Otilonium Bromide as an Antimicrobial: a Structure-Activity Relationship Study

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Introduction (Background)

The general structure of a quaternary ammonium compound (QAC) consist of a positively charged quaternary amine and a lipophilic tail[1] and pictured in figure 1 with a comparison to the compound of interest, Otilonium Bromide (OB). This class of compounds are used in a variety of biomedical and cleaning products[1] such as in figure 2.

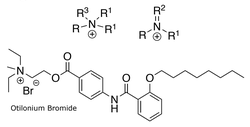


Figure 1. Above are the two general forms of QACs[1]. Below is OB. Notice the quaternary amine warhead to the far left, ester aryl amid linker and long side chain.

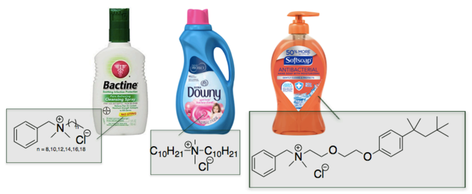


Figure 2. Common household items with QAC active ingredients. From left to right: an antiseptic, surfactant, antibacterial agent.

Each general use of quaternary ammonium compounds have at least one route to accomplish their respective purposes. These possible mechanisms of action can be found in figure 3. The biological pathway of OB has not yet been established, but could be combination of cell membrane disruption and inhibition of nucleic acid synthesis[1]. The structure of OB mimics the phospholipid bilayer, hence selectivity issues over eukaryotic cells like red blood cells.

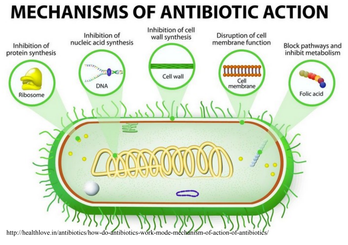


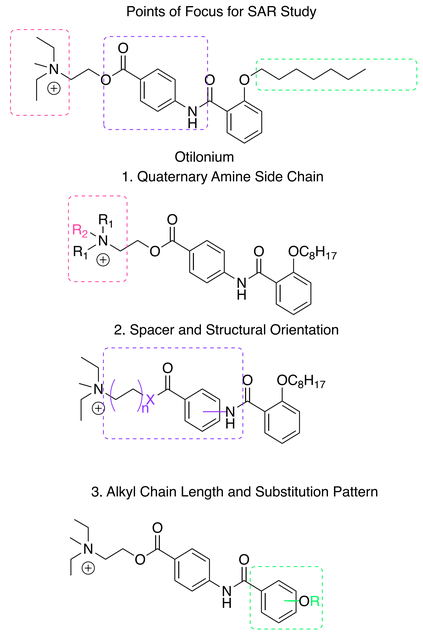
Figure 3. A concise visual of the various major mechanisms of action thought to undergo for antibiotic compounds. The key mechanisms for our work include disruption of cell membrane function and inhibition of nucleic acid synthesis.

Goal

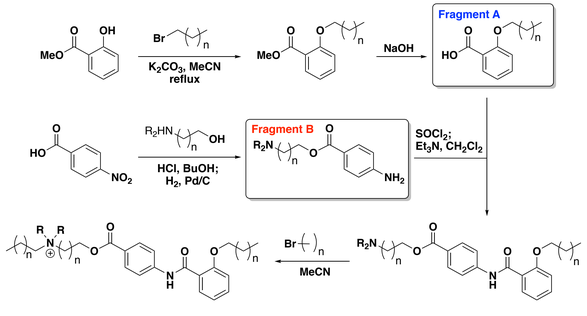
The objective of this project was to synthesize Otilonium Bromide derivatives for a SAR study in order to discover a novel antimicrobial agent, which has a large therapeutic index for hemolytic activity and antimicrobial potency.

Strategy

Maintain minimal inhibition concentrations (MIC) while decreasing hemolysis through a multi-point structure-activity relationship study. This was done by modifying the substituent on the quaternary amine warhead, the chain linker and ester moiety, the substitution of the core aryl groups, and the position and length of the side chain.



General Synthesis



Results

Antimicrobial Activity and Hemolysis of 25 Otilonium Derivatives

SAR Point	Compound	MIC (μg/mL) *S. aureus* Gram+	MIC (μg/mL) *A. baumannii* Gram-	Hemolysis% at 16 μg/mL	Hemolysis% at 32 μg/mL
	Otilonium	4	16	7.0	92
1	072901	4	32	3	85
	072942	2	16	3	91
	072944	2	16	16	32
	072952	2	16	18	99
	073110	8/16	32/64	1.9	13
	073118	4	16	1.8	47
2	072815	4/8	64	3	46
	072954	4	16	4	93
	072955	4	32	7	92
	073113	64	128	0.92	1.3
3	072822	64	128	0	0.2
	073038	4	32	1.5	2.2
1 & 2	073111	4	16/32	1.9	10
	073112	64	128	0.83	0.87
	073117	16	64	0	0.36
1 & 3	149484	8	64	0.27	0.30
	149489	2	16	1.5	5.2
	149491	2	16	0.73	7.9
2 & 3	149540	8	16	3.3	31
	149642	4	32	1.6	19
	149543	32	64	1.6	2.5
1,2,3	149537	8	32	2.3	20
	149538	16	32	1.1	2.8
	149544	8	16	3.4	48
	149545	8	32	1.2	2.7

Table 1. OB was used as a control. 25 analogs were chosen to give a general overview of results and trends between points of modifications, antimicrobial activity and hemolytic activity. Hemolysis percentages rounded to two significant figures. Leads as of December 2017 highlighted in green; as of May 2018 in blue.

Activity and hemolysis generally had expected results and followed the trend of previously reported quaternary ammonium compounds in that as potency increased, hemolysis did as well. This correlation has previously been reasoned with the lack of selectivity for cell penetration of QACs for bacterial cell membrane over eukaryotic cells such as red blood cells. It has been noted that bacteria cell membrane is more electronegative than red blood cells possibly proving some selectivity. However, the Davies group's hypothesis of the mechanism of action of OB and its derivatives on bacterial growth inhibition involving membrane disruption as only a part may be why some results gave only minor decreases in potency while diminishing hemolysis.

Highlighted lead compounds wre chosen based on antimicrobial and hemolysis activity. Further studies for these lead compounds and OB as a control may give insight to the biological pathway and mechanism of action of QACs such as otilonium bromide.

Summary

Established the following through SAR

- **range of lipophilic tail length to minimally decrease potency and greatly decrease hemolysis**
- **Substitution pattern around core aryl in spacer to maintain potency**
- **Chain length between amine and ester gave little change in activities**
- **Alkyl chain length of amine to maintina activity and greatly decrease hemolysis**
- **Bioisosteres of ester linker may be key to diminishing hemolysis and maintaining potency**
- **Heterocyclic quaternary amines may aid in decreasing hemolysis and maintaining potency**

Current Aims

- **Conformational study**
- **Stability study**

References

[1]Jennings MC, Miniole KPC, Wuest WM**. Quaternary Ammonium Compounds: An Antimicrobial Mainstay and Platform for Innovation to Address Bacterial Resistance**. *ACS Infect. Dis. 2015*; 1(7):288-303

Acknowledgements

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