

Small Molecule Inhibitors of *Yersinia pseudotuberculosis* Transcription Factor LcrF (VirF)



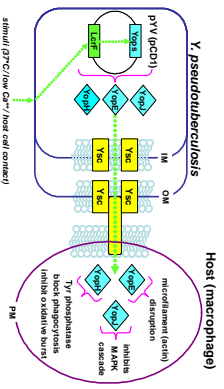
75 Kneeland Street
Boston, MA 02111
E: 617 - 275 - 0040
F: 617 - 275 - 0039

lynnr1-yun@paratekpharm.com | lynnr1-yun@paratekpharm.com
Lynne K. Carnty-Ryan¹, Oak K. Kim¹, Joan Miguel Bada-Lastra¹, Victoria Bartlett¹, Ann Vermet¹, Michael L. Fisher¹, Varunagana Sampathong¹, Cynthia Castillo¹, Stuart B. Levy¹, Joan Messeri¹, Michael N. Aleksuni¹,
¹Paratek Pharmaceuticals, Inc., Boston, MA 02111

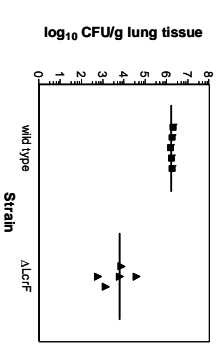
ABSTRACT

Yersinia pseudotuberculosis (Ypb) mutants deficient in type III secretion (T3S) show dramatically attenuated virulence in a mouse model of pneumonia. Expression of the Ypb T3S system is dependent on activation by LcrF (VirF), a transcription factor in the *lcr* operon. In an *in vitro* assay, we used a screen to identify small molecules designed to interfere with the DNA binding activity of LcrF (VirF). All of the compounds lack intrinsic antibacterial activity, as judged by standard MIC assays, and do not show toxicity against mammalian cells. We identified 173 of 799 potential and T3S dependent cytotoxicity toward infected macrophages in whole cell experiments. Selected compounds were used to treat mice prior to and during an acute infection with Ypb bacteria in a pneumonia model. Treatment with a number of compounds substantially reduced the bacterial burden in the lungs in a 3-day mortality. This work demonstrates that treatment with small molecule inhibitors of a bacterial transcription factor can attenuate virulence and thus prevent or limit infection. Since the *lcr* family of transcription factors themselves are well conserved and play central roles in pathogenesis across bacterial genera, the inhibitors could have broad application.

LcrF Regulates Expression of the Type III Secretion System



Virulence Attenuation of LcrF Mutant in a Non-lethal Lung Infection Model



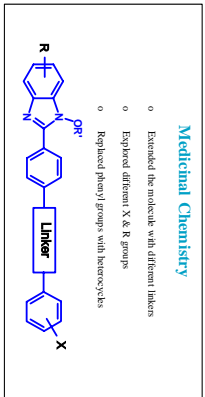
6-8 week old female BALB/c mice were infected intranasally with ~700 CFU of wild type (WT) P2669(BI) or ΔLcrF (ΔM155) *Y. pseudotuberculosis*. Mice were sacrificed 5 days post-infection. Lung tissue were homogenized and serial dilutions were plated (Kroder, Fisher *et al.*, *Molec. & Immun.*, 2007, 75:25-442).

Identification of LcrF Inhibitors

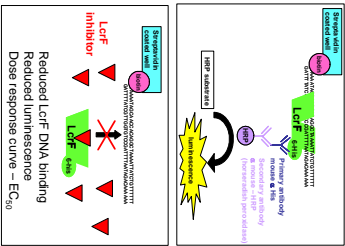
- LcrF is a member of the *lcr* family of transcription regulators
- Defined by 2 conserved helix-turn-helix DNA binding domains
- AcC Family is conserved among bacteria, not found in eukaryotes
- Previously developed small molecule inhibitors of *E. coli* AcC family proteins, Murk & Sosa
- Docked computational chemistry scaffolds to published crystal structure (Droser *et al.*, *PNAS USA* 1998, 95:10413)
- Identified 5 classes of small (<500 Da) molecules with activity *in vitro*
- Pseudotubercularicins in routine MIC panel testing
- Inhibitors are *nonantibacterial* in routine MIC panel testing

Medicinal Chemistry

- Extended the molecule with different linkers
- Explored different X & K groups
- Replaced phenyl groups with heterocycles



