Dublin, 25th of May 2023

Novel antibacterials targeting the transcriptional regulators PrfA and BrtA

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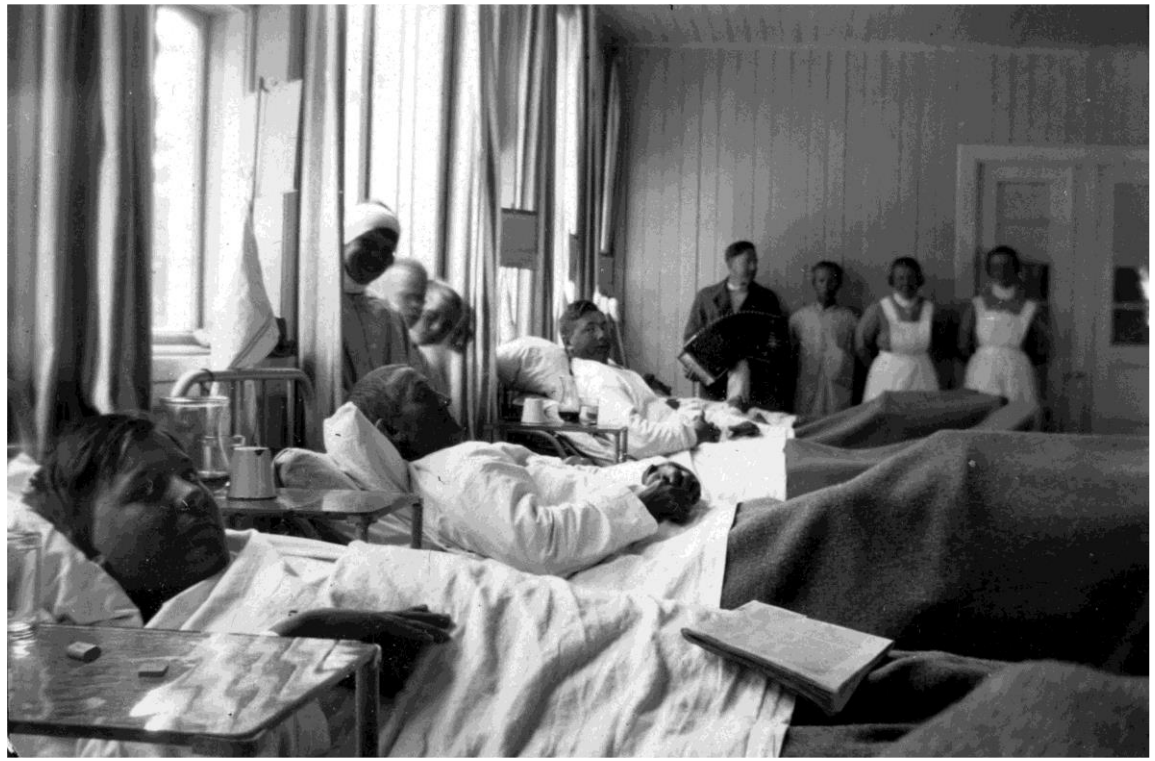
UMEÅ UNIVERSITET

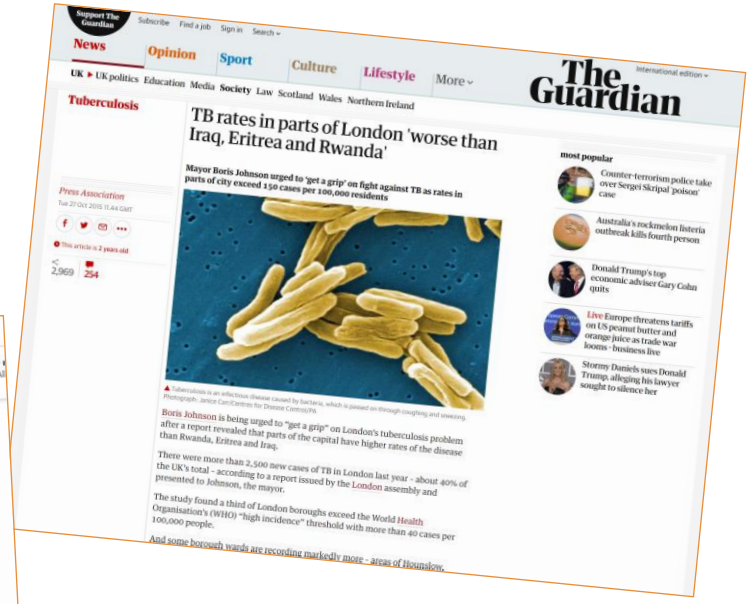
Antibiotics –
Miracle drugs saving billions of lifes

What do antibiotics allow us to do?

