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TITLE: Antiviral Drug Discovery Targeting Zika Virus Protease

PRINCIPAL INVESTIGATOR: Yongcheng Song, PhD

CONTRACTING ORGANIZATION: Baylor College of Medicine

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14. ABSTRACT
Zika virus, transmitted primarily by mosquitos, could become endemic in the tropical and subtropical regions including the southern states and territories of the United States. It could cause catastrophic consequences to the public health, such as microcephaly (small brain/head) of newborns. However, there are no antiviral drugs or vaccines for Zika infection. Zika virus protease (ZVpro) is a viral protein that is essential for viral replication. ZVpro is therefore a drug target. The overall goal of this project is to use a combination of rational inhibitor design, medicinal chemistry, X-ray crystallography and antiviral activity testing to discover small-molecule inhibitors of ZVpro, which are potential drug candidates for Zika infection. During the second funding period, although the overall progress has been delayed due to the COVID-19 pandemic, our activities and results have been satisfactory, showing our potent ZVpro inhibitors are non-cytotoxic and have strong in vitro and in vivo anti-ZIKV activity. We will perform the experiments in accordance with the approved SOW to achieve the goals of the project in the next funding period.

15. SUBJECT TERMS
Zika virus, Antiviral, NS2B-NS3 protease, Small-molecule inhibitor, Medicinal chemistry

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

TABLE OF CONTENTS

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

Zika virus (ZIKV), transmitted primarily by mosquitos, could become endemic in the tropical and subtropical regions including the southern states and territories of the United States. It could cause catastrophic consequences to the public health, including microcephaly (small brain/head) of newborns and Guillain-Barre syndrome. However, there have been no antiviral drugs or vaccines for the prevention and treatment of ZIKV infection. Zika virus protease (ZVpro) is a viral protein that is essential for viral replication. ZVpro is therefore a drug target for ZIKV infection. The overall goal of this project is to use a combination of rational inhibitor design, medicinal chemistry, X-ray crystallography and antiviral activity testing to discover and develop potent and selective small-molecule inhibitors of ZVpro. These compounds are potential drug candidates to treat and prevent Zika infections.

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

Zika virus, Antiviral, NS2B-NS3 protease, Small-molecule inhibitor, Medicinal chemistry

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.

There are 4 major goals/tasks of the projects:
The major Task 1 is to use medicinal chemistry to develop potent ZVpro inhibitors, with the milestones (at Month 36) being ~60% accomplished.
The major Task 2 is to use biochemical and X-ray crystallographic methods to characterize ZVpro inhibitors synthesized in Task 1, with milestones (at Month 36) being ~50% accomplished.
The major Task 3 is to test cellular anti-ZIKV activity as well as cytotoxicity of selected ZVpro inhibitors identified in Task 2, with milestones (at Month 36) being ~50% accomplished.
The major Task 4 is to perform pharmacokinetics, toxicity and in vivo antiviral activity studies, with milestones (at Month 36) being ~50% accomplished

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.

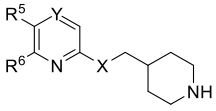
Major Task 1: Medicinal Chemistry development of ZVpro inhibitors. The objective of this Task is to use rational inhibitor design, medicinal chemistry and structure activity relationship (SAR) studies to find potent small-molecule inhibitors of ZVpro.

Subtask 1: Docking studies for designing ZVpro inhibitors. We used a drug discovery software package Schrödinger Suite for the modeling studies, with the ZVpro structure (PDB: 5LC0) being the docking template. Experimental procedure has been described in the last progress report. >300 compounds were docked and molecules showing favorable docking results were chemically synthesized in subtask 2.

Subtask 2: Structure activity relationship (SAR)-guided medicinal chemistry. The general synthesis for medicinal chemistry studies is described in the last progress report. During Year-2, ~80 new compounds were synthesized successfully.

