotinylated (Detection) Antibody

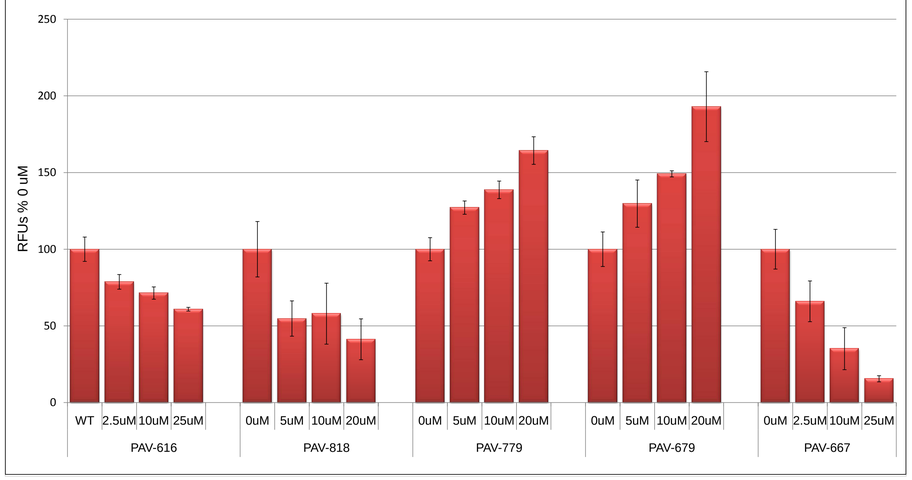
: Immobilized (Coating) Antibody

NCPC/CC:
Native **C**apsid **P**rotein **C**omplex
Completed **C**apsid

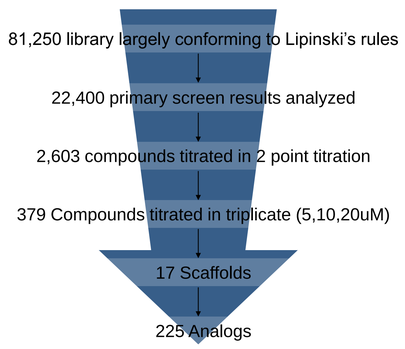
*Non-NCPC/CC formations include aberrant capsid protein complexes and completed capsids, assembly intermediates, and formations that give increased/decreased capture efficiency as compared to NCPC/CC formations



Representative Data from the FLUV NP Plate Assay

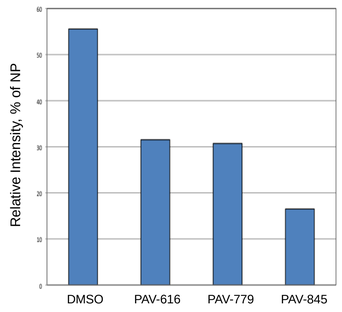


FLUV Nucleoprotein Moderate Throughput Plate Assembly Assay Results

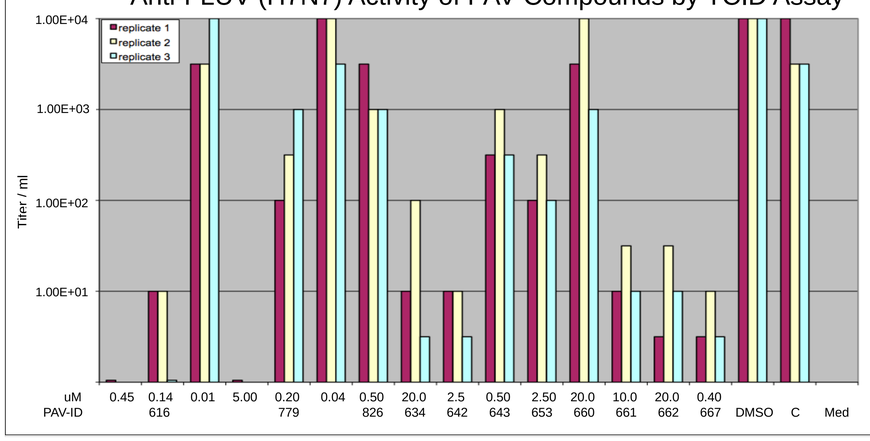


Translation of Nucleoprotein in the Presence of Anti-FLUV Compounds Alter the Sensitivity of NP-Complexes to Protease Digestion

Influenza NP mRNA was translated in the presence of DMSO (vehicle) or 20 uM of PAV-616, PAV-779 or PAV-845. Nucleoprotein complexes were isolated by sucrose gradient velocity sedimentation. The complexes were subjected to proteolytic digestion (0.24 ug/ml proteinase K, at 4ºC for 1 hr), the protease inactivated and the reaction products analyzed by SDS-PAGE. Densitometry was used to determine the degree of nucleoprotein digestion. Four samples were analyzed for each condition. The results indicate that the structure of the NP complexes is altered by the presence of PAV compounds, presumably by influence on host factors assisting assembly. (This experiment was performed by Nicole Wolcott, CUBRC, SUNY Buffalo, Buffalo NY.



