

A Novel Anti-virulence Approach for Treatment of Pneumonia Caused by *Pseudomonas aeruginosa*

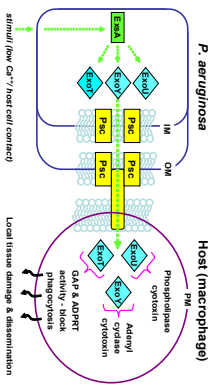
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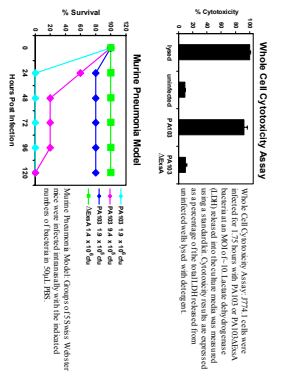
ABSTRACT

Background: Novel therapies for the prevention and treatment of infectious diseases are needed to address the problems of antibiotic resistance. In *P. aeruginosa* (PA), the MexA/MexC family transcription factor ExsA is an important virulence regulator of the type III secretion (T3S) system. We developed small molecule inhibitors of ExsA that block the T3S system and reduce the virulence of PA. ExsA protein components were additionally screened for inhibition of TTS dependent cytotoxicity toward infected J774 cells. A PA mouse model of acute lethal pneumonia was used to assess the efficacy of the compounds. **Results:** Benzimidazole derivatives were screened for inhibition of ExsA activity. A series of benzimidazole derivatives were identified that block ExsA activity and reduce the virulence of PA in a mouse pneumonia model. In the severe PA mouse pneumonia model, there was a significant increase in survival of mice (4-fold p<0.05) treated daily IP for four days post-infection with compound P005301 over vehicle alone. **Conclusion:** Targeting MexA-like proteins with small molecule inhibitors is a novel approach to anti-infective chemotherapy. The identification of MexA-like drugs is a novel approach to anti-infective chemotherapy. The identification of MexA-like drugs is a novel approach to anti-infective chemotherapy. The identification of MexA-like drugs is a novel approach to anti-infective chemotherapy.

ExsA Regulate Expression of the Type III Secretion System



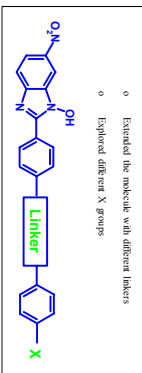
ExsA Mutants are Attirant in Whole Cell and Animal Models of Infection



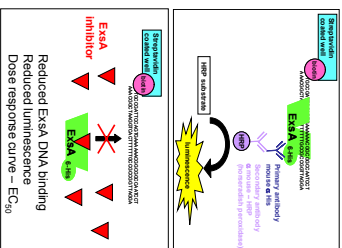
Identification of ExsA Inhibitors

- ExsA is a member of the MexA/MexC family of transcription regulators
- Defined by 2 conserved heteromultimeric DNA binding domains
- The MexA/MexC family is conserved among bacteria, not found in eukaryotes
- Several MexA/MexC family proteins regulate virulence factor expression in bacterial pathogens (Mason et al., 2001, *MB* 69:186-195; Casaz et al., 2006, *Microbiology* 152:2493-2500)
- Paradek previously developed small molecule inhibitors of *E. coli* AnxC family proteins (Muller, Boby and Suss (Dobson et al., *Biorganic & Med Chem Letters*, in press)
- Docked combinatorial chemistry scaffold to published crystal structure of *E. coli* MexA DNA binding domain (Dobson et al., *Nat Commun* 1998, 9:5104-13)
- Paradek hydroxy benzimidazoles