Subtask 3: SAR and quantitative SAR (QSAR) studies. Inhibitory activity against Zika NS2B-NS3 protease (ZVpro) and Zika virus replication of synthesized compounds have been tested using the methods described below. Structure and activities of representative compounds are summarized in Table 1, from which main SARs can be deducted. Compounds **1** was initially identified to be novel inhibitors of ZVpro with IC_{50} of 21.7 μM in our compound screening. Compound **2** with a bulkier *tert*-butyl group shows significantly improved activity with IC_{50} of 3.1 μM. Compound **9** was found to be a potent inhibitor of ZVpro with IC_{50} of 200 nM. Changing the -O- linkage at 2-position to an -NH- in compound **8** (IC_{50}: 400 nM) resulted in a 2-fold activity reduction. Changing the central pyrazine ring in **8** to a pyridine in compound **6** (IC_{50}: 790 nM) further reduced the potency. As compared with compound **7** (IC_{50}: 530 nM) with a *N*-methyl secondary amine or compound **4** (IC_{50}: 1.1 μM) with an amide at the 5-position, the primary amine in compound **9** is more favored. Changing the furan-3-yl group in **9** to a pyrazol-4-yl in compound **5** (IC_{50}: 710 nM) or a fused pyrrole ring in compound **3** (IC_{50}: 1.1 μM) also decrease the inhibitory activity. Additional SARs will be analyzed for new compounds.

Table 1. Structures and activity of compounds **1**-**9**.



For **1**-**5**, **7**, **9**: X = O, Y = N
For **6**: X = NH, Y = CH
For **8**: X = NH, Y = N

Compound	R^5	R^6	ZVpro IC_{50} (μM)	ZIKV-FLR EC_{68} (μM)
1	4-Br-Ph	4-Br-Ph	21.7 ± 1.5	>10 [a]
2	4-*t*-Bu-Ph	4-*t*-Bu-Ph	3.14 ± 0.09	5.0
3	4-(NH_2CH_2)-Ph	Indol-5-yl	1.12 ± 0.07	2.50
4	4-(NH_2CO)-Ph	4-(furan-3-yl)-Ph	1.05 ± 0.07	2.50
5	4-(NH_2CH_2)-Ph	4-(pyrazol-4-yl)-Ph	0.71 ± 0.07	2.50
6	4-(NH_2CH_2)-Ph	4-(furan-3-yl)-Ph	0.79 ± 0.03	1.20
7	4-($MeNHCH_2$)-Ph	4-(furan-3-yl)-Ph	0.53 ± 0.06	1.20
8	4-(NH_2CH_2)-Ph	4-(furan-3-yl)-Ph	0.40 ± 0.05	1.20
9	4-(NH_2CH_2)-Ph	4-(furan-3-yl)-Ph	0.20 ± 0.01	0.30-0.60

[a] EC_{68} cannot be determined because **1** showed cytotoxicity at >10 μM. All other compounds had no significant cytotoxicity.

The overall progress for Task 1 has been delayed due to the COVID-19 pandemic, which caused our institute to essentially shut down research activities 3/20-5/20/2020. Despite this, our activities and results have been satisfactory, with ~200 compounds being designed and synthesized for the first 2 funding years (out of 300 compounds proposed for the project). Several structure-activity relationships have been concluded. The goal and milestone (at Month 36) for the Task have not been fully met.

Major Task 2: Biochemical and X-ray crystallographic characterization of ZVpro inhibitors. The objective of this Task is to perform enzyme inhibition, X-ray crystallographic and other biochemical studies to characterize compounds made in Task 1, which will be used for rational design and SAR studies in Task 1 to find compounds with improved potency.

Subtask 1: Expression and inhibition of ZVpro. Expression, purification of recombinant ZVpro as well as the biochemical assay to determine the activity and inhibition of ZVpro have been described in the last progress report. New batches of recombinant ZVpro were obtained and compounds synthesized in Task 1 were tested for their inhibitory activities against ZVpro, among which potent small-molecule inhibitors (such as compound **9**) were identified with IC_{50} as low as 200 nM. Activity of selected compounds are shown in Table 2.

Moreover, using similar methods, we expressed and obtained recombinant NS2B-NS3 proteases of dengue serotype-2, -3 and West Nile viruses. These and Zika viruses belong to the same family of Flavivirus and are important human pathogens with similar virology. Activity of selected compounds against these viral proteases were also tested and results are shown in Table 2. In general, ZVpro inhibitors exhibited comparable inhibitory activities against other Flavivirus proteases.

