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PRINCIPAL INVESTIGATOR: Martin Conda-Sheridan

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Martin Conda Sheridan, PhD
E-Mail: martin.condasheridan@unmc.edu

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42nd and Emile, Omaha, NE
68198

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14. ABSTRACT
Bacterial infections are a serious threat to human health. During the award period we focused on the synthesis of some phenazines with potential antimicrobial activity. Our hypothesis is the conjugation of amines, polyamines and peptides to the phenazines will enhance their activity. We achieve the synthesis of several new phenazines containing diverse functional groups. Most of the new compounds presented modest antibacterial activity, thus, a structural optimization is being performed using the obtained data. The attachment of amines to the active phenazines proved difficult due to a rearrangement that took places. Thus, we focused in optimizing the structure of potential peptides that can be added to the phenazines. We prepared and evaluated some intriguing new peptides that presented potent activity against a panel of gram positive and gram negative pathogens. We also identified some key features that give rise to their activity. Over the next year we will focus on creating and testing heterocycle-peptide conjugates as novel antibacterials.

15. SUBJECT TERMS
Phenazines, antibacterials, antimicrobial peptides, antibacterial hybrids, gram positive, gram negative, cylindrical proteases

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

The Infectious Diseases Society of America has listed bacterial infections as "1 of the 3 greatest threats to human health." The objective of this project is to modify heterocyclic molecules with antibacterial properties to enhance their activity against gram positive and gram negative bacteria. Specifically, we plan to connect the prepared compounds to primary amines or peptides, both strategies that have been reported to enhance penetration inside bacteria. We expect our compounds will eliminate a series of dangerous pathogens including: *Staphylococcus aureus, Escherichia coli, Pseudomona aeruginosa, Klebsiella pneumoniae,* and *Acinetobacter baumannii*.

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

Antimicrobial peptides; phenazines; cylindrical proteases; gram negative bacteria, bacteria membrane permeability; drug resistant bacteria

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.

The major goals of the project are

- Synthesis, purification and characterization of phenazines linked to peptides and amines
  - These includes the synthesis of various phenazines and antimicrobial peptides to identify proper leads that can later be connected to each other
- The antimicrobial evaluation of the compounds
  - The includes testing the activity of phenazine-peptide complexes, phenazines, and antimicrobial peptides
- Their toxicity evaluation
  - These focuses in testing toxicity against human cells and a wax moth larva (simple in vivo model that does not require University approval)
- Note: based on the synthetic progress and preliminary results, we included another set of heterocycles (containing the pyridine core) to be tested and modified. Likewise, we decided to expand testing by including: chlamydia trachomatis, which is a gram negative bacteria, within the bacteria to be evaluated.

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

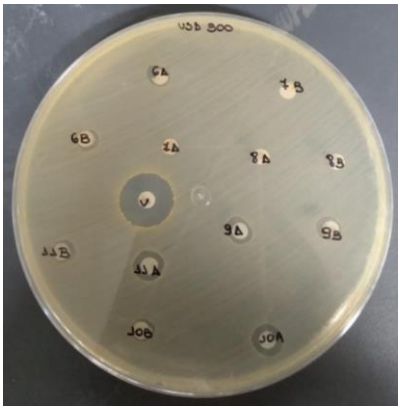
completion, the emphasis in reporting in this section should shift from reporting activities to reporting accomplishments.

We had three major seatbacks: (**1**) personnel turnover due to better job offers, which affected continuity (this was described in the NCE letter). Both postdocs receive offers for high profile medicinal chemistry labs (Dr. Dennis Liotta and Dr. Donald Durden). Thus, even though it hurt progress I see this as a positive because it fostered the careers of two young scientists. (**2**) Unpredicted, and unforeseen, synthetic challenges with side chain functionalization of the phenazines. As detailed in the previous report, adding an amine side chain lead to an intramolecular rearrangement that yielded and inactive adduct. (**3**) The COVID-19 pandemic that resulted in the shutdown of the research enterprise (university wide policy) for ~4 months. This also affected the testing of some of the molecules since this experiment will be performed at a collaborators' lab.

However, we are very happy with the progress of the project and the achievements so far. The major activities included the synthesis and purification of peptides and small molecules. For example, we have recently reported the synthesis, purification, and evaluation of new antimicrobial peptides (https://doi.org/10.1016/j.peptides.2019.170119). This researched was funded with this award. Based on that precedent, we decided to continue the optimization of the peptides with the objective of identifying more potent antimicrobial entities.

