Structural characterisation and inhibition of Arenavirus replication complex elements: assembly, function and inhibition of embedded nucleases

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Structural characterisation and inhibition of Arenavirus replication complex elements: assembly, function and inhibition of embedded nucleases.


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## INTRODUCTION

Arenaviruses, belonging to a family of emerging enveloped segmented and ambisense RNA viruses associated with neurological and hemorrhagic diseases in humans. Arenavirus transcription and genome replication are catalyzed by a ribonucleoprotein replicase complex NP-L. After penetration, L protein initiates transcription to produce NP and L mRNAs[1]. The primary transcription is the result of a cap-snatching mechanism ensured by an endonuclease domain associated to the L polypeptide. As the concentration of NP in the cell increases, genome segments are replicated, to produce full-length copies (cRNA).

## RESULTS

### ARENAVIRUSES

Arenaviruses pipe-line

[Diagram of Arenavirus pipe-line]

- Digestion products analyzed in urea PAGE
- FP signal recorded during 30 min, every 30s. RNA substrate concentration (100 nM). ExoN concentration (0.1 μM to 1.6 μM). Average ± SEM of three independent experiments.

### VIRON STRUCTURE

Assessment of inhibition and cell viability

Scatter plot showing the % of inhibition in the presence of the inhibitor

### NP characterization

Arenavirus Nucleoprotein Architecture

Structural model fitting structural data

### Structural similarities with Narvirus In ExoN MOA & Inhibition by ion chelation

C-terminal ExoN multimerisation tail

Arepavirus exoN

### Screening Methodology

1. Activity measured by FP for LCMV ExoN
2. Screening of 113 compounds (20 μM) against LCMV ExoN by FP.
3. Assessing IC₅₀, of compound ID by FP

**FP data validated by RNA visualization in gel**

- In vitro
- In cellulo
- Assessing inhibition and cell viability

### ARENAVIRUSES

- **VIRION STRUCTURE**
  - **GENOME ORGANIZATION**
  - **ENDONUCLEASE INVOLVEMENT IN viral LIFE CYCLE**

### CRITICAL COMPARISONS

Global comprehension of biological system is critical to achieve proper understanding of the MOA of an enzyme and to develop inhibition strategy. Over the last decade we have characterized several structural and activities of the key proteins of Arenaviral replication complex, both apo and in complex with inhibitors targeting the two nucleases. Compounds targeting both viral transcription and viral impairment of innate immunity present lesser the chances of cell resistant mutations.

We proved the transversal approach studies that fluorescence polarization (FP) method is reliable, sensitive to monitor nuclease activity and feasible in 384-well plates. The method is robust, rapid and non-destructive allowing the visualization of the RNA substrate degradation using gels as orthogonal method of confirmation. We identified several hit inhibitors of vir alarenavirus ExoN activity and determined by fluorescence polarization their IC₅₀ at a micromolar level. Then we validated their activities in cell culture assays and we found compounds displaying efficiency at micromolar level against Junin virus a new world Arenavirus. These results highlight the effectiveness of translational approach in the screening of compounds libraries for the discovery of antivirals.

**REFERENCES**


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