Table 2. IC_{50} (μM) of compounds **3**-**9** against Flavivirus proteases.

Compound	ZVpro	Dengue-2 protease	Dengue-3 protease	West Nile protease
3	1.12 ± 0.07	0.64 ± 0.03	0.54 ± 0.01	0.93 ± 0.03
4	1.05 ± 0.07	0.98 ± 0.02	0.93 ± 0.02	1.34 ± 0.01
5	0.71 ± 0.07	0.21 ± 0.04	0.20 ± 0.07	0.12 ± 0.02
6	0.79 ± 0.03	0.86 ± 0.01	0.86 ± 0.02	1.27 ± 0.03
7	0.53 ± 0.06	0.73 ± 0.02	0.53 ± 0.01	0.87 ± 0.02
8	0.40 ± 0.05	0.29 ± 0.06	0.21 ± 0.07	0.51 ± 0.04
9	0.20 ± 0.01	0.59 ± 0.02	0.52 ± 0.06	0.78 ± 0.02

Subtask 2: Enzyme selectivity for ZVpro. As high selectivity is required for these compounds to be less toxic or interfering to normal physiology, potent ZVpro inhibitor compound **9** was tested for its activity against a panel of 5 selected human proteases. They play important physiological roles and were chosen from 4 protease families with different modes of action, including serine proteases trypsin and DPP4 (dipeptidyl peptidase 4), aspartic protease pepsin, cysteine protease caspase-3, and metalloprotease MMP-8 (matrix metalloprotease 8). We used commercially available assay kits of these proteases to determine the inhibitory activities. As shown in Table 3, compound **9** had no inhibitory activities against these human proteases. These results show that compound **9** is a broadly active inhibitor of Flavivirus proteases with a high selectivity.

Table 3. Inhibitory activity of compound **9** against 5 human proteases**.**

	IC_{50} values (μM) against human proteases (% inhibition at 10 μM)				
	Trypsin	DPP4	Pepsin	Caspase-3	MMP-8
Cpd **9**	>10 (8.3%)	>10 (26%)	>10 (35%)	>10 (30%)	>10 (9.3%)

Subtask 3: X-ray crystallography of ZVpro in complex with selected inhibitors. Methods for crystallization, data collection and structure determination and refinement have been described in the last progress report. We determined X-ray structures of the related dengue-2 NS2B-NS3 protease, which has a highly similar 3-D structure to ZVpro, in complex with compounds **5**, **8** and **9** at 2.7-3.0 Å. The three structures are very similar to each other, with each asymmetric unit containing two inhibitor-bound proteins. Similar to the apo-protein, the protease-inhibitor complexes adopt an open conformation, with NS2B binding partially to NS3. The inhibitor-bound NS3 does not deviate significantly from the apo- or substrate-bound protein, except that the residues 152-164 are disordered (with no observed electron density) upon inhibitor binding. In contrast, the U-shaped peptide segment is well organized in both the apo- and substrate-bound NS3. In the latter case, residues 152-164 constitute part of the S1 and S2 pockets of the active site and have interactions with the substrate. Inhibitor binding pushes the loops 71-75 and 117-122 outwards by ~1.3 Å and 3 Å. All of these movements remodel the surface of NS3 and create a deep, L-shaped pocket that accommodates the inhibitors. The compounds are allosteric inhibitors, which do not occupy the substrate binding site. Mechanistically, these inhibitors bind to and stabilize the protease in the open conformation, which prevents NS2B from folding into the active site as well as the binding of the substrate. The central pyrazine ring of **9** is located at the junction of the L-shaped pocket. The furanylphenyl group is deeply inserted into the pocket with favorable hydrophobic interactions. The 2- and 5-substituents occupy a deep surface groove, having mostly hydrophobic interactions. The positively charged -NH_2 of compound **9** has hydrogen-bond and electrostatic interactions with Asp75, one of the protease catalytic triad. Crystallization and structure determination of ZVpro in complex with our inhibitors are on-going.

Subtask 4: Other biochemical/biophysical characterization of selected ZVpro inhibitors. In accordance with the approved SOW, we will start this in Year-3.