- Treatment of infectious diseases, like tuberculosis



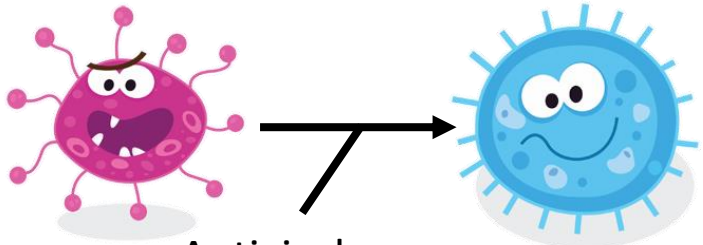
Antibiotic resistance is one of the biggest threats to global health



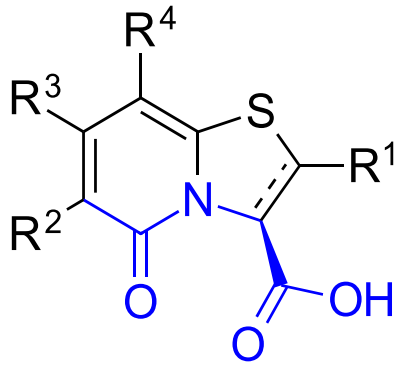
What are the alternatives to old antibiotics?

- Antivirulence drugs
- New antibacterials
- ??

Antivirulence drug:
Targeting bacterial virulence to disarm pathogens

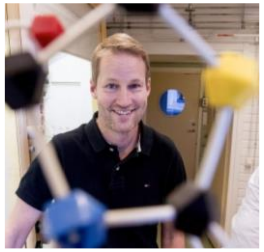


Antivirulence drug



Heterocyclic 2-pyridones

> 1500 synthesized by group of Fredrik Almqvist



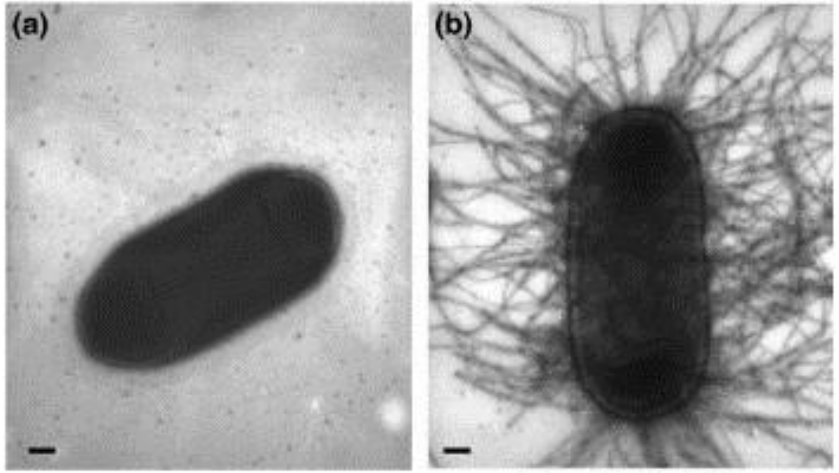
Fredrik Almqvist
(Umeå Univ. Umeå)

Scott Hultgren
(Wash. Univ St. Louis, US)

Uropathogenic *E. coli*:

