Facially Amphiphilic Cationic Antimicrobial Agents Derived from Bile Acids

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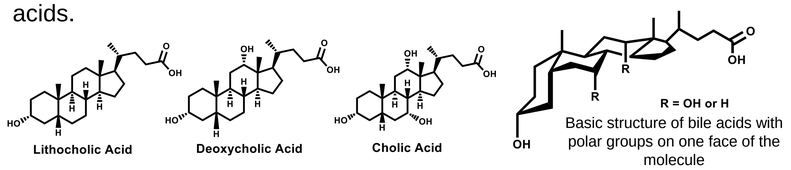
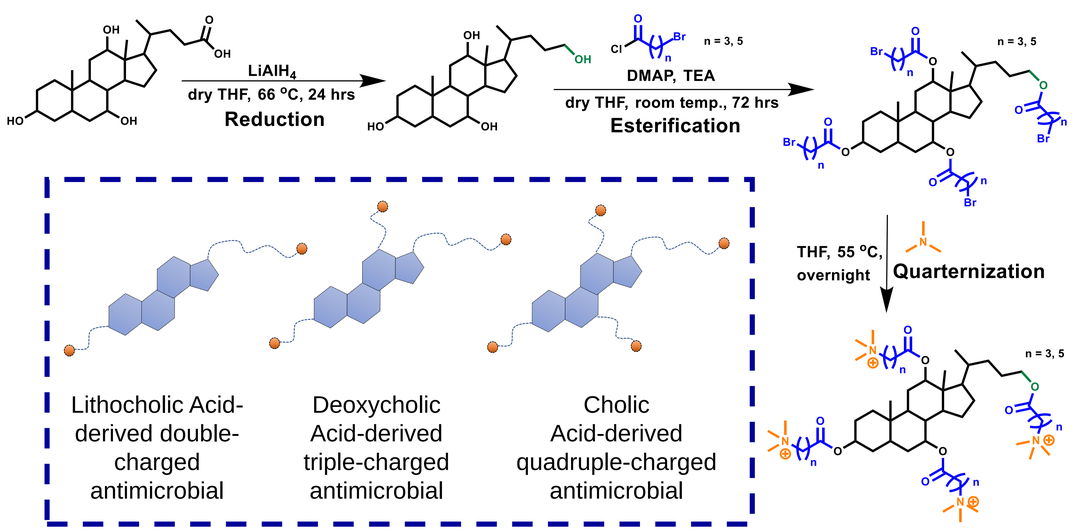
Bacterial Resistance & Natural Compounds

The U.S. has over 2.8 million antibiotic-resistant bacterial infections annually, resulting in over 35,000 deaths and costing over 4.6 billion dollars every year. As antimicrobial-resistant bacterial infections become more common throughout the world, developing new antimicrobials becomes increasingly important.

In addition to synthesizing a variety of new antimicrobials, we consider utilizing naturally-occurring compounds inspired by antimicrobial peptides (AMPs). Regardless of their biological origin, all AMPs have common features such as positively charged (cationic) groups and an amphiphilic structure. They physically perturb the membranes, which leads to death or inhibits further growth of bacteria. Inspired by these naturally occurring AMPs, our research is focused on developing synthetic mimics from bile acids.



Chemical Synthesis: Methods and Results



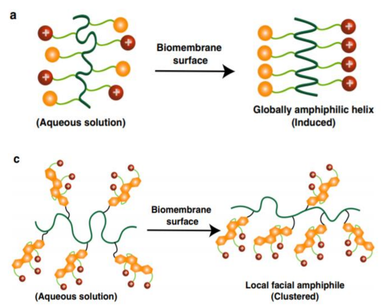
Each of the synthesis steps was performed on cholic acid, deoxycholic acid, and lithocholic acid to give the final molecules with 2, 3, and 4 charges respectively.

Characterization:
- Infrared Spectrometry,
- Proton Nuclear Magnetic Resonance,
- Zeta Potential,
- Dynamic Light Scattering

	LCA (2 charges)	DCA (3 charges)	CA (4 charges)
Size	145.9 nm	242.0 nm	249.5 nm
Zeta Potential	41.9 mV	45.9 mV	48.4 mV