Anti-FLUV (H7N7) Activity of PAV Compounds by TCID Assay



A Lead Series With Robust SAR is Active Against Multiple FLUV Strains

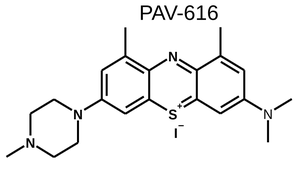
PAV-667
EC_{50} < 0.01 µM
EC_{99} > 0.10 µM
CC_{50} > 0.10 µM
SI = 10

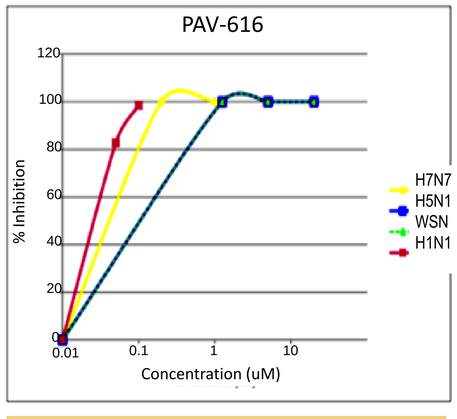
PAV-742
EC_{50} = 0.25 µM
EC_{99} = 2.0 µM
CC_{50} = 6.0 µM
SI = 24

PAV-779
EC_{50} = 0.05 µM
EC_{99} = 0.15 µM
CC_{50} > 10 µM
SI >200

PAV-616
EC_{50} = 0.02 - 0.1 µM
EC_{99} = 0.08 – 1 µM
CC_{50} > 10 µM
SI >500

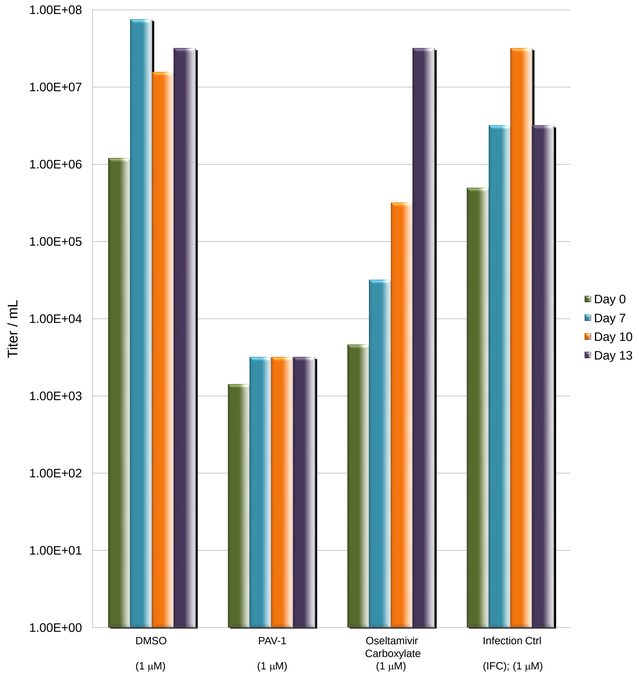
PAV-616





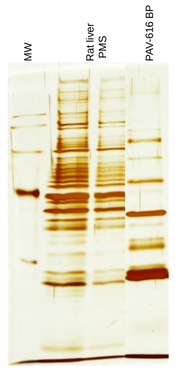
- Lead series PAV-8667 shows robust SAR in live virus assay
- PAV-616 is active against multiple strains of the influenza virus
- Lead series PAV-8667 has excellent drug-like properties
- Further series optimization is underway for PK, Safety and animal efficacy studies

Comparison of Oseltamivir Carboxylate vs. PAV-1 For Influenza Resistance Development

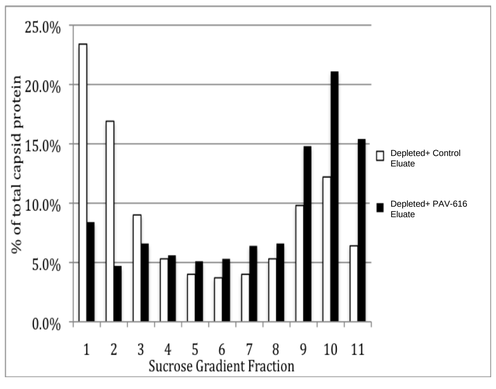


The serial passage of the WSN strain of FLUV was performed daily on MDCK cell in the presence of antiviral agents (PAV-1 and the active form of Tamiflu) at 1 mM concentration. Over the course of 2 weeks, the FLUV developed full resistance to Tamiflu. In contrast, PAV-1 inhibited the virus at comparable levels from day 1 through day 13. The study was run with several Prosetta compounds (with only PAV-1 shown) targeting the FLUV, and all have displayed a lack of resistance development.

PAV-616 Binding Proteins Promote FLUV Nucleoprotein Oligomeric Complex Formation



Proteins captured by PAV-616 Sepharose from a rat liver post-mitochondrial supernatant (PMS) fraction were eluted by soluble PAV-616, analyzed by 12% SDS-PAGE and stained with silver.



Influenza virus nucleoprotein mRNA was translated in vitro for 1 hour in a translation system that was depleted of PAV-616 binding proteins. Puromycin was then added to cause chain termination and the sample was then placed on ice. Aliquots were taken, then supplemented with either control column eluate or the PAV-616 eluate. Both samples were then further incubated, sedimented through gradients of sucrose, fractionated and the proteins analyzed by SDS-PAGE. The distribution of the NP polypeptides was determined by densitometry.

Summary and Future Directions

1. Prosetta has developed broad pathway-wide screens for viral nucleocapsid assembly modulators including influenza virus. Hits from this assay have been validated by live virus cell culture testing of inhibitors on H1N1, H5N1, H7N7, H3N2 and WSN strains.
2. Prosetta has successfully optimized lead small-molecule series to improve both efficacy and toxicity profiles.
3. Preliminary results show no influenza virus resistance to Prosetta compounds to date in head to head time course with oseltamivir carboxylate as a control.
4. Prosetta will continue with SAR optimization while moving forward into animal efficacy studies.
5. Prosetta has identified potential macromolecular targets of the most active anti-influenza compounds.
6. Prosetta will pursue studies to determine point of action of active compounds in the influenza nucleoprotein assembly pathway and identify protein targets that directly contact active compounds.

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