The overall progress for Task 2 has been delayed due to the COVID-19 pandemic, which caused our institute as well as Synchrotron facility to close for 2-3 months. Despite this, the activities and results have been satisfactory, with potent ZVpro inhibitors (IC_{50} as low as 200 nM) identified and crystal structures of protease-inhibitor complexes determined. The goal and milestone for the Task have not been fully met.

Major Task 3: To test cellular anti-ZIKV activity and cytotoxicity of selected potent ZVpro inhibitors. The objective of this Aim is to perform cell-based assays to determine cytotoxicity and anti-ZIKV activity of potent ZVpro inhibitors.

Subtask 1: Perform cytotoxicity testing of selected inhibitors. Cytotoxicity assays against mammalian cells Vero and U87 have been described in the last progress report. We selected ~30 potent ZVpro inhibitors and tested their cytotoxicity. Most of these compounds, such as compounds 1-9 in Table 1 do not significantly inhibit proliferation of these cells with EC50s of more than 10 μM.

Subtask 2: Perform cellular antiviral activity testing of selected inhibitors. The assays for evaluating antiviral activity of our compounds have been described in the last progress report. We selected ~20 potent ZVpro inhibitors without significant cytotoxicity to mammalian cells and tested their anti-ZIKV activity in human brain cells U87, using both qPCR (to quantify ZIKV RNA) and end-point dilution assays (to determine tissue culture infective dose 50, or TCID50). Most of these compounds showed good to strong antiviral activity against ZIKV (FLR strain), as representatively shown in Table 1 with EC_{68} (concentration at which viral replication is inhibited by 68%) values of 0.3-5 μM. In addition, the most potent compound 9 was also tested and shown comparable antiviral activity (EC_{68} = 600 nM) against the HN16 strain of Zika virus. It also exhibited strong antiviral activity against replication of dengue serotype 2 virus (strain K0049). These results show our inhibitors targeting ZVpro (that is conserved among Flavivirus family) has a broad antiviral activity against Zika (and potentially dengue and other Flavivirus) infection.

Although the overall progress for the Task 3 has been delayed due to the COVID-19 pandemic, our activities and results have been satisfactory, showing our potent ZVpro inhibitors are non-cytotoxic and have potent and broad antiviral activity against Zika and dengue virus infections. The goal and milestone for the Task have not been fully met.

Major Task 4: To test PK/Tox and in vivo anti-ZIKV activity of selected potent ZVpro inhibitors. The objective of this Aim is to perform a series of in vitro and in vivo PK/Tox testing to select good drug candidates and test their in vivo antiviral activity in a mouse model of ZIKV infection.

Subtask 1: PK/Tox and brain distribution testing of selected inhibitors. Since none of ZVpro inhibitors have met the criteria for PK/Tox evaluation, we will perform this task for qualified compounds in the next funding period.

Subtask 2: To test in vivo anti-ZIKV activity of selected ZVpro inhibitors. A mouse model of ZIKV infection which is suited for evaluating antiviral activity of our compounds has been developed and described in the last progress report. In vivo anti-ZIKV activity of compound **9** was evaluated in this mouse model. We found that intraperitoneal (ip) injection of 100 $TCID_{50}$ of ZIKV-FLR caused rapid viral replication and death of the mice in ~10 days. Started 1h before inoculation of ZIKV, ip treatment with compound **9** (15 mg/kg/12h) for 24h (when ZIKV replication is in a rapidly growing phase) reduced ZIKV RNA copies in both plasma and brains of the mice by 96% and 98%. Treatment at 30 and 20 mg/kg/day for 3 days significantly prolonged the survival of ZIKV-infected mice, with the average values for the control, 20 and 30 mg/kg groups (N=12) being 11.7, 13.7 and 15.1 days. These results show that compound **9** can effectively inhibit ZIKV replication in vivo.

Although the overall progress for the Task 4 has been delayed due to the COVID-19 pandemic, our activities and results have been satisfactory, showing our potent ZVpro inhibitor compound 9 possesses strong anti-ZIKV activity in a mouse model of Zika infection. The goal and milestone for the Task have not been fully met.

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.

Nothing to report.

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.

Nothing to report.

