AWARD NUMBER: W81XWH-16-1-0692

TITLE: Selective AAK1 and GAK Inhibitors for Combating Dengue and Other Emerging Viral Infections

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REPORT DATE: October 2018

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From - To)*
October 2018	Annual	15 Sep 2017-14 Sep 2018

4. TITLE AND SUBTITLE
Selective AAK1 and GAK Inhibitors for Combating Dengue and Other Emerging Viral Infections

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-16-1-0692

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Geneva Foundation
Tacoma, WA 98402

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research
And Material Command
Fort Detrick, MD 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)
USAMRMC

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT
We discovered an Achilles' heel of unrelated viruses: a requirement for AP2-associated protein kinase 1 (AAK1) and cyclin G-associated kinase (GAK), host kinases that regulate clathrin adaptor proteins-mediated pathways. Our data point to AAK1 and GAK as "master regulators" of viral infection and attractive targets for broad-spectrum antivirals. We discovered that approved anticancer drugs that target these kinases; sunitinib and erlotinib, potently inhibit replication of multiple viruses *in vitro* and reduce mortality in mice infected with DENV and EBOV. This approach is now being advanced to the clinic for both of these indications. Nevertheless, while sunitinib and erlotinib are quite potent inhibitors of AAK1 or GAK, respectively, they are not selective and are therefore associated with toxicity resulting from inhibition of other host cell kinases. The goals of this proposal are to: optimize novel, chemically distinct, selective lead AAK1 and GAK inhibitors targeting validated virus-host interactions and already demonstrating great promise against DENV, and advance their development to a near-IND stage. This approach would also protect against biothreat agents from eight viral families, including EBOV and CHIKV.

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USARMRMC
Unclassified	Unclassified	Unclassified	Unclassified		19b. TELEPHONE NUMBER *(include area code)*

TABLE OF CONTENTS

	Page
1. Introduction	1
2. Keywords	1
3. Accomplishments	1
4. Impact	3
5. Changes/Problems	4
6. Products	6
7. Participants & Other Collaborating Organizations	8
8. Special Reporting Requirements	10
9. Appendices	11

1. **INTRODUCTION:** *Narrative that briefly (one paragraph) describes the subject, purpose and scope of the research.*

The Dye lab will be responsible for the *in vitro* and *in vivo* testing of selective AAK1 and GAK inhibitors against filoviruses and alphaviruses. Our efforts will provide the efficacy data for the selective AAK1 and GAK inhibitors in cell culture and rodent models of authentic filovirus and alphavirus infection.

2. **KEYWORDS:** *Provide a brief list of keywords (limit to 20 words).*

Broad-spectrum, anti-viral, filovirus, Ebola virus, Marburg virus, alphavirus, chikungunya virus, mouse model

3. **ACCOMPLISHMENTS:** *The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction.*

What were the major goals of the project?

List the major goals of the project as stated in the approved SOW. If the application listed milestones/target dates for important activities or phases of the project, identify these dates and show actual completion dates or the percentage of completion.

Our overall goal is to develop broad spectrum antiviral drugs with a high genetic barrier to resistance by targeting host proteins that are critical to the life cycle of multiple viruses. The major goals of the Dye lab:

1. Evaluate novel inhibitors rapidly for efficacy against multiple viruses; 2. Use efficacy and viability data gathered to feed into the compound optimization process; 3. Use our *in vitro* assays to assist in ranking compounds for preclinical studies; and 4. Demonstrate efficacy with no toxicity of lead compound(s) in rodent models of filovirus and alphavirus disease.

What was accomplished under these goals?