+ 2-pyridones

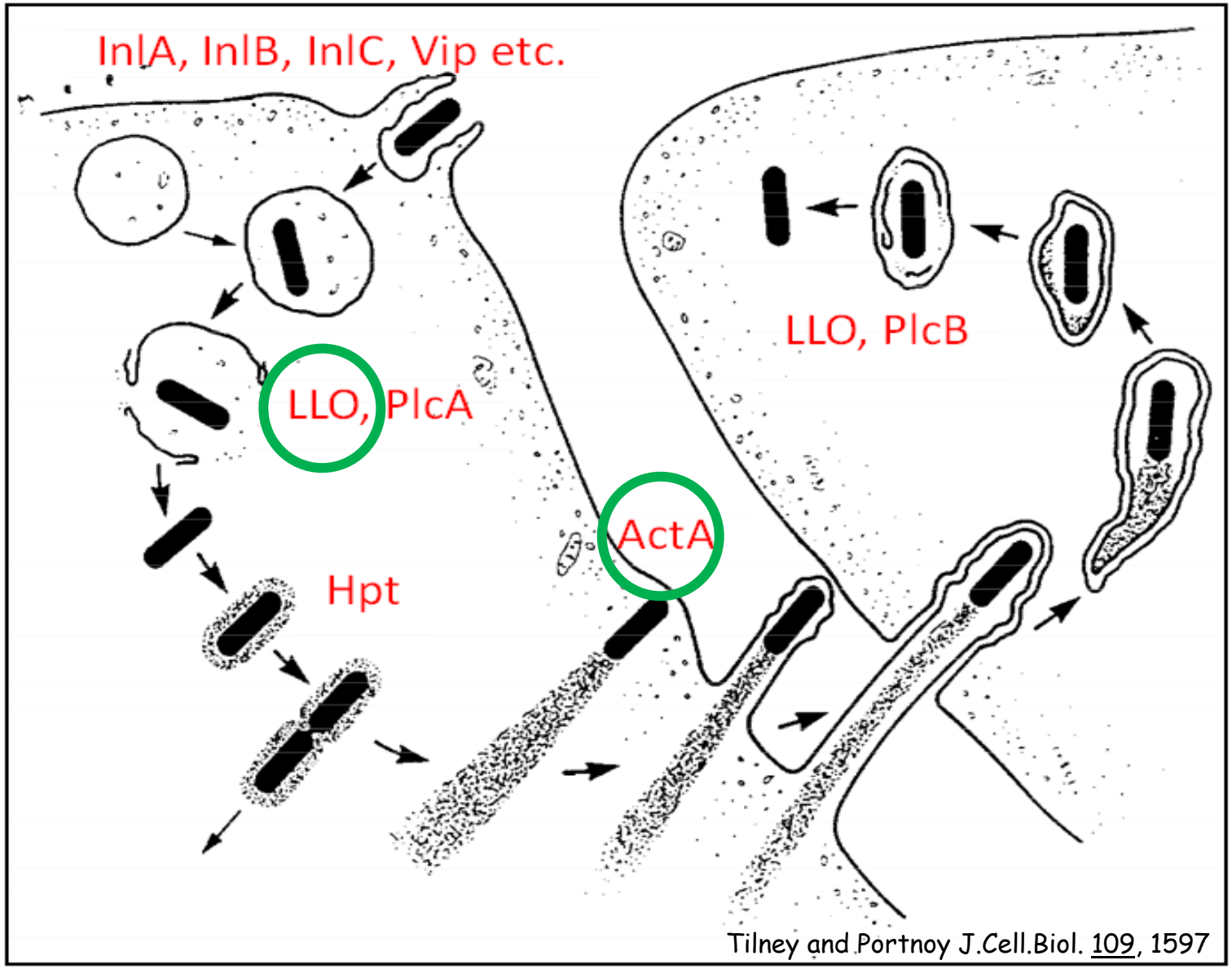
- 2-pyridones



Current Opinion in Pharmacology

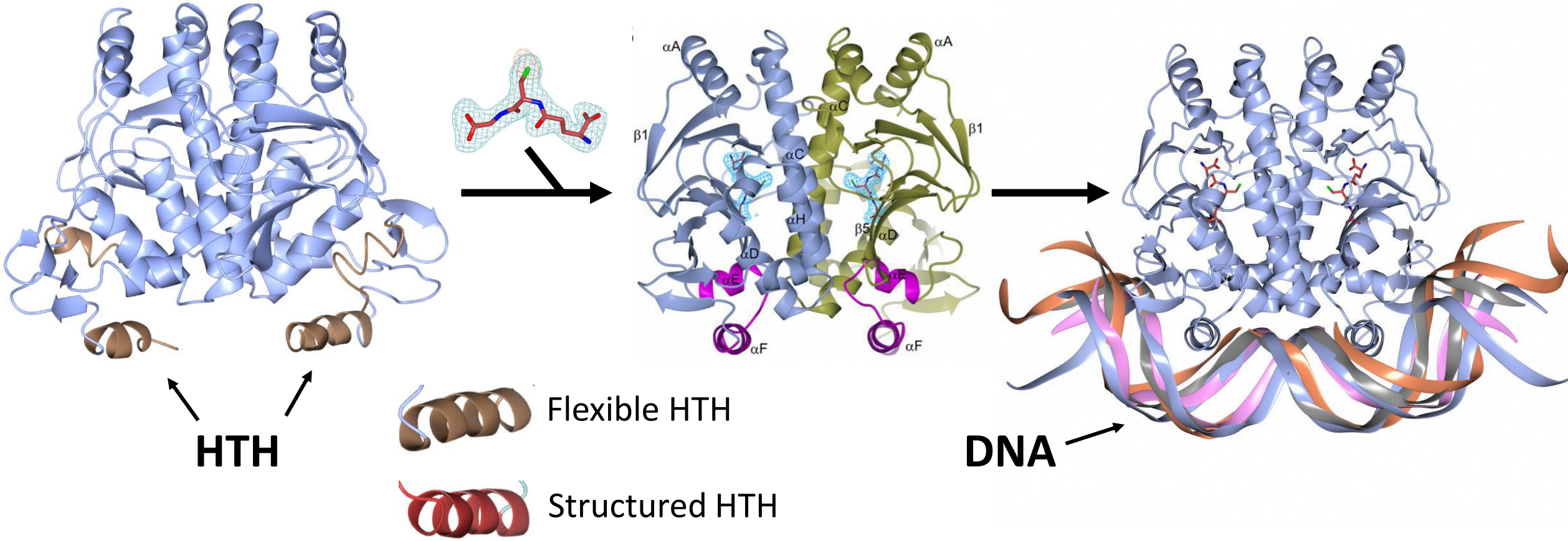
Pinkner *et al.*, PNAS, 2006
Greene *et al.*, mBio, 2014