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 funding period, we will perform the experiments we proposed in accordance with the approved SOW to achieve the goals of the project, using a combination of rational inhibitor design, synthetic medicinal chemistry, biochemistry, X-ray crystallography and in vitro and in vivo testing of biological activities and toxicities of potent ZVpro inhibitors.

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:*

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.

Although the overall progress has been delayed due to the COVID-19 pandemic, our activities and results have been satisfactory as described in the Accomplishment section. We expect there would be no negative impact to our overall accomplishment of this project.

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

Nothing to report.

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).*

Li, X.; Song, Y. Proteolysis-Targeting Chimera (PROTAC) for Targeted Protein Degradation and Cancer Therapy. *J. Hematol. Oncol.* **2020**, 13(1):50. (Published and acknowledged this DoD grant award). A copy of reprint is attached.

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.*

Nothing to report.

- **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.

PCT application entitled "Novel inhibitors of Flavivirus protease for prevention and treatment of Zika, dengue and other Flavivirus infections" has been filed on April 9, 2020. A copy is attached.

- **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".

Name: Song, Yongcheng
Role: PI
Researcher Identifier (e.g. ORCID ID): 0000-0003-2611-2476
Person Months: 4
Contribution to Project: As PI, Dr. Song is responsible for all aspects of the studies proposed, including experimental design, data analysis, postdoc training and manuscript preparation.
Funding Support:

Name: Rico-Hesse, Rebecca
Role: co-Investigator
Researcher Identifier (e.g. ORCID ID): 0000-0001-6216-1000
Person Months: 1
Contribution to Project: Dr. Rico-Hesse helped the PI design experiments for Aims 3 and 4, train personnel and analyze results.
Funding Support:

Name: Li, Xin
Role: Postdoc
Researcher Identifier (e.g. ORCID ID): 0000-0002-0189-4371
Person Months: 10
Contribution to Project: Dr. Li worked with Dr. Song to perform organic synthesis, including optimize synthetic route, and medicinal chemistry development of novel inhibitors of ZIKV protease.
Funding Support:

Name: Nie, Shen-You
Role: Postdoc
Researcher Identifier (e.g. ORCID ID): 0000-0002-7396-8114
Person Months: 1
Contribution to Project: Dr. Nie performed organic synthesis, including optimize synthetic route, and medicinal chemistry development of novel inhibitors of ZIKV protease.
Funding Support:

Name: Lin, Yi Lun
Role: Postdoc
Researcher Identifier (e.g. ORCID ID): 0000-0002-8540-4260
Person Months: 2
Contribution to Project:
Funding Support: Dr. Lin performed protein crystallization, X-ray data collection and structure determination of Zika protease.

Name: Yao, Yuan
Role: Research Associate
Researcher Identifier (e.g. ORCID ID): 0000-0002-4543-2988
Person Months: 12
Contribution to Project: Dr. Yao performed biochemical and biological activity testing of ZVpro inhibitors.

Funding Support:

Name: Wu, Fangrui
Role: Research Faculty
Researcher Identifier (e.g. ORCID ID): 0000-0001-8141-3584
Person Months: 2
Contribution to Project: Dr. Wu performed biochemical activity testing of ZVpro inhibitors.
Funding Support:

Name: Wu, Xiaowei
Role: Postdoc
Researcher Identifier (e.g. ORCID ID): 0000-0002-2157-2847
Person Months: 4
Contribution to Project: Dr. Wu performed molecular modeling and organic synthesis of novel inhibitors of ZIKV protease.
Funding Support:

Name: Zhang, Yinjie
Role: Postdoc
Researcher Identifier (e.g. ORCID ID): 0000-0002-4009-0771
Person Months: 2
Contribution to Project: Dr. Zhang performed biochemical activity testing of ZVpro inhibitors.
Funding Support:

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.

See attached updated Other Support forms (with changes noted) for the PI and co-Investigator. These changes have no impact on the funded project.

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:
<u>Organization Name:</u>
<u>Location of Organization: (if foreign location list country)</u>
<u>Partner's contribution to the project</u> (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.*

Nothing to report.

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.*

1. A copy of the published article.
2. A copy of the patent application.
3. Other Support forms for the PI and co-Investigator.