We evaluated the antimicrobial activity of various peptides based on the structure of the natural peptide citropin 1.1 using a disk diffusion assay. The peptides were tested against a panel of bacteria: *S. aureus* (HU25 and USA 300), S. epidermis, *E. coli*, *P. aeruginosa*, *K. pneumoniae*, and *A. baumannii*. Based on the disk method DAN-1-13 was one of the most promising leads (**Figure 1**). The sequence of the peptide is Sar_Leu_1-Nap_Lys_Val_Ile_Arg_Lys_Val_Ala_Lys_Val_Ile_Gly_Gly_Leu. 1-Nap = Naphtyl side chain linked at carbon 1

Figure 1. *Staphylococcus aureus* USA300 (MRSA strain). The AMPs: 4. DAN -1-13, 5. DAN -1-71A, 6. DAN -1-71B, 9. SYD -1-5, 10. AJP -1-1 and 11. HHX -2-28 showed activity against this strain. We used vancomycin "V" as a positive control.



Thereafter, we performed a broth microdilution assay to determine the minimal inhibitory concentration (MIC) values of the most active antimicrobial peptides. The best lead, DAN-1-13 has a MIC value of 4

Figure 2. Broth microdilution assay in triplicate. **Columns 1-3**; DAN-1-13 serial microdilution. **Columns 5-7;** DAN-1-71A serial microdilution. **Column 9.** Blank (culture medium only). **Column 11.** Negative control (medium and inoculum); in the last four wells, DMSO was applied to assess solvent-related toxicity. **Column 12.** Positive control (Vancomycin microdilution)

μg/mL against *S. aureus* USA 300 (**Figure 2**). In this assay, the compound was more potent than the vancomycin control. The figure also shows data for DAN-1-71A, which has the following sequence: Sar_Leu_Trp_Lys_Val_Ile_Arg_Lys_Val_Aib_Lys_Val_Ile_Gly_Gly_Leu (Aib= 2-Amino isobutyric acid, which is used to promote α-helix formation).

In order to validate the presented results, we determined the minimum bactericidal concentration (MBC) of the peptides. Birefly; we planted one dilution in a petri dish to observe growth of bacteria. For example, DAN-1-13 presented an MBC of 8 μg/mL against *S. aureus* USA 300 (**Figure 3**).

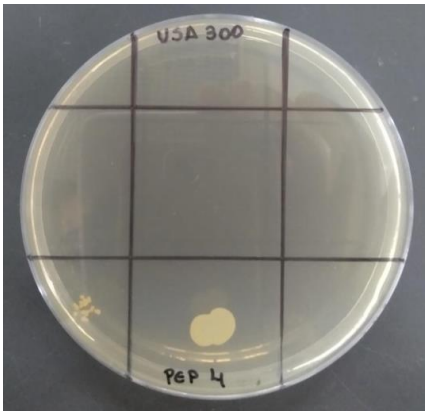


Figure 3. MBC of DAN-1-13 against USA300. One line of each triplicate was selected and applied on a Petri plate. From the left to the right, the first well "A" until "H". The first well of positive control was applied in the last space.

In addition, we have continued with the preparation of peptides to find better leads (we think this must be a continuous process until we find the best therapeutic entity). We have prepared 18 new entities, 10 are pure and fully characterized, the rest are in the purification or characterization stages. In all cases we used the same conditions and reagents: Rink Amide Am resin and Oxyma/DIC/DIPEA or HATU/DIPEA as coupling reagents (for special amino acids we used PYBOP/DIPEA. Deprotections were performed using 4-methyl-piperidine 20% in DMF. The reactions of coupling and deprotection were performed in a Biotage microwave: 75°C, 5 minutes reaction time, with 2 min of pre-stirring. If the coupling reactions were difficult, we performed them using a reaction time of 7 min with 3 min of pre-stirring. **Figure 4** shows the structure one of the new peptides