Many virulence genes in *Listeria monocytogenes* are regulated by the transcriptional activator PrfA



Glutathione act as a co-factor to activate PrfA, restructuring the flexible HTH of PrfA to allow DNA-binding

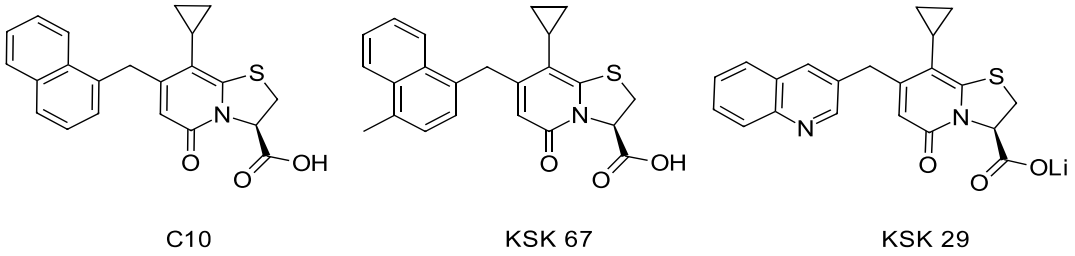
Glutathione binds at the entrance of an intra-protein tunnel site

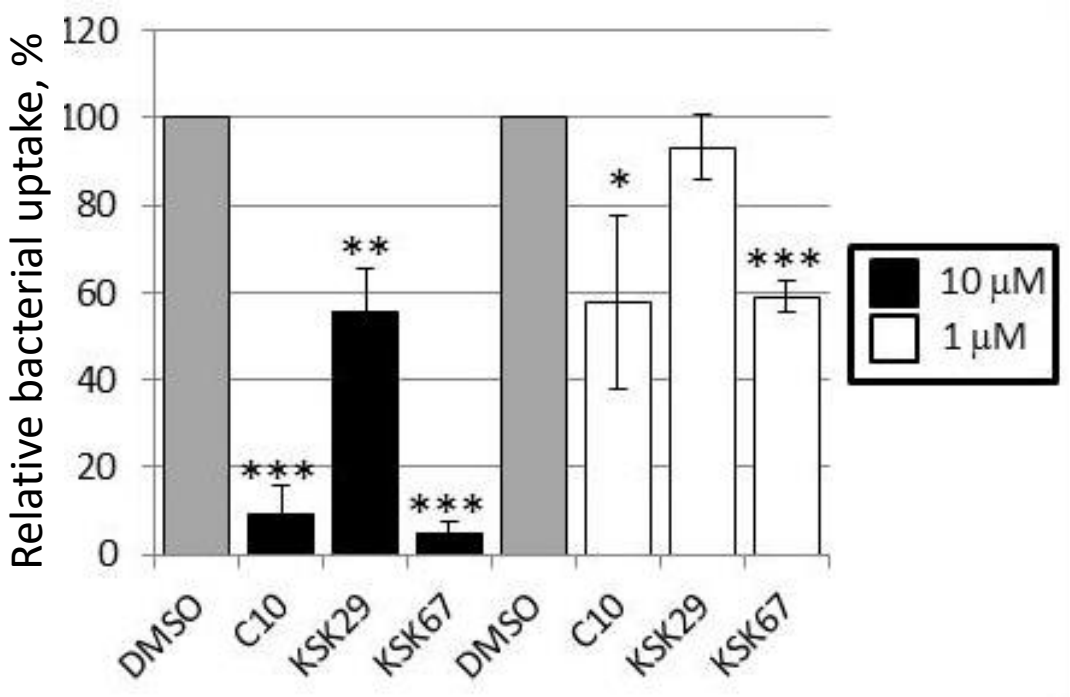


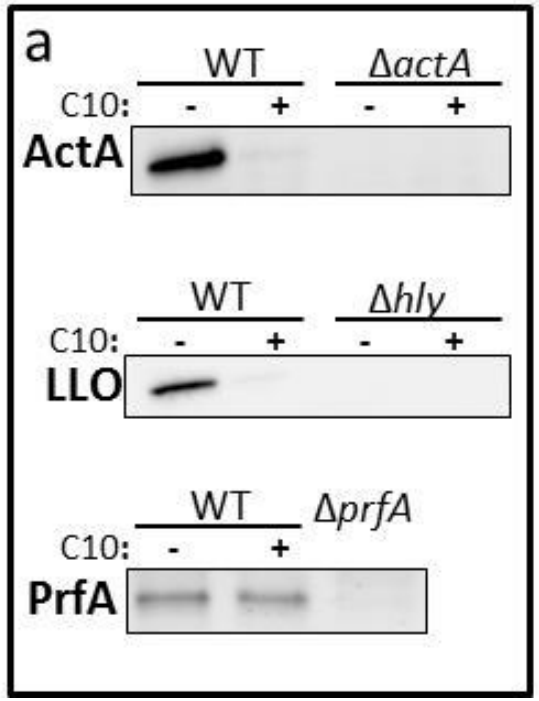
Reniere *et al*., 2015 Nature; Hall *et al*., 2016 PNAS

Can 2-pyridones affect *L. monocytogenes* pathogenicity?