For this reporting period describe: 1) major activities; 2) specific objectives; 3) significant results or key outcomes, including major findings, developments, or conclusions (both positive and negative); and/or 4) other achievements. Include a discussion of stated goals not met. Description shall include pertinent data and graphs in sufficient detail to explain any significant results achieved. A succinct description of the methodology used shall be provided. As the project progresses to completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

This year, USAMRIID has tested eight candidate inhibitors, five of which had not yet been examined *in vitro* against any filovirus or alphavirus. Inhibitors were evaluated for effects on *in vitro* viability and inhibitory capacity against Ebola virus (EBOV), Marburg virus (MARV) and/or chikungunya virus (CHIKV). For viability assessment, compounds were applied to cells at a range of doses, and a commercially

available kit was used to measure ATP content as a marker of viability in uninfected cells. In select experiments, viability was also measured by a second method, to allow for more direct comparison with collaborators. Compounds/doses that do not decrease viability below 70% that of vehicle are considered inhibitory without toxicity. Briefly, cells were pretreated with inhibitor for 1h (filoviruses) or 0-48 h (chikungunya virus), incubated with virus for one hour without inhibitor present, washed, and then media containing inhibitor was added back to cells for the remainder of the experiment. At indicated time points, supernatants were collected and frozen at -80^{o}C, plates were fixed and removed from the suite, and virus infection was measured by immunofluorescence using a benchtop high content imaging system. A summary of all compounds testing to date is found in Table 1 (Appendix A). The following compounds were evaluated against EBOV *in vitro*: 2608, 2787, 2791, 7-z-oxozeaenol, RMC34, RMC35, RMC36, and RMC76. All of these compounds demonstrated activity at some level against

EBOV (Figures 1 through 3, and data not shown, Appendix B). 2608, 2787 and 7-z-oxozeaenol were tested *in vitro* against MARV and all showed efficacy (Figures 1-2 and data not shown, Appendix B). Finally, 2787 and 2608 were tested *in vitro* against CHIKV. 2608 demonstrated efficacy against CHIKV (Figure 3 and data not shown, Appendix B). While 2787 initially appeared promising, after expanding dose curves and treatment times, 2787 demonstrates unacceptable toxicity in our system at this time. Bases on these data, we are currently establishing dosing parameters to move forward with a mouse experiment evaluating the protective capacity of 7-z-oxozeaenol against Ebola virus disease in mice. The remainder of these data will also be used to prioritize optimization of compounds for animal studies going forward.

What opportunities for training and professional development has the project provided?
If the project was not intended to provide training and professional development opportunities or there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe opportunities for training and professional development provided to anyone who worked on the project or anyone who was involved in the activities supported by the project. "Training" activities are those in which individuals with advanced professional skills and experience assist others in attaining greater proficiency. Training activities may include, for example, courses or one-on-one work with a mentor. "Professional development" activities result in increased knowledge or skill in one's area of expertise and may include workshops, conferences, seminars, study groups, and individual study. Include participation in conferences, workshops, and seminars not listed under major activities.

This year, a junior member of the Dye lab had the opportunity to present our work at a scientific conference. Ms. Danielle Dorosky presented a poster at the American Association of Immunologists Annual Meeting in Austin, Texas in May of 2018.

How were the results disseminated to communities of interest?
If there is nothing significant to report during this reporting period, state "Nothing to Report." Describe how the results were disseminated to communities of interest. Include any outreach activities that were undertaken to reach members of communities who are not usually aware of these project activities, for the purpose of enhancing public understanding and increasing interest in learning and careers in science, technology, and the humanities.

In addition to results were presented at a scientific conference (see above), this work resulted in the following publication in 2018 (see below).

What do you plan to do during the next reporting period to accomplish the goals?
If this is the final report, state "Nothing to Report."

Describe briefly what you plan to do during the next reporting period to accomplish the goals and objectives.

During the next year, we will continue to confirm lead hits and test modifications of lead candidates *in vitro*, and begin evaluating the efficacy of lead candidates in rodent models of infection.

4. IMPACT: D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how findings, results, techniques that were developed or extended, or other products from the project made an impact or are likely to make an impact on the base of knowledge, theory, and research in the principal disciplinary field(s) of the project. Summarize using language that an intelligent lay audience can understand (Scientific American style).

Nothing to report.