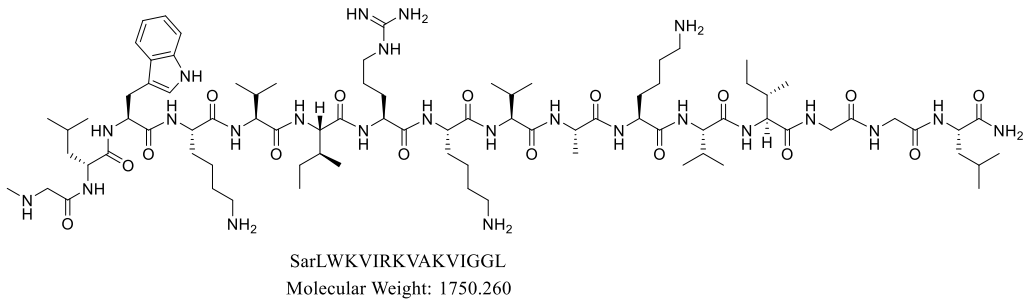
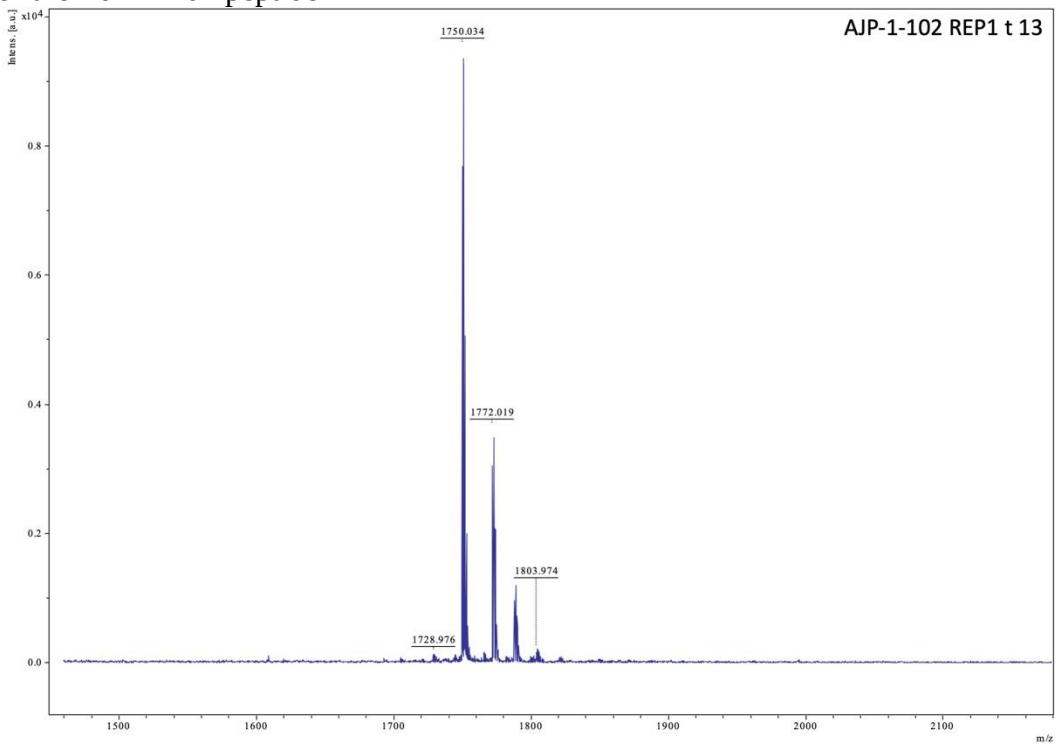
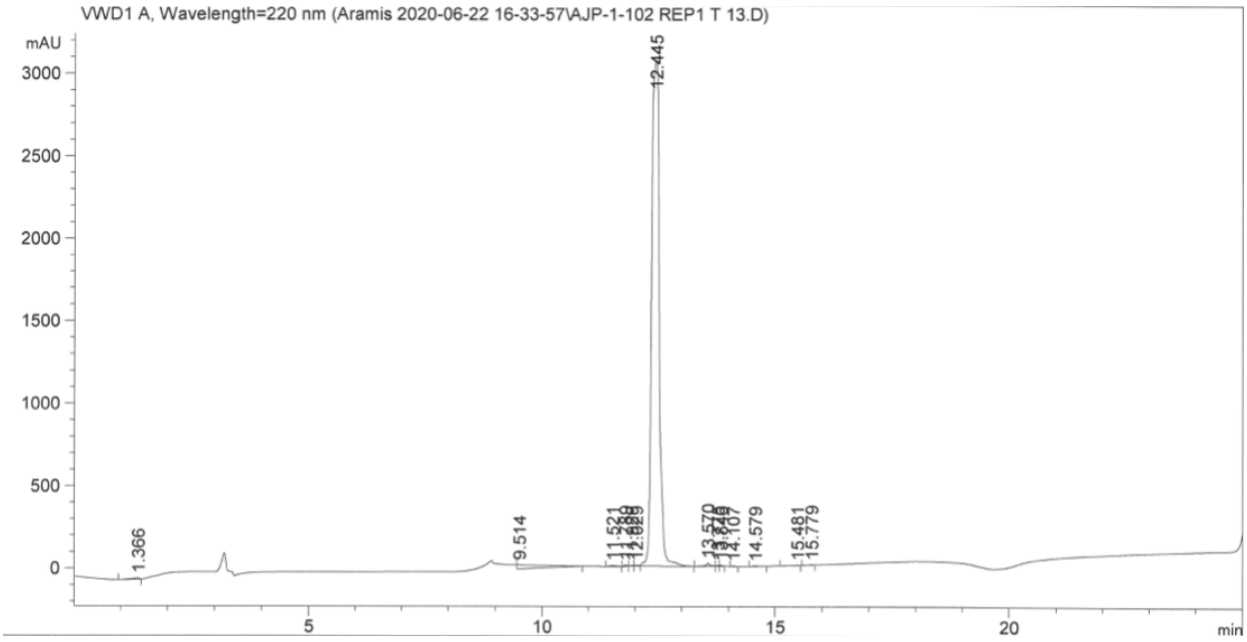


