Identification of small molecules with antibiotic activity in *M. smegmatis*

Mikaela Seemann, Dr. Meghan Blackledge
Department of Chemistry, High Point University

INTRODUCTION

Several species within the *mycobacterium* genus cause infection in humans with symptoms such as skin lesions and nodule buildup in the lungs [1]. Current treatment options for mycobacterial infections require daily medication intake for six to nine months and cause significant side effects including nausea, loss of appetite, and headache [2]. Since these treatment challenges often lead to patient non-compliance, there is a need for effective novel drugs with less side affects [2]. The Blackledge lab identified compounds that inhibit the PASTA kinase Stk1 in methicillin resistant Staphylococcus aureus (MRSA). Mycobacteria contain a homologous PASTA kinase, PknB, that is essential for survival, so small molecule PknB inhibitors are under investigation as novel antimycobacterial therapies [3]. Several compounds with different molecular scaffolds were initially screened in *Mycobacterium smegmatis*. The phenanthroline class was identified from this initial screen to show antibiotic activity in *M. smegmatis*. Further studies of this class of molecules were initiated to develop a structure-activity relationship (SAR) in *M. smegmatis*. Then the phenanthrolines were screened for activity in other *mycobacterium* species such as *M. abscessus.*

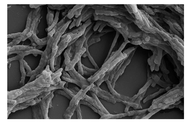


Figure 1. SEM image of *M. smegmatis*

METHODS

A standard microdilution assay was used to evaluate compound antibiotic activity against *M. smegmatis.* Bacteria culture was grown in 7H9 media. For *M. smegmatis*, the assay plate was incubated for 48 hours at 37°C. Turbidity in the well indicated bacterial growth. For *M. abscessus*, AlamarBlue as added to the assay plate wells after 72 hours of incubation. Plates were incubated for another 48 hours. The negative control provided the coloration standard for no bacterial growth. Minimum inhibitory concentration (MIC) was recorded as the lowest well concentration that showed inhibited bacterial growth. Final MIC was calculated as the average value replicated across three trials.

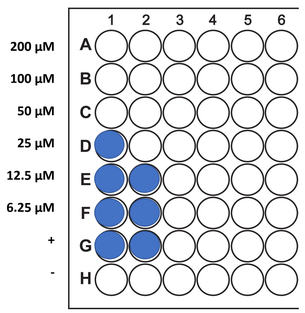


Figure 2. Plate layout of MIC assay

Figure 3. Picture of stained assay plate for *M. abscessus*

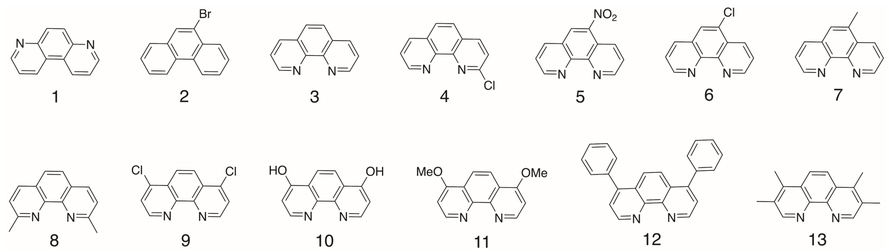


Figure 4. Structures of phenanthrolines and phenanthroline derivatives investigated in this study.

RESULTS AND DISCUSSION

Table 1. Minimum inhibitory concentrations (MIC) of tested compounds against *M. smegmatis*

Compound	MIC (µM)	Compound	MIC (µM)
1	>200	**8**	100
2	>200	**9**	3.125 / 6.25
3	3.125 / 6.25	**10**	25
4	100	**11**	>200
5	12.5	**12**	12.5
6	6.25	**13**	12.5
7	3.125 / 6.25		

Table 2. MIC of tested compounds against *M. abscessus*

Compound	MIC (µM)
3	50
4	>200
5	25
6	100
7	50
8	>200
9	>200

The 1,10-phenanthroline scaffold shows the most antibiotic activity. Substitution at positions 2 and 9 on the 1,10-phenanthroline scaffold are detrimental to antibiotic activity. As seen in compounds **5-7,** increasing the electron donating character of the substituent at the 5-position correlated with lowered MIC values (Table 1). For 4,7-disubstituted 1,10-phenanthrolines, the MIC of compound **9** shows that the electronegative chlorine atoms neither interfered with nor improved the antibiotic activity. Abrogated activity was observed in compounds **10** and **11** with alkyl substituents on positions 3,4,7 and 8.