What was the impact on other disciplines?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how the findings, results, or techniques that were developed or improved, or other products from the project made an impact or are likely to make an impact on other disciplines.

Nothing to report.

What was the impact on technology transfer?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe ways in which the project made an impact, or is likely to make an impact, on commercial technology or public use, including:

- *transfer of results to entities in government or industry;*
- *instances where the research has led to the initiation of a start-up company; or*
- *adoption of new practices.*

Nothing to report.

What was the impact on society beyond science and technology?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

- *improving public knowledge, attitudes, skills, and abilities;*
- *changing behavior, practices, decision making, policies (including regulatory policies), or social actions; or*
- *improving social, economic, civic, or environmental conditions.*

Nothing to report.

5. CHANGES/PROBLEMS: *The PD/PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency grants official whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:*

Changes in approach and reasons for change
Describe any changes in approach during the reporting period and reasons for these changes. Remember that significant changes in objectives and scope require prior approval of the agency.

Nothing to report.

Actual or anticipated problems or delays and actions or plans to resolve them
Describe problems or delays encountered during the reporting period and actions or plans to resolve them.

Due to the shutdown of containment labs at USAMRIID (beginning in May 2018 and ongoing; BSL-3 labs opened 10/09/2018 on a limited basis; estimated date of BSL-4 opening is early 2019), our efficacy evaluation of compounds in vitro ceased, and no in vivo efficacy work was initiated. We have used this time to refine viability assays

and assess compound toxicity in mice. We have initiated work to begin evaluating compound in BSL-3 with the next two weeks, and will be ready to evaluate compounds at BSL-4 as soon as containment is open.

Changes that had a significant impact on expenditures

Describe changes during the reporting period that may have had a significant impact on expenditures, for example, delays in hiring staff or favorable developments that enable meeting objectives at less cost than anticipated.

Nothing to report.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Describe significant deviations, unexpected outcomes, or changes in approved protocols for the use or care of human subjects, vertebrate animals, biohazards, and/or select agents during the reporting period. If required, were these changes approved by the applicable institution committee (or equivalent) and reported to the agency? Also specify the applicable Institutional Review Board/Institutional Animal Care and Use Committee approval dates.

Significant changes in use or care of human subjects

N/A

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. PRODUCTS: *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

- **Publications, conference papers, and presentations**
  Report only the major publication(s) resulting from the work under this award.

  Journal publications. *List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Optimization of Isothiazolo[4,3- b]pyridine-Based Inhibitors of Cyclin G Associated Kinase (GAK) with Broad-Spectrum Antiviral Activity. Pu SY, Wouters R, Schor S, Rozenski J, Barouch-Bentov R, Prugar LI, O'Brien CM, Brannan JM, Dye JM, Herdewijn P, De Jonghe S, Einav S. J Med Chem. 2018 Jul 16

  Books or other non-periodical, one-time publications. *Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like. Identify for each one-time publication: author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (e.g., book, thesis or dissertation); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).*

  Nothing to report.

  Other publications, conference papers and presentations. *Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication as noted above. List presentations made during the last year (international, national, local societies, military meetings, etc.). Use an asterisk (*) if presentation produced a manuscript.*

  See above.

- **Website(s) or other Internet site(s)**
  List the URL for any Internet site(s) that disseminates the results of the research activities. A short description of each site should be provided. It is not necessary to include the publications already specified above in this section.

  Nothing to report.

- **Technologies or techniques**
  Identify technologies or techniques that resulted from the research activities. Describe the technologies or techniques were shared.

  Nothing to report.

- **Inventions, patent applications, and/or licenses**
  Identify inventions, patent applications with date, and/or licenses that have resulted from the research. Submission of this information as part of an interim research performance progress report is not a substitute for any other invention reporting required under the terms and conditions of an award.

  Nothing to report.