Figure 4. Structure of a a potential antimicrobial peptide; AJP-1-102

As has been the case, the peptides are purified using high performance liquid chromatography (LC) and characterized by mass spectrometry (usually MALDI). Below is the MALDI trace showing the presence of the AJP-1-102 peptide

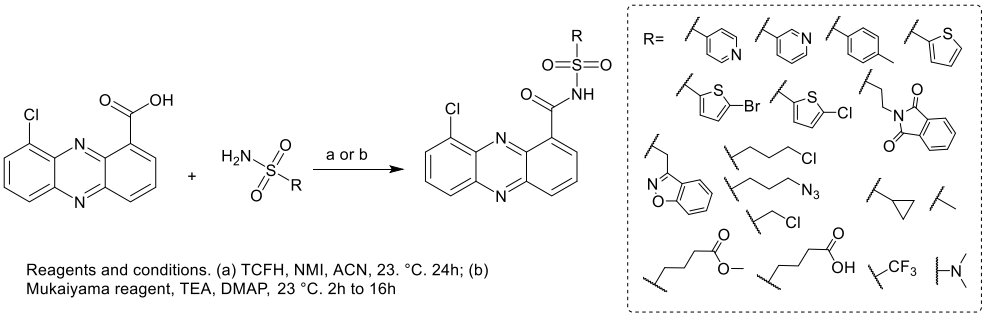


In order to assess the purity, we injected the fractions obtained by the high performance LC experiment in an analytical HPLC using a water/Acetonitrile gradient run. The spectrum shows AJP-1-102 is 96% pure and can be used for testing. We have selected 95% as an acceptable purity for the peptides (this is based on the journal of medicinal chemistry guidelines for purity).



Furthermore, we prepared a library of 27 phenazine compound as shown below (**Scheme 1**). In some cases, the objective was to further understand antibacterial action. In others, to identify a moiety that can be further functionalized to link the molecules to the peptides.

Scheme 1. Synthetic route used to prepare phenazines.



Reagents and conditions. (a) TCFH, NMI, ACN, 23. °C. 24h; (b) Mukaiyama reagent, TEA, DMAP, 23 °C. 2h to 16h

The general synthetic methods were as follows:

Method A: A phenazine derivative (0.05 g, 0.018-0.20 mmol) and the appropriate sulfonamide derivative (1.2 eq) were dissolved in anhydrous acetonitrile (7 mL) using a sonicator bath, then NMI (85 µL, 0.025mmol) and TCFH (88 mg, 0.07 mmol) were added. The formed clear yellow solution was stirred at room temperature for 2 h. The formed bright yellow precipitate was filtered, washed with acetonitrile, and purified by column chromatography to afford the desired product.

Method B: A phenazine derivative (0.18 mmol), DMAP (15 mg, 10 mol%), and a sulfonamide derivative (0.2 mmol) in DCM (3 mL) were stirred for 10 minutes. Then, triethylamine (100µL, 0.8 mmol) was added dropwise, and the reaction mixture was stirred at room temperature for 16 h. After the reaction completion, the mixture was poured over 10 mL of DCM and washed with 2N HCl (5mL x 3). The resulted organic layer was dried over sodium sulfate and purified using column chromatography.

The structure and the proton nuclear magnetic resonance (^{1}H NMR) spectrum of a model phenazine compound is shown below in **Figure 5**.