Facial Amphiphilicity: Membrane Interactions

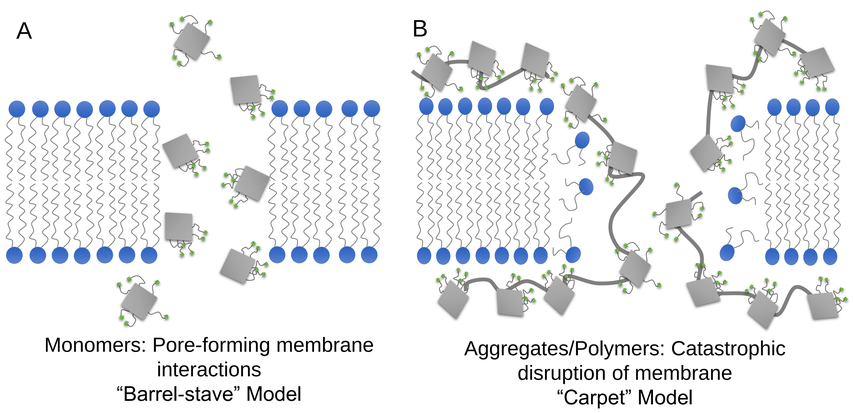
- Based off of naturally-occurring antimicrobial peptides
- Balance of hydrophilic and hydrophobic faces
- Favorable interactions with the bacterial membrane
- Local facial amphiphilicity avoids entropic cost of global facial amphiphilicity



Approach: Polymers or Monomers?

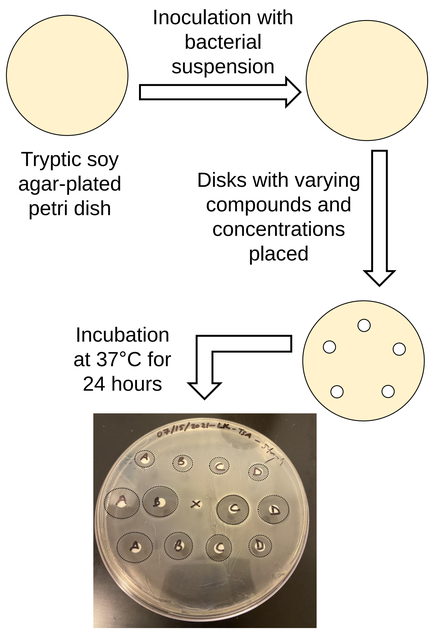
Bile acids are chosen as starting materials because they are:
1. Naturally abundant and biocompatible
2. Local facial amphiphilicity helps overcome entropic barrier

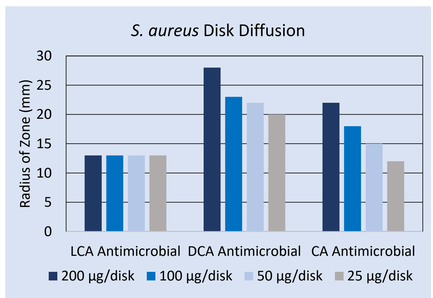
Individual monomers, as opposed to polymers or aggregates of monomers, may show better selectivity for bacterial cells over mammalian cells, leading to lower levels of cytotoxicity.



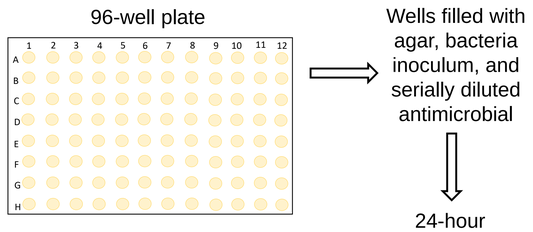
Microbiology: Methods and Results

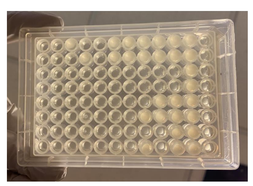
Disk Diffusion Assay:



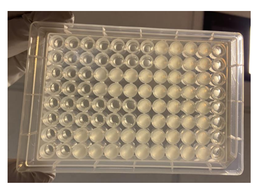


Minimum Inhibitory Concentration:





MIC of *S. aureus* (Gram-positive)



MIC of *E. coli* (Gram-negative)

	Chain Length	MIC (µg/mL) S. Aureus (ATCC 29213)	E. Coli (ATCC 25922)	P. Aeruginosa (ATCC 27853)
LCA-based Antimicrobial	4	1.0	3.9	125.0
	6	0.5	3.9	62.5
DCA-based Antimicrobial	4	1.0	31.0	>250.0
	6	0.5	16.0	125.0
CA-based Antimicrobial	4	16.0	125.0	>250.0
	6	62.5	250.0	>250.0

Conclusions/Future Directions

The lithocholic acid-derived antimicrobial with a 6-carbon chain performed better than the other antimicrobial compounds in MIC testing. Cholic acid antimicrobials consistently performed poorly. Our main takeaways are:
- Balancing hydrophobic to hydrophilic portions (cholic acid may be too hydrophilic) to ensure favorable membrane interaction
- Differences in interactions with gram-positive and gram-negative bacteria
- Increased flexibility for interaction from longer carbon chains

As the project continues, we will:
- Repeat disk diffusion and MIC testing with different media
- Compare monomer performance to polymer performance
- Fine-tune the balance between number of charged groups and length of carbon chain

References

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Acknowledgements

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