Large collaborative project with group Fredrik Almqvist and group Elisabeth Sauer-Eriksson at Umeå University





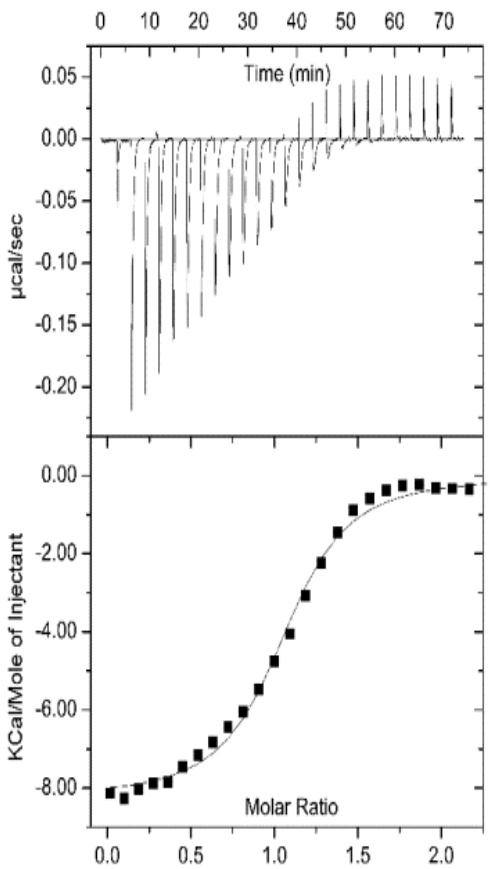


Good *et al.*, Cell Chemical Biology, 2016

The 2-pyridones bind directly to PrfA, preventing PrfA from binding DNA

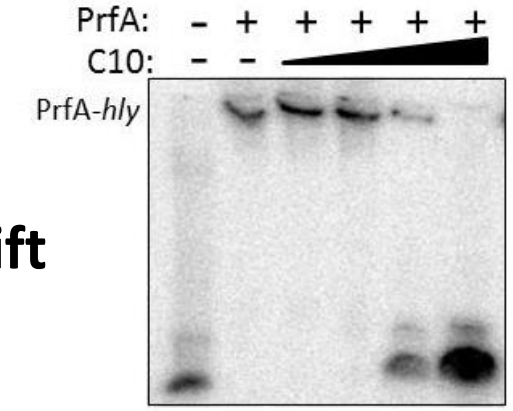