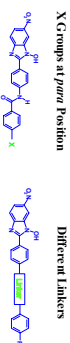
Medicinal Chemistry



ExsA Inhibition in Cell Free DNA Binding Assay



Exploring the Structure – Activity Relationship

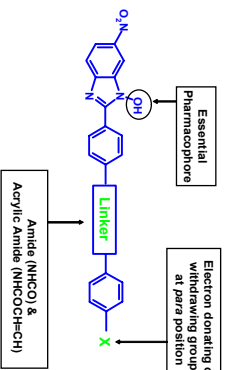


Compound	X	ExsA Ed50 (nM)	Linker	ExsA Ed50 (nM)
P005172	H	6.0	HO(CH2)2	~8.0
P005173	OH	6.0	HO(CH2)2	~8.0
P005174	OMe	6.2	HO(CH2)2	~8.0
P005175	F	7.2	HO(CH2)2	~8.0
P005176	Cl	7.2	HO(CH2)2	~8.0
P005177	Br	7.2	HO(CH2)2	~8.0
P005178	I	8.7	HO(CH2)2	~8.0
P005179	NH2	9.1	HO(CH2)2	~8.0
P005180	NO2	10.1	HO(CH2)2	~8.0
P005181	SO2Me	10.8	HO(CH2)2	~8.0
P005182	SO2Et	12.0	HO(CH2)2	~8.0
P005183	SO2iPr	13.3	HO(CH2)2	~8.0
P005184	SO2nBu	20.7	HO(CH2)2	~8.0
P005185	SO2tBu	21.2	HO(CH2)2	~8.0
P005186	SO2C6H5	20.9	HO(CH2)2	~8.0
P005187	SO2C6H4	34.3	HO(CH2)2	~8.0
P005188	SO2C6H3	45.0	HO(CH2)2	~8.0
P005189	SO2C6H2	45.0	HO(CH2)2	~8.0
P005190	SO2C6H	25.0	HO(CH2)2	~8.0

Acrylic Amide Linker Series

Compound	X/Y	ExsA Ed50 (nM)
P005191	H	2.4
P005192	OH	2.4
P005193	OMe	2.4
P005194	F	2.4
P005195	Cl	2.4
P005196	Br	2.4
P005197	I	2.4
P005198	NH2	2.4
P005199	NO2	2.4
P005200	SO2Me	2.4
P005201	SO2Et	2.4
P005202	SO2iPr	2.4
P005203	SO2nBu	2.4
P005204	SO2tBu	2.4
P005205	SO2C6H5	2.4
P005206	SO2C6H4	2.4
P005207	SO2C6H3	2.4
P005208	SO2C6H2	2.4
P005209	SO2C6H	2.4

Structure - Activity Relationship Overview



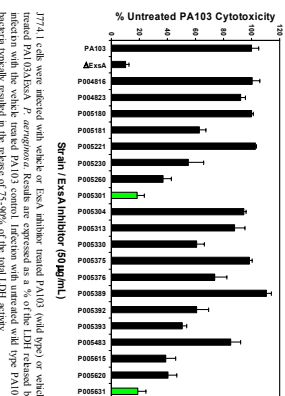
ExsA Inhibitors are Non-Antibacterial

Compound	ExsA Ed50 (nM)	PA103 MIC (μg/ml)
P005172	6.0	>128
P005173	6.0	>128
P005174	6.2	>128
P005175	7.2	>128
P005176	7.2	>128
P005177	7.2	>128
P005178	8.7	>128
P005179	9.1	>128
P005180	10.1	>128
P005181	10.8	>128
P005182	12.0	>128
P005183	13.3	>128
P005184	20.7	>128
P005185	21.2	>128
P005186	20.9	>128
P005187	34.3	>128
P005188	45.0	>128
P005189	45.0	>128
P005190	25.0	>128

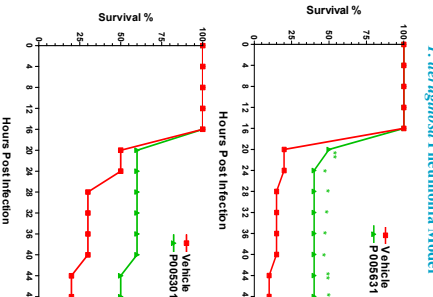
Screen Compounds for Inhibition of *P. aeruginosa* Cytotoxicity

- Type III Secretion dependent cytotoxicity - ExsA mutant positive control
- Cells will type III secretion under Type III Secretion blocking conditions with ExsA inhibitor or equal volume vehicle
- Infect J774 cells in the presence of ExsA inhibitor - measure lactate dehydrogenase (LDH) release

Cytotoxicity of *P. aeruginosa* toward J774.1 Cells +/- ExsA Inhibitors



Efficacy of ExsA Inhibitors in a Lethal *P. aeruginosa* Pneumonia Model



CONCLUSIONS

- The MexA/MexC family transcription factor ExsA is required for full virulence in tissue culture and animal models of *P. aeruginosa* infection.
- Treatment with ExsA inhibitors attenuates virulence and protects animals from *P. aeruginosa* pneumonia.
- ExsA inhibitors are non-antibacterial and thus not under the same selection pressure for resistance as traditional antibiotics.
- Inhibitors that act against multiple members of the MexA/MexC family of transcription factors may further inhibit *P. aeruginosa* virulence and could be used as broad spectrum agents against multiple bacterial pathogens.

Efficacy of P005301 and P005301, prototypic ExsA inhibitors, vs. *Pseudomonas aeruginosa* PA103 in a mouse lethal pneumonia model (IP organisms inoculated intranasally). P005301 was assessed at various times post infection. A statistically significant difference was noted between the untreated (vehicle) and the P005301 treated groups. ** p<0.05, * p<0.1 by Chi-Square analysis, n = 22 mice/group. P005301 was administered IP at 25 mg/kg at 18, 1, 5, 20, 26, and 44 hours post-infection. Mortality was assessed at various times post infection, n = 6-8 mice/group.