Antibacterial Properties of Novel Eumelanin-Inspired Phenylene Indolyne Derivatives

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Abstract

Eumelanin Inspired Phenylene Indolyne (EIPE) derivatives EIPE-1 and EIPE-HCl are novel compounds. The unique characteristic of EIPE is its structure serving as scaffolding for functional groups that may have antibacterial properties. The purpose of this study is to determine if EIPE derivatives possess antibacterial properties. Both gram-positive and gram-negative organisms will be screened against EIPE derivatives using a standardized Kirby Bauer disk agar diffusion assay. Only a few gram-positive organisms exhibited susceptibility to EIPE-1. These results showed that EIPE-1 does have antibacterial properties against some pathogenic gram-positive organisms.

Introduction

Hypothesis

- Intrinsic resistance to EIPE-1 is due to the general impermeability properties of the gram-negative outer membrane for hydrophobic substances.

Background

- EIPE is a novel compound with a eumelanin inspired core and unique sites for functional group scaffolding.
- EIPE-1 is hydrophobic and EIPE-HCl is hydrophilic.
- Gram-negative bacteria have an outer membrane that generally excludes hydrophobic molecules.
- Gram-positive bacteria lack an outer membrane.

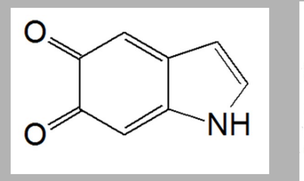

Figure 1. eumelanin inspired core

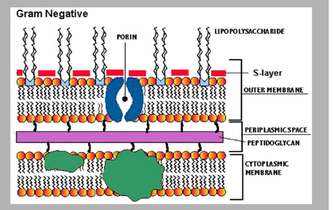

Figure 2. Gram-negative outer membrane

Methods

Standardized Disk Agar Diffusion Bioassay
Selected gram-positive and gram-negative bacteria were screened for susceptibility to EIPE-1 and EIPE-HCl using a standardized disk agar diffusion bioassay routinely practiced in our lab.

Table 1. Susceptibility of Test Strains to EIPE-1 and EIPE-HCl

Organism	Inhibition Zone Diameter (mm)[a] ± SD							
	Control[b]	EIPE-1 (25µg)	Control	EIPE-1 (50µg)	Control	EIPE-1 (250µg)	Control	EIPE-HCl (25µg)
Gram-negative								
E. coli ATCC 25922	0.0	0.0	ND[c]	ND	ND	ND	0.0	0.0
E. coli K12 413	0.0	0.0	ND	ND	ND	ND	0.0	0.0
P. moltocida P-1581	0.0	0.0	ND	ND	0.0[e]	3.91	0.0	0.0
P. aeruginosa ATCC 27853	0.0	0.0	ND	ND	ND	ND	0.0	0.0
S. entomophila ATCC 43705	0.0	0.0	ND	ND	ND	ND	0.0	0.0
S. fonticola ATCC 9844	0.0	0.0	ND	ND	ND	ND	0.0	0.0
S. marcescens CO1-A	0.0	0.0	ND	ND	ND	ND	0.0	0.0
S. marcescens ATCC 13880	0.0	0.0	ND	ND	ND	ND	0.0	0.0
S. marcescens 8100	0.0	0.0	ND	ND	ND	ND	0.0	0.0
S. marcescens ATCC db11	0.0	0.0	ND	ND	ND	ND	0.0	0.0
S. odorifera ATCC 53077	0.0	0.0	ND	ND	ND	ND	0.0	0.0
S. plymuthica ATCC 18	0.0	0.0	ND	ND	ND	ND	0.0	0.0
S. rubidaea ATCC 27593	0.0	0.0	ND	ND	ND	ND	0.0	0.0
Gram-positive								
B. cereus	0.0	0.0	ND	ND	ND	ND	0.0	0.0
B. subtilis ATCC 6633	0.0	1.1 ± 0.2	0.0	3.3 ± 0.1	0.0	1.8 ± 0.3	0.0	0.0
D. radiophilus	0.0	0.0	ND	ND	ND	ND	0.0	0.0
E. faecalis	0.0	0.0	ND	ND	ND	ND	ND	ND
L. Lactis	0.0	0.4 ± 0.6	ND	ND	ND	ND	0.0	0.0
M. luteus	0.0	1.0 ± 1.0	ND	ND	0.0	2.2 ± 1.9	0.0	0.0
S. aureus ATCC 25923	0.0	1.6 ± 0.1	0.0	3.1 ± 0.2	0.0	2.3 ± 0.2	0.0	0.0
S. aureus ATCC 29213	0.0	2.2 ± 0.6	ND	ND	0.0	2.9 ± 0.3	0.0	0.0
S. aureus SFL 8	0.0	2.4 ± 1.3	ND	ND	0.0	2.4 ± 0.3	0.0	0.0
S. aureus SFL 64	0.0	2.5 ± 1.5	ND	ND	0.0	2.5 ± 0.2	0.0	0.0
S. aureus T-5706	0.6 ± 0.9	2.1 ± 0.3	ND	ND	0.0	2.2 ± 0.2	0.0	0.0
S. epidermidis	0.0	2.6 ± 0.3	ND	ND	0.0	3.3 ± 0.5	0.0	0.0