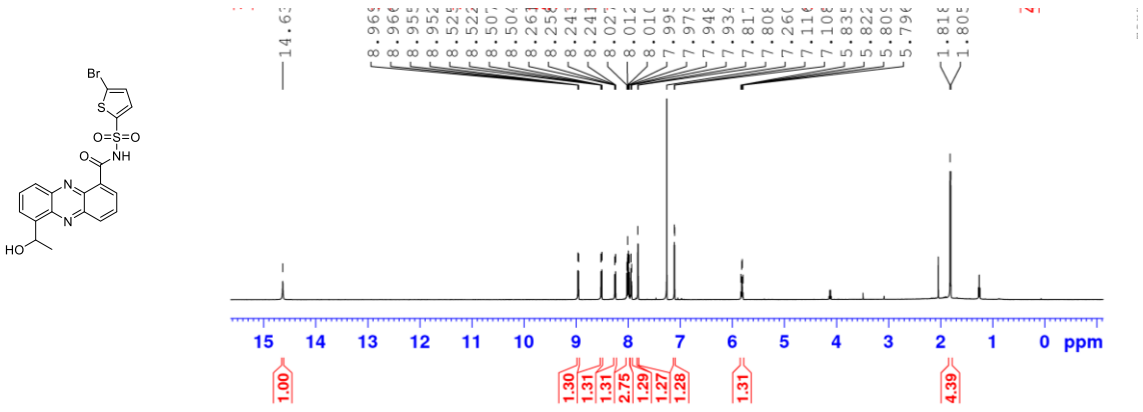
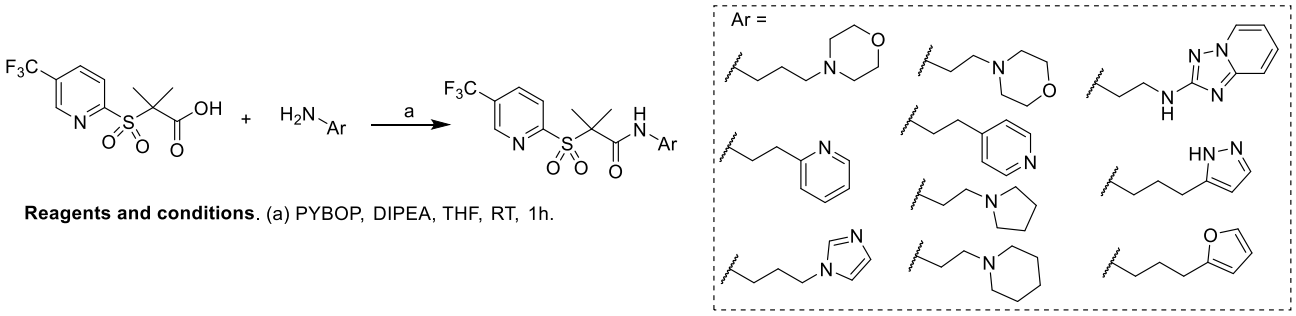


Figure 5. Structure of a novel phenazine and NMR spectrum confirming the identity of the molecule

Given the difficulties with phenazine functionalization, we decided to explore an alternative heterocyclic core. The compounds, termed ACPs, activate cylindrical proteases, a promising new antibacterial target. We have prepared and characterized 25 new molecules as shown in **Scheme 2**.

Scheme 2. Synthesis and structure of some ACp derivatives



As is the case with all heterocycles, we characterized them by NMR (Figure 6) and MS.

