Efficacy of repurposed ClpXP protease inhibitors in *Bacillus anthracis* Sterne

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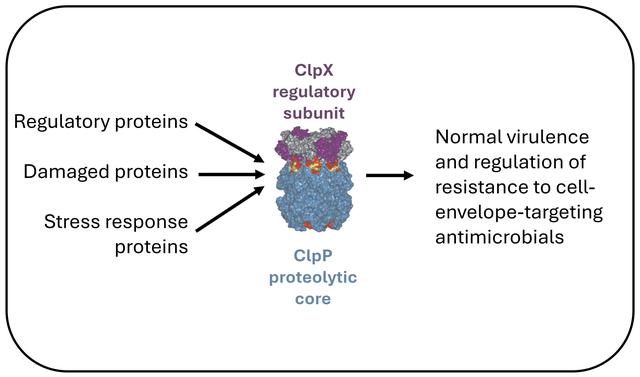
Background

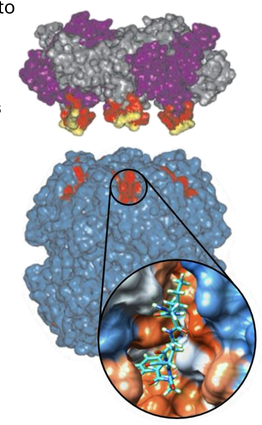
As increasing antimicrobial resistance continues to limit treatment options for bacterial infections, several new approaches have sought to avoid the challenges faced by traditional antibiotics. One such approach is targeting virulence factors, which are necessary for pathogens to evade host defenses and establish infection but not for survival outside the host. This strategy could provide an effective form of treatment while reducing selective pressures for bacteria to evolve resistance mechanisms and limiting damage to populations of non-pathogenic bacteria. ClpXP is a highly conserved proteolytic complex consisting of the ClpP core and the ATP-dependent regulatory protein ClpX. This complex plays a critical role in post-translational regulation of the proteome and is essential for virulence in several bacterial pathogens. In *B. anthracis,* deletion of *clpx* also results in increased susceptibility to cell-envelope-targeting antibiotics such as penicillin.



Joshi et al. 2004 Nat Struct Mol Biol.; Aljghami et al. 2022 Front Mol Biosci.

Approach

- Use structure-based drug repurposing to inhibit formation of the ClpXP complex
- We targeted a site in ClpP normally bound by a tripeptide 'IGF' in ClpX; this interaction is essential for complex stabilization
- 4,636 candidate compounds were screened *in silico* and 5 high-affinity compounds were selected for further testing
- We then evaluated whether inhibitors were able to mimic the phenotype of a *Δclpx* mutant in *B. anthracis*



- Checkerboard assays using varying concentrations of each inhibitor with penicillin were used to examine synergistic reactions using FIC index calculations

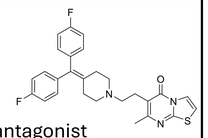
FIC index = $(MIC_{AB}/MIC_A) + (MIC_{BA}/MIC_B)$

- MIC_{AB} is the minimum inhibitory concentration (MIC) of drug A tested in combination, MIC_A is the MIC of drug A tested alone, MIC_{BA} is the MIC of drug B tested in combination and MIC_B is the MIC of drug B tested alone.

Kim et al. 2001 Nat Struct Mol Biol.

Tested Compounds

Ritanserin

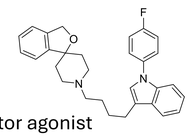


Binding Affinity: -9.5 kcal/mol

- 5-HT receptor antagonist
- Evaluated for treatment of psychiatric conditions.
- Commercial development discontinued after phase III clinical trials

Wiesbeck et al. 1999 Alcohol Clin Exp Res.

Siramesine

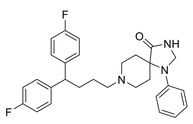


Binding Affinity: -9.4 kcal/mol

- Sigma-2 receptor agonist
- Potential as an anti-cancer agent and anxiolytic
- Underwent phase II clinical trials for treatment of anxiety

Česen et al. 2013 Cell Death Dis. Heading 2001 Curr Opin Investig Drugs.

Fluspirilene



Binding Affinity: -9.1 kcal/mol

- Dopamine D_2 receptor antagonist and regulator of CDK2 activity
- Phase III clinical trials for schizophrenia were terminated

Chouinard et al. 1986 J Clin Psychopharmacol.

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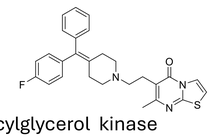
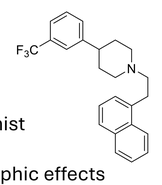


Binding Affinity: -10.1 kcal/mol

- Inhibitor of diacylglycerol kinase
- 5-HT receptor antagonist
- Weak antagonist of dopamine D_2 and histamine H_1 receptors

de Chaffoy de Courcelles et al. 1985 J Biol Chem.