- **Other Products**
  Identify any other reportable outcomes that were developed under this project. Reportable outcomes are defined as a research result that is or relates to a product, scientific advance, or research tool that makes a meaningful contribution toward the understanding, prevention, diagnosis, prognosis, treatment and /or rehabilitation of a disease, injury or condition, or to improve the quality of life. Examples include:
  - *data or databases;*
  - *physical collections;*
  - *audio or video products;*
  - *software;*
  - *models;*
  - *educational aids or curricula;*
  - *instruments or equipment;*
  - *research material (e.g., Germplasm; cell lines, DNA probes, animal models);*
  - *clinical interventions;*
  - *new business creation; and*
  - *other.*

  Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?
Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change".

Example:

Name:	*Mary Smith*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	*1234567*
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Smith has performed work in the area of combined error-control and constrained coding.*
Funding Support:	*The Ford Foundation (Complete only if the funding support is provided from other than this award.)*

Name:	Dr. Jennifer Brannan
Project Role:	Senior Research Scientist
Nearest person month worked:	6
Name:	Dr. Spencer Stonier
Project Role:	Senior Research Scientist
Nearest person month worked:	4

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active. Annotate this information so it is clear what has changed from the previous submission. Submission of other support information is not necessary for pending changes or for changes in the level of effort for active support reported previously. The awarding agency may require prior written approval if a change in active other support significantly impacts the effort on the project that is the subject of the project report.

Title:	Assessing and Characterizing the Protective Efficacy of Convalescent Human Plasma from Ebola Virus Survivors as a Treatment for Patients with Acute Ebola Virus Disease
POP:	10/01/2014-09/30/2017
Change in Support:	Previously active, but now closed.

Title:	Development and Production of Panfilovirus Therapeutic Monoclonal Antibodies
POP:	09/17/2015 − 09/16/2017
Change in Support:	Previously active, but now closed.
Title:	Structure-guided Redesign of Monoclonal Antibodies Targeting Conserved Filovirus Epitopes
POP:	07/01/2016-06/30/2018
Change in Support:	Previously active, but now closed.
Title:	Optimization of Broad-spectrum Inhibitors of Filovirus Infection that Antagonize the NPC1-GP Interaction
POP:	12/15/2017-12/14/2020
Change in Support:	Previously pending, but now active.
Title:	Inhibitors of Virus Glycoprotein-LAMP1 Receptor Binding for Lassa Virus Therapy
POP:	01/15/2018-12/31/2019
Change in Support:	Previously pending, but now active.
Title:	Synergistic Panfiloviral Entry Inhibiting Antibody Drug Conjugates
POP:	02/08/2018-01/31/2020
Change in Support:	Previously pending, but now active.
Title:	Development of CM-SV1, a Monoclonal Antibody Treatment for Sudan Virus
POP:	02/16/2018-01/31/2020
Change in Support:	Previously pending, but now active.
Title:	Development of VEEV – Specific Antibodies for Therapeutic Purposes
POP:	07/01/2018-06/09/2019
Change in Support:	Previously pending, but now active.
Title:	Advanced Preclinical Development and Production of Master Seed Virus of GEO-LM01, a Novel MBA-VLP Vaccine Against Lassa Fever
POP:	10/01/2018-09/30/2020
Change in Support:	Previously pending, but now active.

What other organizations were involved as partners?
If there is nothing significant to report during this reporting period, state "Nothing to Report."

Describe partner organizations – academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) – that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

Provide the following information for each partnership:
Organization Name:
Location of Organization: (if foreign location list country)
Partner's contribution to the project (identify one or more)

- *Financial support;*
- *In-kind support (e.g., partner makes software, computers, equipment, etc., available to project staff);*
- *Facilities (e.g., project staff use the partner's facilities for project activities);*
- *Collaboration (e.g., partner's staff work with project staff on the project);*
- *Personnel exchanges (e.g., project staff and/or partner's staff use each other's facilities, work at each other's site); and*
- *Other.*

Organization Name:	Geneva Foundation
Location of Organization:	Tacoma, WA
Partner's contribution to project:	Administrative Support
Organization Name:	Stanford University
Location of Organization:	Stanford, CA
Partner's contribution to project:	Collaboration