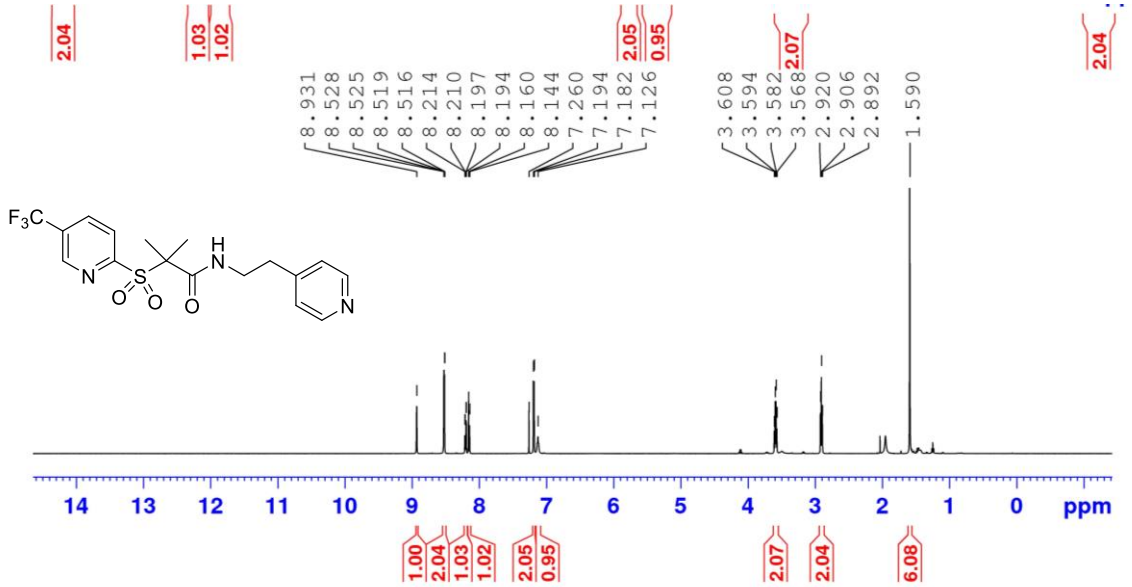


Figure 6. Structure of an ACP compound and NMR spectrum confirming its identity

As seen in **Figure 7**, the ACP compounds have the ability to degrade casein, which is a biomarker for activation of the ClpP protease. Interestingly, even though activation of the ClpP system was observed, the assays did not show antimicrobial effect with MIC values > 128 µg/mL against the panel of tested bacteria

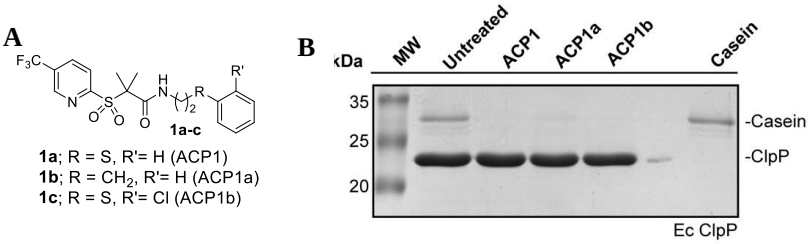


Figure 7. **A)** Structures of ClpP activators. **B)** I*n vitro* protease assay (SDS PAGE, 50 µg/mL) shows the degradation of casein upon addition of the compounds, which indicates activation of *E. coli* ClpP.

(this has been seen by us and the Houry group: https://doi.org/10.1016/j.chembiol.2011.07.023). It has been postulated this is the result of efflux pumps and limited penetration across the membrane of G-ve pathogens. Thus, these heterocycles are perfect to test the working hypothesis of this grant.

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.

The people that benefited the most form the grant were the graduate studenta involved in the project:
Mohamed Seleem, MS in organic chemistry, PhD student. Part of the data generated in this project was used to write a UNCM fellowship/assistantship. In fact, he was awarded the "Program of Excellence Assistantship," which provided 2 years of funding during the PhD studies (it is expected he will graduate at the end of the fellowship). A key component of the fellowship is the participation in professional development workshops. The success rate of the fellowship is 31% (16 applicants from the department of pharmaceutical sciences, 5 awards). He also gave a talk at the Midwest regional meeting of the American Chemical Society (October 2019) entitled: Development of ClpP2 activators to treat chlamydial infections. In addition, he has become an expert in antimicrobial testing and cell work. He is learning in silico drug design (mainly docking and calculation of properties) using various software: MOE, pymol, chimera, molinspiration, glide, and autodockViva.
Luana the Campos, MS in computational chemistry, PhD student. Ms Campos joined the project last September. She taught Mr Seleem computational chemistry and at the same time started learning organic synthesis. She has prepared several compounds that need to be evaluated.

Other people that have been involved on the synthesis of peptides and molecules are Dr. Audifas Matus Meza, Mr. Aramis Pereira (MS in biochemistry, technician, will become a PhD student but has issues with his visa); Dr. Nathalia Rodrigues de Almeida (currently a faculty at the University of Nebraska at Omaha, a success story. She taught diverse techniques to the students). All of them have enhanced skills in peptide or organic chemistry and microbiology, either learning from me or collaborators across campus (Scot Ouellette and Kenneth Bayles labs at UNMC)

I held regular personal meetings (weekly, plus two joint meetings involving the whole group) with all pthe ersonnel involved in the project and show experimental techniques to them. Mr Seleem, Mr Pereira, and Ms Campos have received additional training. We have attended several workshops mainly from Agilenbt, Teledyne (https://www.teledyneisco.com/en-us/chromatography/seminars-and-webinars?utm_source=Pardot&utm_medium=email&utm_campaign=C_2006_Peptide%20from%20Analytical%20Webinar) and Chemical Computing group (https://www.chemcomp.com/). These workshops focused on chromatography techniques or *in silico* drug design, but essential skills for a medicinal chemist.