Isothermal calorimetry

C10

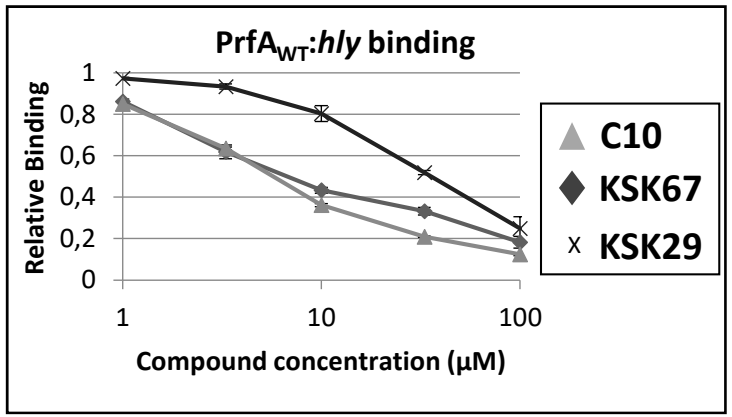


K_D ~1.0 µM

Gelshift



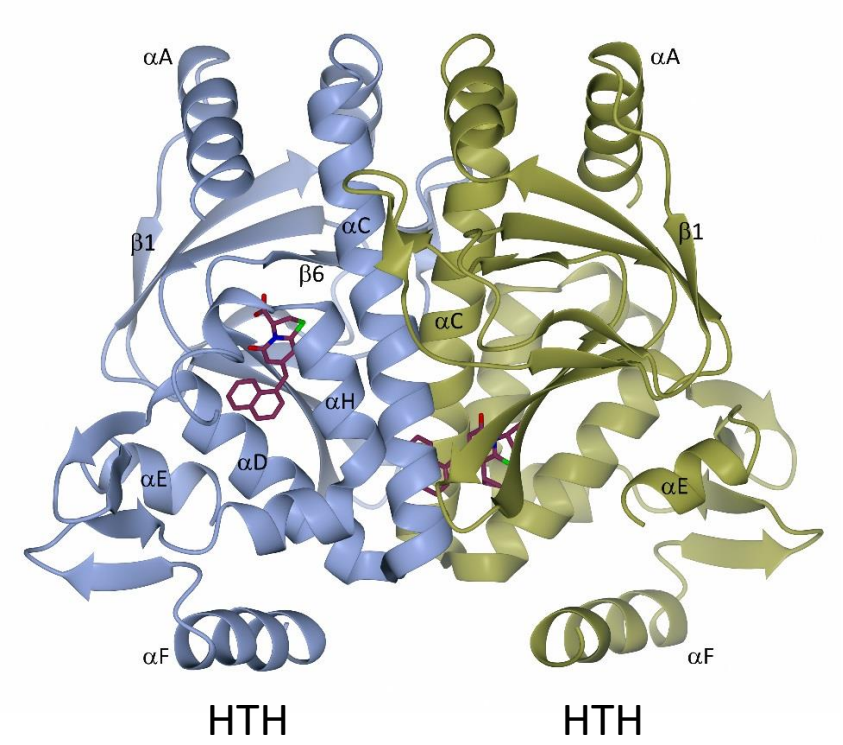
Surface Plasmon Resonance



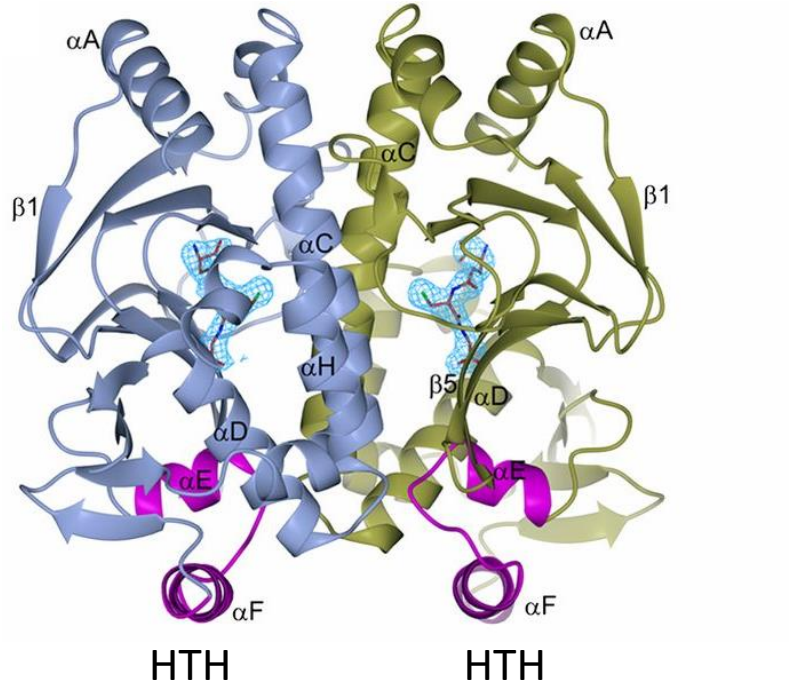
Good *et al.*, Cell Chemical Biology, 2016

How does the 2-pyridone:PrfA interaction look like?

