

Antibacterial activity of synthesized organic small molecules

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Background

Our collaborator, Duminda Liyanage of SOKA University of America, has synthesized eight small organic molecules that have potential antimicrobial activity. These compounds are imine-containing compounds with different substituent groups and are precursor molecules to therapeutic antibiotics. The process of synthesizing these imine-containing compounds is more eco-friendly requiring less waste generated and energy consumption.

The antibiotic activity of these compounds has been tested in a Minimal Inhibitory Concentration (MIC) assay against both Gram-positive and Gram-negative bacteria. The assay was performed using *Escherichia coli*, *Staphylococcus epidermidis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The MICs were taken and analyzed for patterns of how the compounds affected the microbes on day one of being dissolved based on their structure.

Objectives

The goal of this study is to determine whether a group of synthesized small organic molecules has any antibacterial properties.

Methods

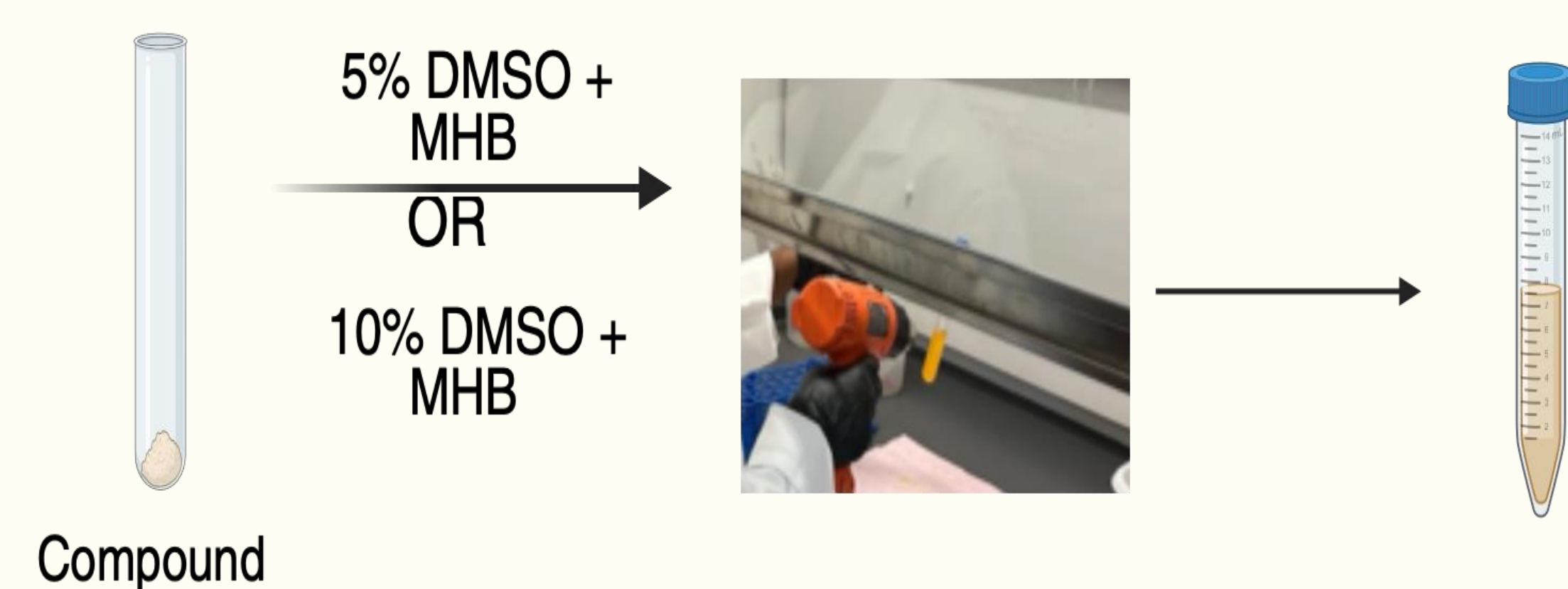


Figure 1. Solubilization of organic compounds. Compounds were resuspended in either 10% or 5% DMSO in MHB depending on their solubility, then dissolved using sonication and heat.