In addition, I request all my students to write proposal to secure external funding. I read the proposals and provide feedback (although I do not write the proposals myself). Also, we have spent a large amount of time navigating funding agencies and mechanism, we discuss the funding calls, what is included in a proposal and general grantsmanship skills. We also discuss career options and useful strategies to achieve career goals (using books, papers, and my experience in detail). In addition, I am in charge of the departmental seminar. I arrange meetings between my group and the speakers presenting in the department to enhance the network of my students and to give them the opportunity of presenting their research (3 15 min presentations followed by Q&A). Perhaps the highlight over the last year were the meetings with two members of the national Academy of Science: William Jorgensen (Yale University) and Jeffrey Moore (Univ of Illinois, Urbana). Having the chance to speak in a small room with some of the brightest scientists in the world has brought tremendous professional growth to my students.

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.

Results were disseminated as peer-reviewed papers (see below), classes, seminars (listed below), and Zoom meetings with collaborators. The synthetic methods we used to prepare the compounds were included in a short class that I gave at the CIBION (Centro de Investigaciones en Bionanociencias) in Buenos Aires, Argentina. I have been invited to give lectures focused on the synthesis of heterocycles (next October, by Zoom) at CIBION and also at University of Itajuba-Minas Gerais, Brazil.

Regarding outreach, once again, we had a troop of Girl Scouts visiting the university. I talked about our research, and available career options with them and their parents. In addition, I taught two 3 hr lectures to the High School Alliance students (a UNMC program focused in motivating high school students, especially minorities and females, to pursue careers in sciences). I described the drug discovery process and used this project as an example of the methods we use to create new drugs. I also went on recruiting trips and used the generated data to showcase my lab and the University. We are planning to create a small video to introduce our results.

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.

We will finish the synthesis and evaluation of the small molecules (the heterocycles), the peptides and their hybrids. At this stage, we mainly need to focus in antimicrobial and mammalian cell evaluation.

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).

We have prepared a library of two classes of compounds (phenazines and pyridines) using established protocols. Once the new heterocycles are evaluated, we will be able to derive structure-activity relationships that can be used to further optimize the molecular structures in order to design new, more potent broad spectrum antibiotics.

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 at the moments. However, once we test the molecules, we can start mechanism of action studies to understand if the compounds kill bacteria using a traditional mechanism or if a new bacterial target has been identified. Please note that, as shown, some of the compounds are potential activators of cylindrical proteases, which has been identified as a novel antimicrobial target Thus, there is potential to influence the area of microbiology.

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.*

This project is exploratory in nature and we do not expect short term outcomes at the public health level (drug approvals take years if not decades). However, the outreach activities have helped to conscientize students and parents of the power of medicinal chemistry in health and every day lives.

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:*

Other than the mentioned synthetic, COVID-19 and personnel challenges, there is nothing to report. As mentioned, we have included another set of heterocyles that suffer the same problems the phenazines encounter but seem to be more amenable for synthetic modifications. Given the new heteroclycles also present antibacterial activity but possess limited penetration into bacteria, we believe they are within the scope of the grant (although the molecules were not originally described on the proposal).

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.

We have fixed most synthetic problems. The issue with testing is related to COVID-19 but once things go back to normal, this issue will be promptly addressed.

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.

Although not of significant impact, personnel turnover coupled to the time to identify and hire replacements forced me to move budget from supplies to personnel and between technicians, postdocs, and students. However, as can be seen in the expenses, the changes were not substantial.

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

Not applicable.

Significant changes in use or care of vertebrate animals

Not applicable.

Significant changes in use of biohazards and/or select agents

Not applicable.

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).*

1. Seleem, M.; Rodrigues de Almeida, N.; Chhonker, Y. S.; Murry, D. J.; Guterres, Z.; Blocker, A. M.; Kuwabara, S.; Fisher, D. J.; Leal, E. S.; Martinefski, M. R.; Bollini, M.; Monge, M. E.; Ouellette, S.; Conda-Sheridan, M. Synthesis and Antichlamydial Activity of Potential Activators of Cylindrical Proteases. J. Med. Chem. 2020, 63, 4370-4387. Published, acknowledgement of federal support: yes
2. Rodriguez de Almeida, N.; Catazaro, J.; Chhonker, Y.; Murry, D.; Powers, R.; **Conda-Sheridan, M**. Understanding Interactions of Citropin 1.1 Analogues with Model Membranes and Their Influence on Biological Activity. *Peptides* **2019**, 170119. Published, acknowledgement of federal support: yes

  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).

Not applicable.