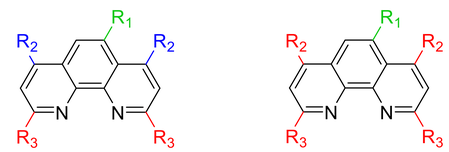
The 1,10-phenanthroline scaffold (**3**) shows reduced antibiotic activity in *M. abscessus*. The nitro group at position 5 improved antibiotic activity from the scaffold (**3**) whereas a chlorine added to the 5-position worsened antibiotic activity. The methyl group did not improve nor detract from antibiotic activity of the scaffold. MICs for compounds **8** and **9** show that substituents in position 2 and 9 or 4 and 7 worsened antibiotic activity compared to the phenanthroline scaffold.

CONCLUSIONS

Substituents at specific locations on the 1,10-phenanthroline ring structure affect antibiotic activity. Increasing the electron withdrawing character of a substituent at position 5 correlates to better antibiotic property whereas electron donating substituents at positions 4 and 7 exhibit lowered antibiotic activity. This preliminary structure-activity relationship data is being utilized to inform our synthetic efforts. At position 5, new electron withdrawing groups with varying steric effects will be added in future derivatives. Derivatives with other electronegative groups on positions 4 and 7 will be synthesized to determine what groups are tolerated for maintaining antibiotic activity.

In *M. abscessus*, the basic scaffold structure did not show effective antibiotic activity. Since the position 5 nitro substituted compound displayed the best antibiotic activity, further SAR studies would involve screening other compounds with electron withdrawing groups at position 5. Another goal is to screen phenanthroline scaffold (**3**) substituted at positions not yet explored such as 3 and 4

***M. smegmatis* SAR** ***M. abscessus* SAR**



R_n = electron donating groups have better activity
R_n = electron withdrawing groups have better activity
R_n = hinders antibiotic activity

REFERENCES

1. Bernut, A.; Herrmann, J.-L.; Ordway, D.; Kremer, L., The Diverse Cellular and Animal Models to Decipher the Physiopathological Traits of Mycobacterium abscessus Infection. *Frontiers in Cellular Microbiology* 2017, 7
2. Blanchard, J. D.; Elias, V.; Cipolla, D.; Gonda, I.; Bermudeza, L. E., Effective treatment of Mycobacterium avium subsp. hominissuis and Mycobacterium abscessus species infections in macrophages, biofilm, and mice by using liposomal ciprofloxacin.
3. Nguyen, T. V. *et al*. "The Discovery of 2-Aminobenzimidazoles that sensitize *Mycobacterium smegmatis* and *M. tuberculosis* to b-lactam antibiotics in a pattern distinct from b-lactamase Inhibitors." Angewandte Chemie (2017).
4. Fisher, Jed F., and Shahriar Mobashery. "B-Lactam Resistance Mechanisms: Gram-Positive Bacteria and Mycobacterium Tuberculosis." Cold Spring Harbor Perspectives in Medicine 6.5 (2016): a025221. PMC. Web. 3 Oct. 2018.

ACKNOWLEDGEMENTS

I would like to acknowledge the following groups for their support and collaboration:
The Blackledge lab
High Point University Undergraduate Research and Creative Works (URCW)
High Point University Summer Undergraduate Research Program in the Sciences (SuRPS)
High Point University Department of Chemistry
High Point University Student Government Association (SGA)