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES:

Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

APPENDIX A

Table 1

Compound	*In vitro* inhibition observed		
	EBOV	MARV	CHIKV
2608	yes	*yes*	yes
2787	yes	yes	no
2791	yes	nd	nd
7-z-oxozeaenol	yes	yes	nd
RMC34	yes	nd	nd
RMC35	yes	nd	nd
RMC36	yes	nd	nd
RMC76	yes	nd	nd

APPENDIX B

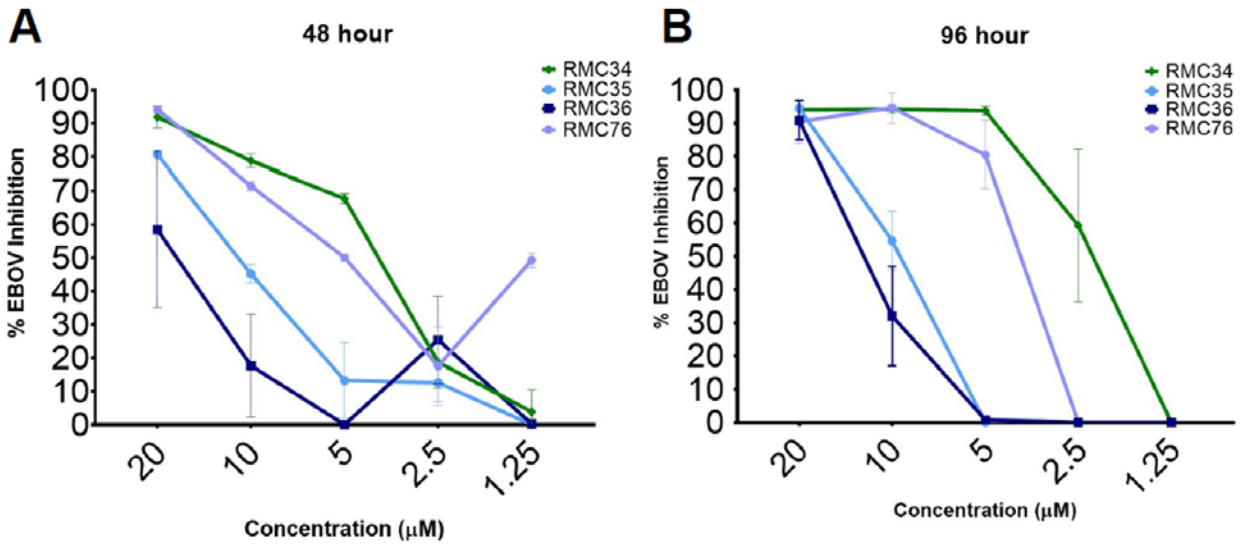

Figure 1: EBOV infection in Huh-7 cells treated with next generation kinase inhibitors. Percent of infected cells, normalized to control, is reported for EBOV infected Huh-7 cells in the presence of inhibitor after 48 (A) and 96 (B) hours.

