Review of the proposal and project objectives.

Overview and rationale of the project:

The progressive loss of efficacy of known antibiotics, the limited progress in the development of new antimicrobials, and the fact that current antibiotics target a limited number of cellular processes, are the main factors why there is a dire need for new antibiotics that can fight treatment-resistant bacterial infections. In particular, antibiotics with a novel mechanism of action would be of high importance.

Why target Caseinolytic Protease P (ClpP)?

The proteolytic subunit of caseinolytic protease (ClpP) is a serine protease involved in the proteolysis of defective and misfolded proteins and has been characterized in several bacteria. In recent years it has been reported that modifications in ClpP function **have been shown to significantly reduce the infectivity and virulence of several pathogenic bacteria**, both Gram+ and Gram-. In 2005, it was reported that the natural cyclic acyldepsipeptides (ADEPs) could inhibit the growth of Gram+ bacteria by inducing unregulated intracellular proteolysis by activating ClpP. In 2019, a collection of new small molecules, called imipridones (ONC-201, ONC-206 and ONC-212) were confirmed to act as activators of human ClpP (HsClpP). In the following years they were also shown to activate bacterial ClpPs, including the ClpP found in *S. aureus* (SaClpP). In particular, the most potent analogue, ONC-212, has been shown to suppress the proliferation of several Gram+ (*Staphylococcus aureus*, *Bacillus subtilis*, and *Enterococcus faecium*) and to a lesser degree Gram- (*Escherichia coli* and *Neisseria gonorrhoeae*) species and have positive effects *in vivo*. Current scientific evidence clearly supports activation of ClpP as a potential pharmacological target to produce antimicrobial activity. Unfortunately, none of these ClpP activators have reached clinical trials as potential antibiotics due in part to their lack of selectivity between activating bacterial ClpP (e.g. SaClpP) and human ClpP.

Based on the above rationale, this project proposed a research plan to identify and develop compounds that could *selectively* activate bacterial ClpP, and to investigate their *in vitro* and *in vivo* antimicrobial profiles.

List of the project goals and an executive summary of the accomplishments the project has made toward achieving these goals.

To attain the objectives of the project, several progressional goals were proposed:

1. Identify new structurally distinct ClpP agonists.
2. Evaluate selectivity between human and bacteria versions of ClpP.
3. Test promising compounds in susceptible and resistant bacteria strains, alone or as combinations.
4. Test most promising compounds against biofilms, alone or as combinations.
5. Improve physicochemical and pharmaceutical properties of hits to generate lead compounds.
6. Test lead compounds in a proof-of-concept *in vivo* models.

The overall objective for this project “strategic lines” was to determine whether selective activators of bacterial ClpP (in particular ClpP of *S. aureus*) could possess antibacterial effects worthy of continued development towards the clinic.