Legend
[a]Diameter of growth inhibition zone minus disk diameter (6.0mm); n=3.
[b]DMSO was used to dissolve EIPE-1, therefore control disks were prepared by impregnating with solvent and allowing to air dry prior to plate application.
[c]ND, not determined.
[d]Values only represented one independent determination.
[e]Only tested one time

Figure 3. Kirby Bauer Disc Agar Diffusion Assay

- EIPE-HCl (25µg) top row
- EIPE-1 (250µg) middle row
- EIPE-1 (25µg) bottom row
- Control on left column, compound on right column
- *B. subtilis* has EIPE-1 (50µg) after EIPE-1 (25µg)

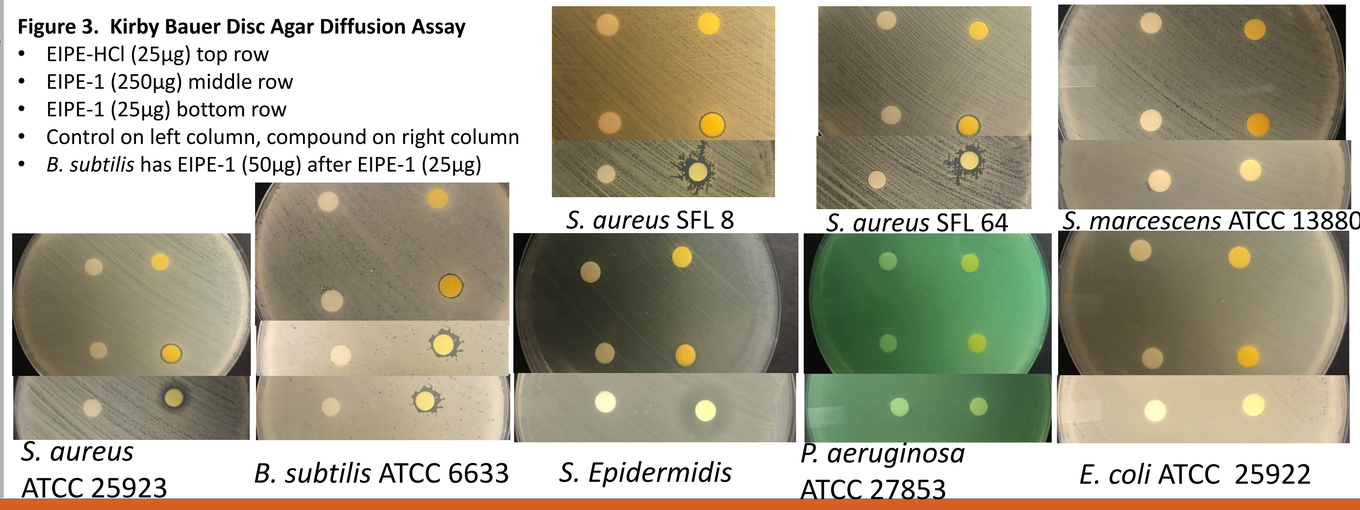

Figure 4. Batch Culture Growth Kinetics

Results Summary

Gram-Negative Organisms

- All organisms demonstrated resistance.

Gram-Positive Organisms

- *B. subtilis* ATCC 6633, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, *S. aureus* T-5706, *S. epidermidis, S. aureus* SFL 8, and *S. aureus* SFL 64 all showed small zones of inhibition against EIPE-1.
- *B. subtilis* ATCC 6633 and *S. aureus* ATCC 25923 exhibited larger zones of inhibition when tested against the EIPE-1 at the 2X potency of EIPE-1.
- 10X Potency of EIPE-1 did not exhibit significantly larger zones.
- *B. cereus*, *D. radiophilus*, *E. facalis, L. lactis*, *M. Luteus* displayed resistance to EIPE.

Conclusions

- EIPE-1 has demonstrated a susceptible gram positive spectrum at 25 µg.
- Mechanism of resistance due to exclusionary properties is unknown, but susceptibility of *P. moltocida* P-1581 against the 250 µg EIPE-1 potency suggests the outer membrane is responsible for resistance.
- Susceptibility is dose dependent.
- EIPE-1 is a novel compound different than the mainstream β-lactam antibiotics.
- The hydrophobic derivative is more effective than the hydrophilic derivative.
- EIPE-1 likely causes bacteriolysis in batch culture growth kinetics.

Future Research

- Minimal inhibitory concentration and minimal bactericidal bioassays would help quantify the efficacy of EIPE-1.
- Batch culture growth kinetic assays should be conducted to find mechanism responsible for resistance.

References

- Clayborn, A.B., S.N. Toofan, and F.R. Champlin. 2011. Journal of Hospital Infection. 77:129-133. DOI: 10.1016.