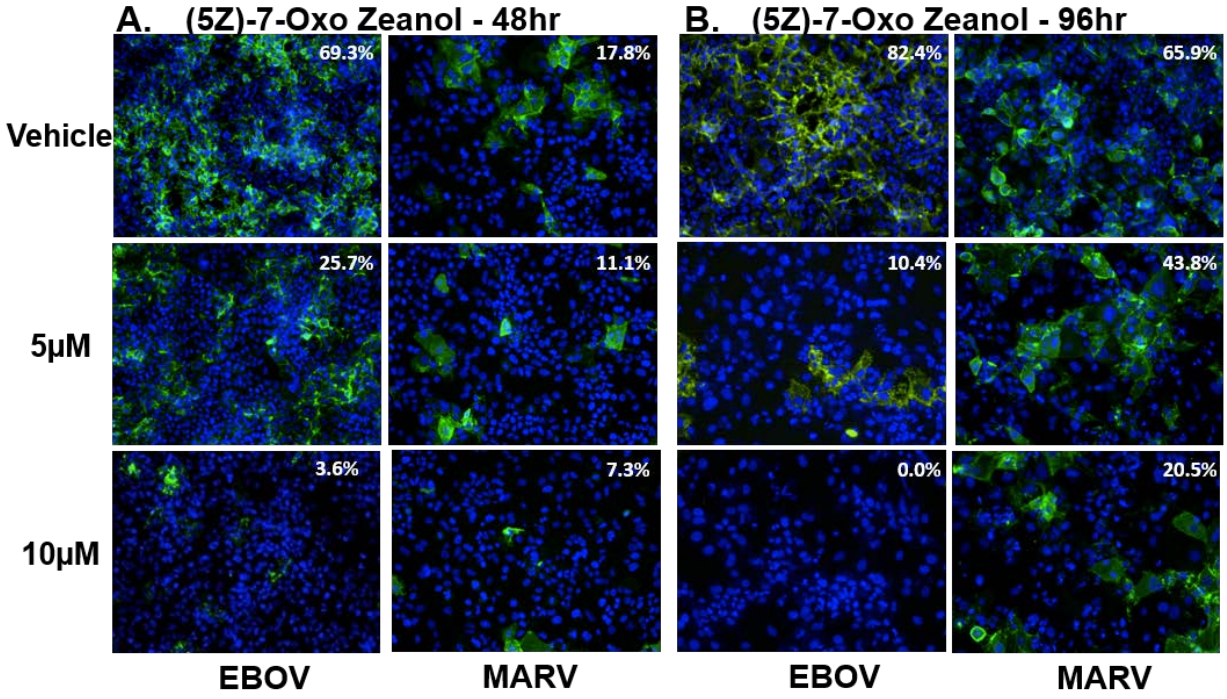

Figure 1: (5Z)-7-Oxo Zeaenol inhibits EBOV and MARV infection in Huh-7 cells. Percent of infected cells, normalized to control, is reported for filovirus infected Huh-7 cells in the presence of inhibitor. Representative images are shown for the 48 (A) and 96-hour (B) time points. Green = virus infected cells; blue = nuclei.

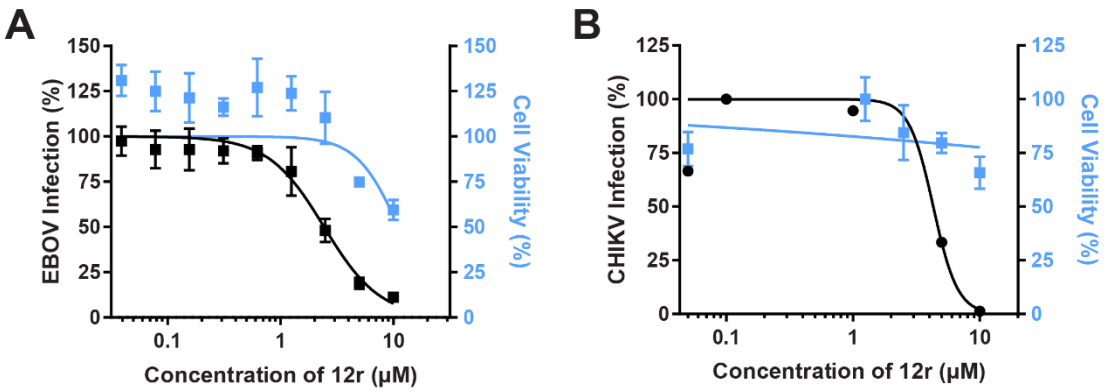

Figure 3: 2608 suppresses EBOV and CHIKV infections. Cell viability (blue) and dose response of EBOV (A) or CHIKV (B) infection (black) to compound 12r measured by immunofluorescence (A) or plaque (B) assays in Huh7 (A) or Vero (B) cells 48 hours after infection. Data are plotted relative to vehicle control. Shown are representative experiments from at least 2 conducted, each with 6 biological replicates; shown are means ± SD.