With 2-pyridone



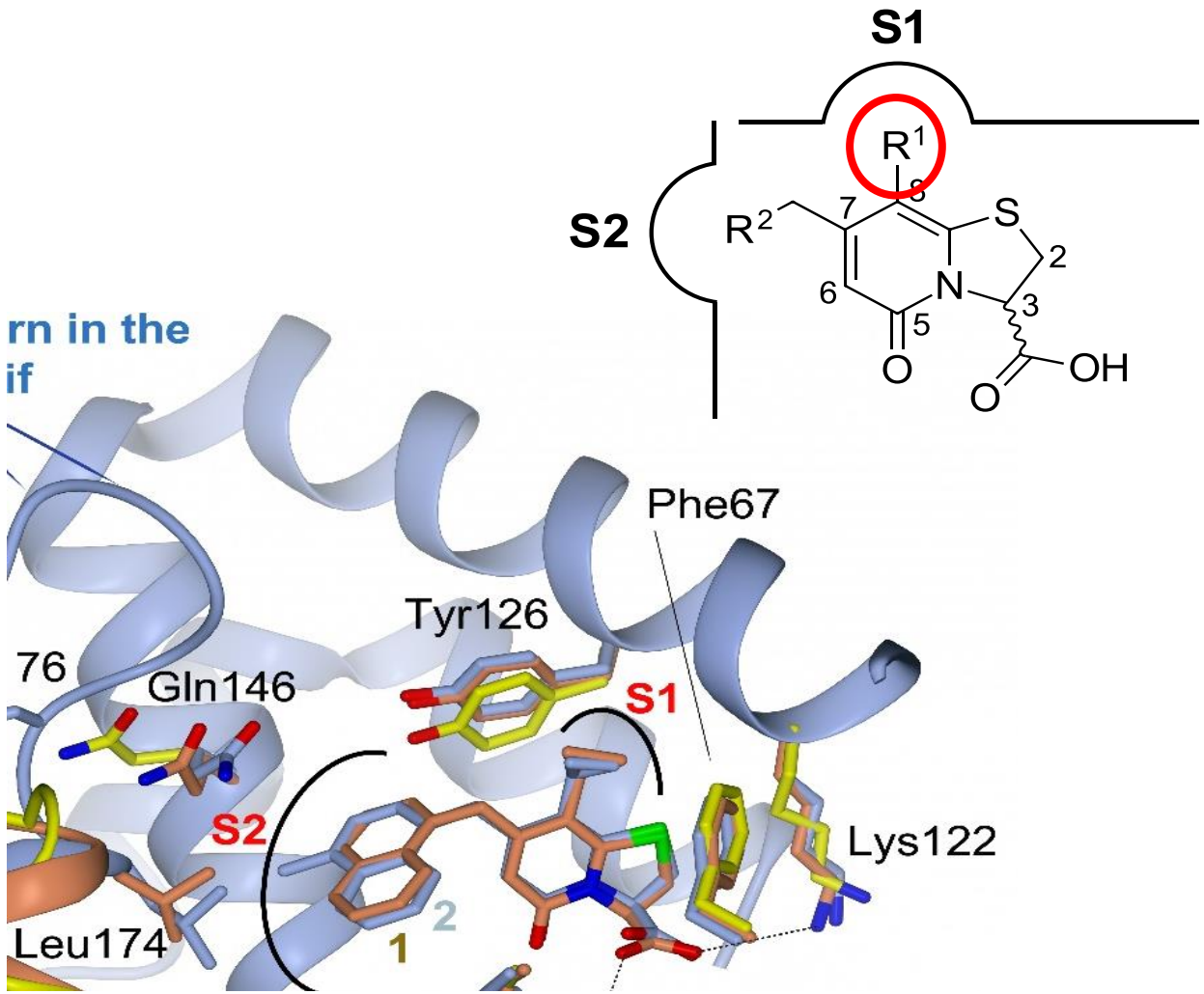
With Glutathione (co-activator)

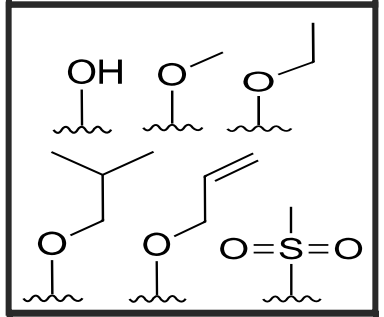
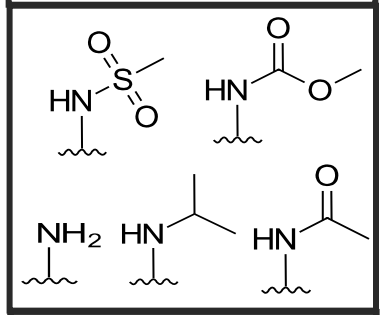
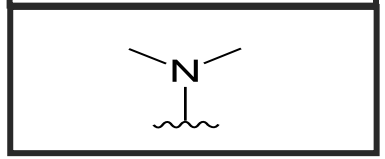


2-pyridones binds to the same site as glutathione, but tilts the HTH to position preventing DNA-binding

Hall *et al*., PNAS, 2016 Good *et al*., Cell Chemical Biology, 2016

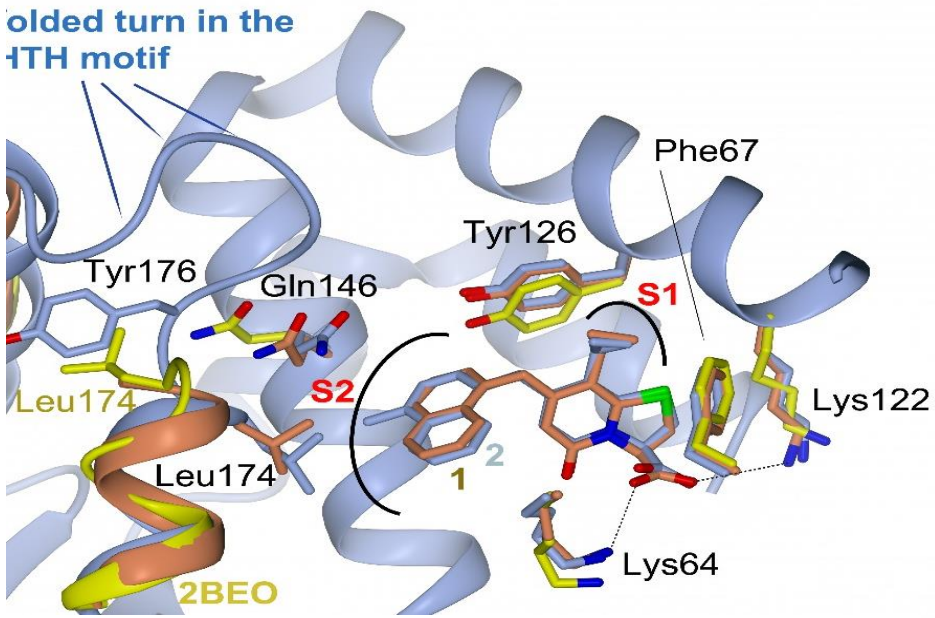
Can the cyclopropyl group at position R1 be substituted by a more ”sticky” group?