Xaliproden

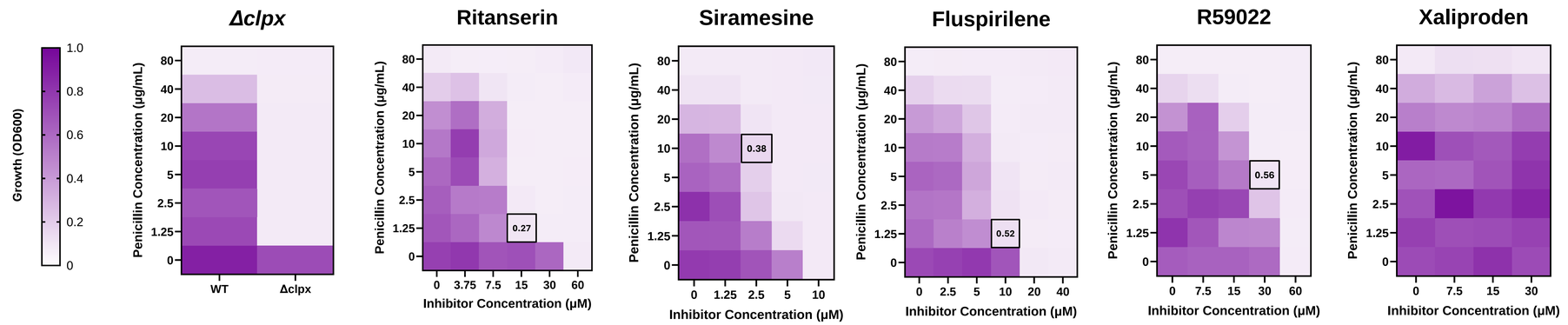


Binding Affinity: -9.2 kcal/mol

- 5-HT receptor agonist studied for potential neurotrophic effects
- Completed phase III clinical trials for treatment of ALS with positive results

Meininger et al. 2004 ALS Other Motor Neuron Disord.

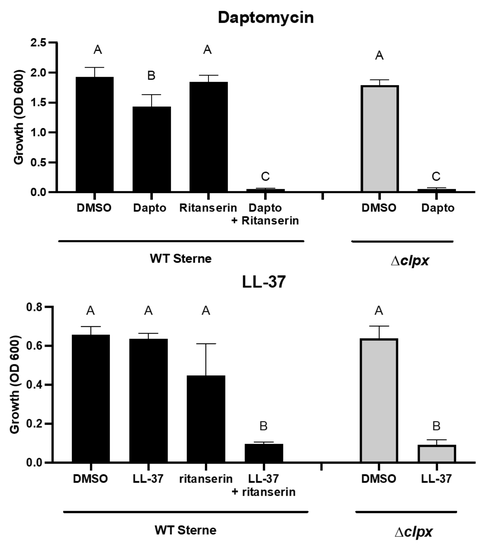
Checkerboard Results



A maximum of 1% DMSO was used as a vehicle to improve solubility of inhibitors.
The lowest FIC value for each inhibitor is displayed above the corresponding well. Values below 0.5 indicate synergistic interaction, while values 0.5 – 4 indicate indifference and those above 4 indicate antagonism.

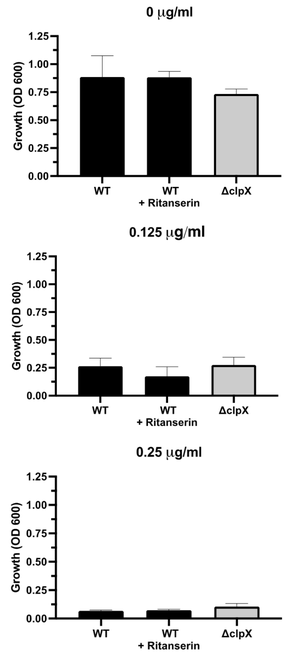
Other Antimicrobials

Cell-Envelope-Targeting Antimicrobials



Statistically significant differences represented by different letters as determined by one-way ANOVA followed by Tukey's post-hoc test.

Non-Cell-Envelope-Targeting Antibiotics: Ciprofloxacin



Conclusions & Future Directions

- Most of the tested compounds are likely inhibiting ClpXP as predicted, however one compound (Xaliproden) could not be evaluated due to solubility limits.
- The inhibition of bacterial growth when used alone suggests that inhibitors have substantial off-target effects.
- This reflects an inherent limitation of the drug repositioning approach.
- Further confirmation of activity will be determined by examining the effect of these compounds on downstream targets of ClpXP.

Acknowledgements:
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