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.*

1. Peptide Amphiphiles, Microbiology and Structural Characteristics of a Promising Class of Biomaterials. Federal Fluminense University, Rio de Janeiro, Brazil, Oct. 25, 2019.
2. Design and Synthesis of Heterocycles as New Antibacterial Agents. Federal University of Itajuba, Minas Gerais, Brazil, Oct. 22, 2019.
3. Peptide Amphiphiles, Microbiology and Structural Characteristics of a Promising Class of Biomaterials. Kansas State University, Oct. 15, 2019.
4. Nanostructures as Antibacterial Agents. Role of Supramolecular Morphology in Biological Action.***** Georgia State University, April 23, 2019.
5. Nanostructures as Antibacterial Agents. Role of Supramolecular Morphology in Biological Action. ***** University of Illinois at Chicago, April 3, 2019.

- **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.

https://www.condasheridanlab.com/ This is my laboratory website. It contains basic information regarding our research, news, publications, contact information, etc

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

Not applicable

- **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.

Not applicable

- **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.*

Not applicable

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:	Martin Conda-Sheridan
Project Role:	PI
Researcher Identifier (e.g. ORCID ID):	0000-0002-3568-2545
Nearest person month worked:	0.9 (7% effort)
Contribution to Project:	Dr. Conda-Sheridan supervised the project. He trained students and postdoctoral researchers on the synthetic aspect of the research, including purification and characterization of molecules. He reviewed the data.
Funding Support:	The remaining of my salary came from the national sicence foundation (NSF-Career), the American Chemical Society-PRF grant, a NIH-COBRE, and the UNMC-college of Pharmacy.

Name:	Mohamed Seleem
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	0000-0003-4379-5133
Nearest person month worked:	7 (58% effort)
Contribution to Project:	Mr. Seleem is involved on the synthesis, characterization, and purification of compounds. He also performs microbiological studies.
Funding Support:	He was funded by this grant.

Name:	Luana Campos
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	0000-0001-8527-688X
Nearest person month worked:	6.7 (56% effort)
Contribution to Project:	Ms. Campos performs the synthesis, characterization, and purification of compounds. She also does in silico studies.
Funding Support:	She was funded by this grant.

Name:	Aramis Pereira
Project Role:	Technician (accepted into graduate school, visa problems)
Researcher Identifier (e.g. ORCID ID):	N/A
Nearest person month worked:	2.3 (19% effort)
Contribution to Project:	Mr. Pereira works on the synthesis and identification of peptides.
Funding Support:	The remaining of his salary (mainly in the area of peptides) came from a NIH-COBRE, and the UNMC-college of Pharmacy.

Name:	Audifas Salvador Matus Meza
Project Role:	Postdoc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	1 (8% effort)
Contribution to Project:	Dr. Matus Mesa trained students on the synthetic aspect of the research, including purification and characterization of molecules.
Funding Support:	The remaining of his salary (working in other small molecules antibacterials) came from the NIH-COBRE grant.

Name:	Nathali Rodrigues de Almeida
Project Role:	Postdoc
Researcher Identifier (e.g. ORCID ID):	0000-0002-9552-1233
Nearest person month worked:	3 (25% effort)
Contribution to Project:	Dr. Almeida trained students on microbiology.
Funding Support:	This grant.

We have also established collaborations with Dr. Fabio Alves (University of Fluminense) and Dr. Mohamed Seleem (same name than my student) (Virginia Tech). They have shown interest in evaluation our compounds but do to the pandemic, things are in stand by at the moment.

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.

New grant:
1941731- National Science Foundation-CAREER 03/01/20-02/28/25
DESIGN AND UNDERSTANDING OF COMPLEX BIOMATERIALS
The goal of this CAREER proposal is to create nature-inspired self-assembling biomaterials with tunable properties that are stimuli sensitive and whose supramolecular architecture can be predicted with the aid of novel software tools.
Role: Conda-Sheridan, PI

Expired grant:
57434-DNI7, American Chemical Society-Petroleum Research Fund 09/01/17-08/31/19
The Evolution of Self-Assembled Organic Materials
The goal of this project is to develop a fundamental understanding of the relationship between molecular and supramolecular structure and material properties.
Role: Conda-Sheridan, PI

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.

Organization Name: Community for Open Antimicrobial Drug Discovery (CO-ADD) -The University of Queensland

- **Location of Organization:** 306 Carmody Rd, The University of Queensland, St. Lucia, QLD, 4072, Australia
- **Partner's contribution to the projec**t They performed the biological evaluation of selected molecules against ESKAPE pathogens (E. coli, K. pneumoniae, A. baumannii, P. aeruginosa, S. aureus (MRSA)) and fungi C. neoformans and C. albicans.
- **Financial support;** Wellcome Trust

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.