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SJSU Undergraduate Research Grants

Uncovering targets of antivirulence compounds in *Pseudomonas aeruginosa* via photoaffinity labeling

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Abstract

Pseudomonas aeruginosa (PA) is an opportunistic Gram-negative bacterium affecting patients with weakened immune systems like burn victims and surgical implant patients.[1] We have pursued an antivirulence strategy to combat this pathogen by inhibiting pyocyanin, a redox-active virulence factor.[2]

Inhibition of pyocyanin renders the bacterium more benign, which should allow the host to clear the infection more easily.[3] We are interested in using an approach with a photoaffinity label (PAL) to characterize the molecular target(s) of our inhibitors. Understanding the mechanism of action will aid in the development of the compounds as therapeutics.

Synthetic Route

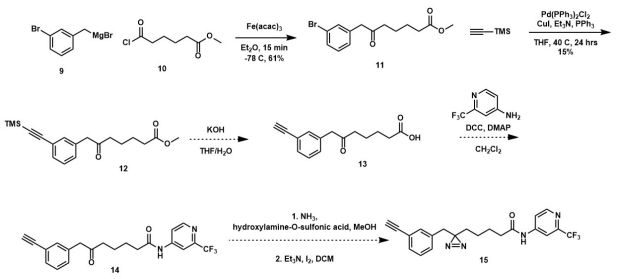

Figure 2 – Planned synthetic route for preparation of the inhibitor (**14**) and PAL analog (**15**). I have successfully synthesized compound **12** thus far and am working to optimize the Sonogashira reaction.

Research Questions

- What alkyne positions lead to the best inhibition efficacy?
- What ketone positions reduce efficacy substantially?
- Which atom linker maintains the highest inhibition efficacy?
- What route will be effective to make the target PAL analog?

Project Activities

We found the optimal position to place the ketone and alkyne (functional groups necessary for photoaffinity labeling) by conducting pyocyanin assays (**Figure 1**). The first two steps of the multistep synthetic route were carried out and characterized by NMR (**Figure 2**).

Future Directions

Further optimization of the first two steps will be necessary; conditions for planned steps will also likely need optimization. Additionally, the inhibitor (**14**) and PAL analog (**15**) will be assayed for efficacy. Finally, copper-click, affinity chromatography, and HRMS will be used to isolate and characterize targets.

Optimal Functional Group Site

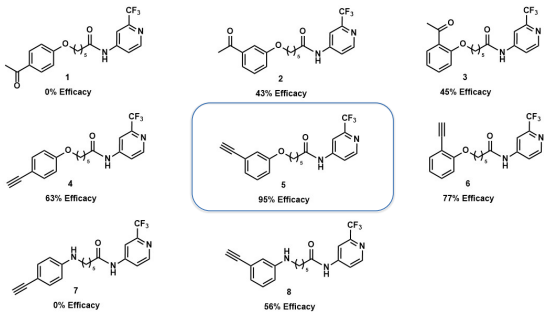

Figure 1 – Pyocyanin inhibitors were assayed at 100 µm in wild-type PA. Pyocyanin inhibition efficacy for different ketone or alkyne placement were determined qualitatively using UV/Vis. Compound 5 (ether-linked aryl alkyne) maintained the best ability to block pyocyanin production.

Citations

1. Gellatly, S. L.; Hancock, R. E. *Pathog. Dis.*, **2013**, *67*, 159.
2. Miller, L. C.; O'Loughlin, C. T.; Zhang, Z.; Siryaporn, A.; Silpe, J. E.; Bassler, B. L.; Semmelhack, M. F., *J. Med. Chem.,* **2015,** *58*, 1298.
3. Lau, L. W.; Hassett, R. H.; Kong, F.; Mavrodi, D. *Infect. Immun.*, **2004**, *72*, 4275.

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