Methods

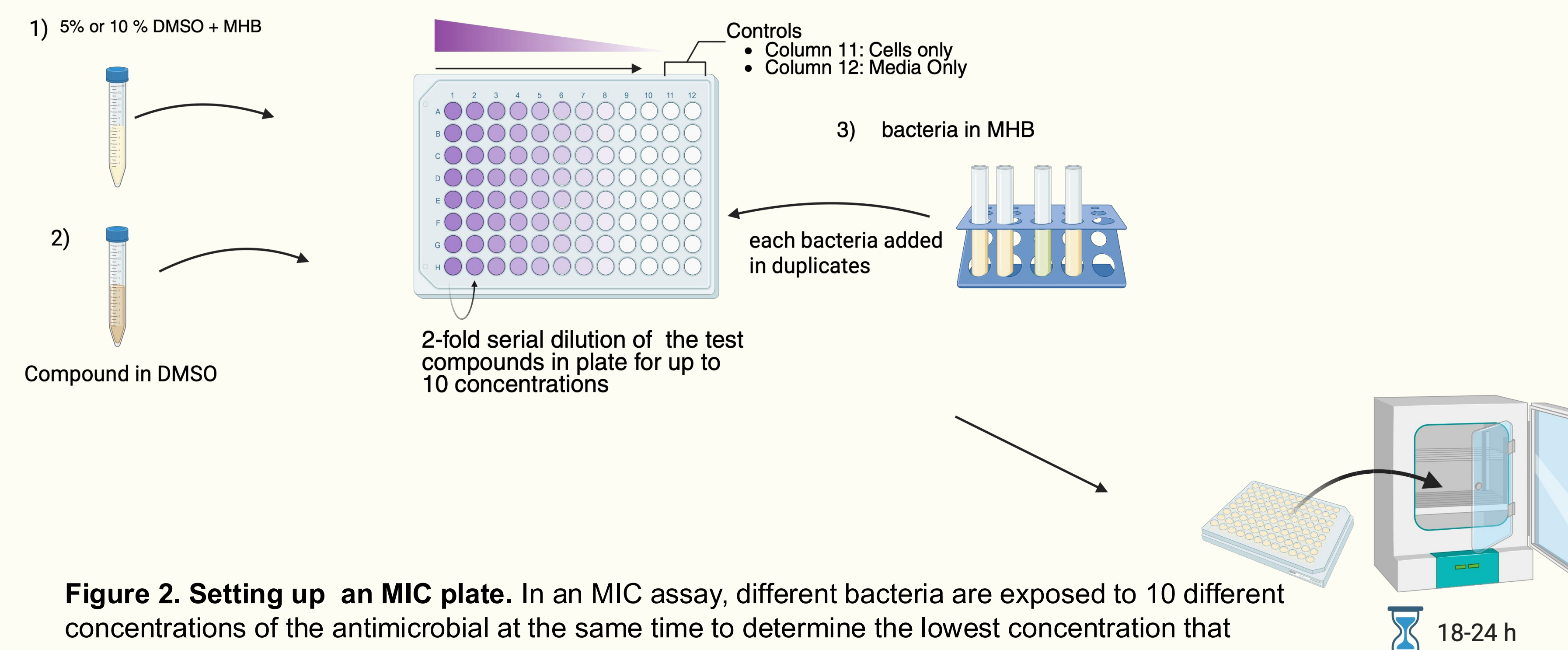


Figure 2. Setting up an MIC plate. In an MIC assay, different bacteria are exposed to 10 different concentrations of the antimicrobial at the same time to determine the lowest concentration that inhibits growth. A 2-fold serial dilution of a compound was set up in a 96-well microtiter plate using the appropriate MHB/DMSO solution as the diluent. Each bacterial strain was added at a final concentration of 5×10^5 cfu/ml in duplicate rows to the plate. Each plate also contained a control column without compound (media and cells only), and a media only column to control for contamination. The plates are incubated at 37 °C for 18-24 hours.

Results

Compound	Conc. Range tested	<i>E. coli</i>	<i>K. Pneumoniae</i>	<i>P. aeruginosa</i>	<i>S. epidermidis</i>
15	0.25 - 0.00035 mg/mL	>0.25mg/mL	0.25mg/mL	0.25 mg/mL	0.06mg/mL / 0.125mg/mL
16	1.25 - 0.0015 mg/mL	0.6mg/mL	1.25 mg/mL	1.25 mg/mL	0.3 mg/mL
18	0.5 - 0.0007 mg/mL	>0.5 mg/mL	>0.5 mg/mL	>0.5 mg/mL	>0.5 mg/mL
42	2.5 - 0.0035 mg/mL	2.5 mg/mL	2.5 mg/mL	2.5 mg/mL	2.5 mg/mL
43	2.5 - 0.0035 mg/mL	0.6 mg/mL	0.15 mg/mL / 0.07 mg/mL	0.3 mg/mL	0.07 mg/mL / 0.035 mg/mL
45	1.25 - 0.0015 mg/mL	1.25 mg/mL	>1.25 mg/mL	0.3 mg/mL / 0.6 mg/mL	>1.25 mg/mL
80	1.25 - 0.0015 mg/mL	1.25 mg/mL	0.6 mg/mL	1.25 mg/mL	0.07 mg/mL
81	0.5 - 0.0007 mg/mL	>0.5 mg/mL	0.5 mg/mL	>0.5 mg/mL	0.5 mg/mL

No sensitivity Lower sensitivity Higher sensitivity

Figure 3. The Minimum Inhibitory Concentration for each bacterial strain on day 1 (day of compound solubilization). The MIC is the lowest concentration of compound that inhibits bacterial growth. Green highlighting indicates that MIC is higher than the highest concentration that was soluble in the plate, blue indicates the MIC was a 2-fold decrease from the highest concentration, and yellow indicates MICs that were more than 2-fold decrease from top of range. Two MIC values in a square indicates a difference in MIC in the duplicates in the plate. The ">" indicates that the MIC is greater than the highest concentration of the compound that was soluble. Compounds that are highlighted in the same color are structurally related.

Conclusions & Next Steps

The compounds all exhibited some antimicrobial effect against some of the bacteria except for compound 18. The compounds that had the strongest antimicrobial effects were compounds 43, 80, and 16. Their MICs show that a lower concentration compound was required to inhibit the growth of bacteria.

Of the related compounds, compound 16 showed to be more bactericidal than compound 15 and compound 43 was more bactericidal than compound 42. Compound 80 was more bactericidal than compound 81.

S. epidermidis was the most sensitive to the greatest number of compounds, specifically 43, 80, 16, and 15 as it took less compound to inhibit the growth.

Future experiments include analysis of additional compounds that are related to the compound groups shown here, as well as analyzing the stability of the antimicrobial activity of the compounds beyond Day 1.

References

Bayeh, Y., Mohammed, F., Gebrezgiabher, M., Elemo, F., Getachew, M. and Thomas, M. (2020) Synthesis, Characterization and Antibacterial Activities of Polydentate Schiff Bases, Based on Salicylaldehyde. *Advances in Biological Chemistry*, 10, 127-139.

Some figures created in <https://BioRender.com>

Acknowledgements

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