LcrF Inhibition in Cell Free DNA Binding Assay



Exploring the Structure - Activity Relationship

X Groups at para Position

Compound	X	LC50 (μM)	LC50 (nM)
PRO0712	H	216	216
PRO0200	Me	609	609
PRO0201	Me	109	109
PRO0418	F	114	114
PRO0313	Me	122	122
PRO0203	CO ₂ Me	129	129
PRO0412	CO ₂ Me	143	143
PRO0424	CH ₃	151	151

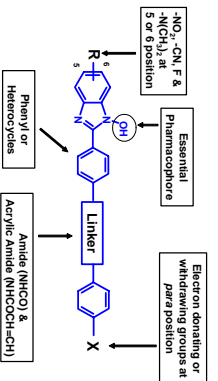
X Groups at meta/ortho Position

Compound	X	LC50 (μM)	LC50 (nM)
PRO0206	H	11.4	11.4
PRO0207	H	29.0	29.0
PRO0041	H	597.3	597.3
PRO0412	CO ₂ Me	15.1	15.1
PRO0209	formyl	27.5	27.5
PRO0240	formyl	597.9	597.9

Different Linkers

Compound	Linker	LC50 (μM)	LC50 (nM)
PRO0206	Linker A	11.4	11.4
PRO0207	Linker B	41.5	41.5
PRO0208	Linker C	59.5	59.5
PRO0209	Linker D	59.4	59.4
PRO0210	Linker E	3.9	3.9
PRO0211	Linker F	4.6	4.6
PRO0212	Linker G	4.28	4.28
PRO0213	Linker H	11.7	11.7
PRO0214	Linker I	13.1	13.1

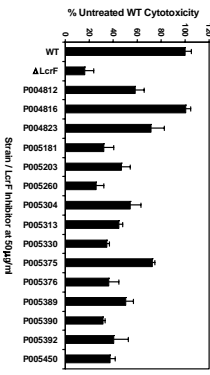
Structure - Activity Relationship Overview



Screen Compounds for Inhibition of *Y. pseudotuberculosis* Cytotoxicity

- Type III secretion dependent cytotoxicity - ΔLcrF mutant positive control
- Culture wild type bacteria under Type III Secretion inducing conditions with LcrF inhibitor or equal volume vehicle
- Inject J7741 cells in the presence of inhibitor - measure lactate dehydrogenase (LDH) release

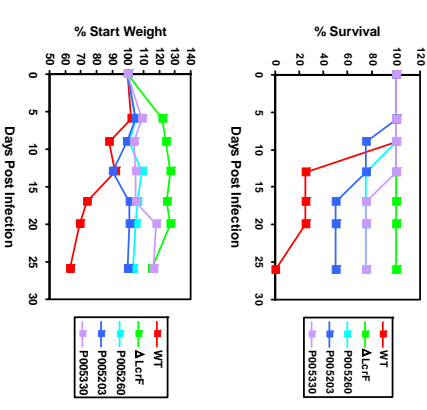
Cytotoxicity of *Y. pseudotuberculosis* Toward J7741 Cells +/- LcrF Inhibitors



Efficacy of LcrF Inhibitors in a Non-lethal Lung Infection Model

J7741 cells were infected with vehicle or LcrF inhibitor treated wild type (WT) Ypb(BI) or vehicle treated ΔLcrF (ΔM155) *Y. pseudotuberculosis*. Results are expressed as a % of the bacteria growth yielded in the release of 25-50% of the total LDH activity.

Efficacy of LcrF Inhibitors in a Lethal *Y. pseudotuberculosis* Pneumonia Model



CONCLUSION

- Groups of 4 CD1 mice (7-8 week old males) were dosed subcutaneously with either vehicle or compound (25 mg/kg) 1 day prior to infection, at the time of infection (0h), at 8h, and then daily for 8 days following intranasal infection with ~120 CFU of wild type (WT) P2669(BI) or ΔLcrF (ΔM155) *Y. pseudotuberculosis*. Mice that % survival data for P005260 and P005330 are on top of each other.
- LcrF is required for full virulence in tissue culture and animal models of *Y. pseudotuberculosis* infection.
- Treatment with LcrF inhibitor attenuates virulence and protects animals from *Y. pseudotuberculosis* infection.
- LcrF inhibitors are nonantibacterial and thus not under the same selection pressure for resistance as traditional antibiotics.
- Inhibitors that act against multiple members of the *lcr* family of transcription factors are more effective against multiple bacterial pathogens.
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