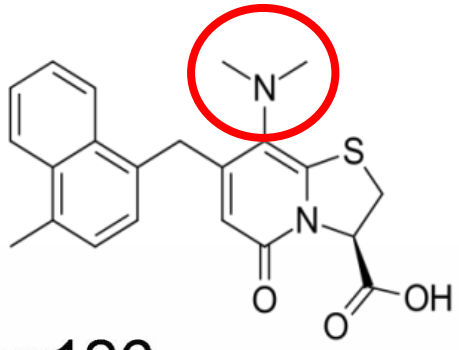
Substitutions (R1)	Effectiveness (Blocked uptake, IC_{50})
	~2 µM
	>20 µM
	>20 µM
	<0.5 µM

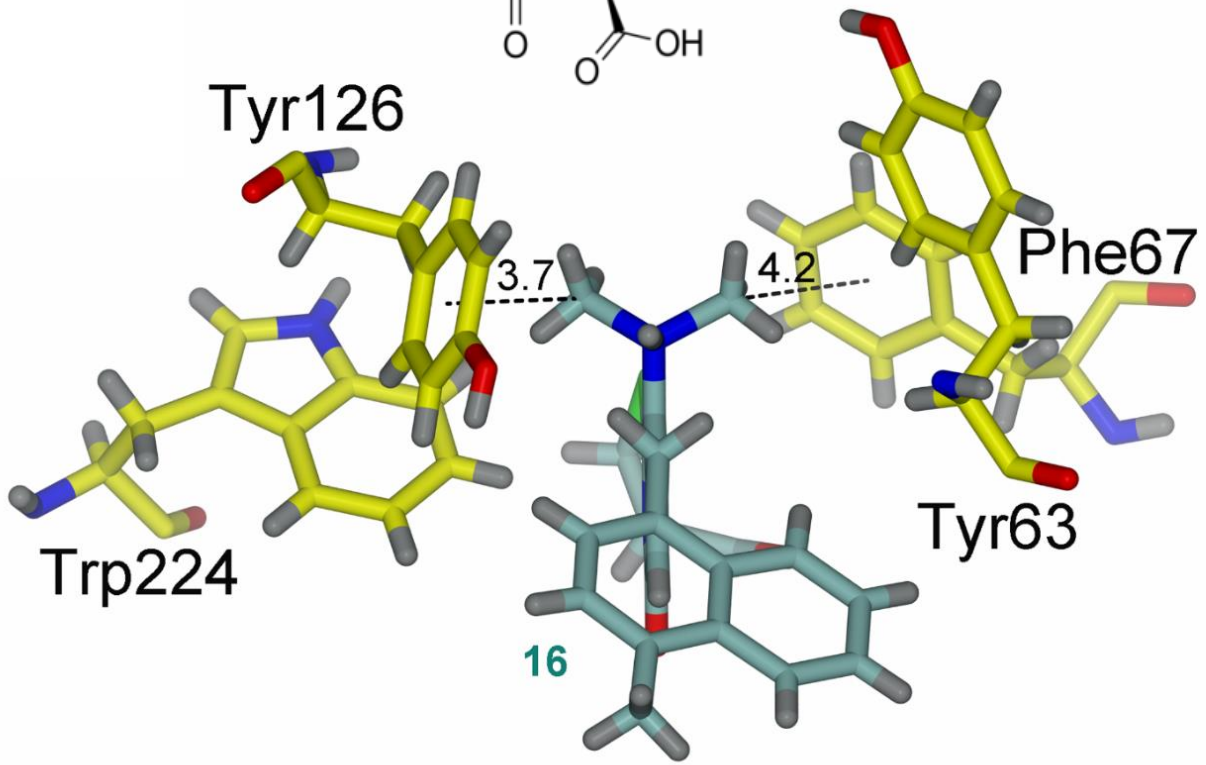
Kulén *et al.*, J Med Chem, 2018

Why does the dimethyl amine group show better properties compared with the cyclopropyl group?



MK206



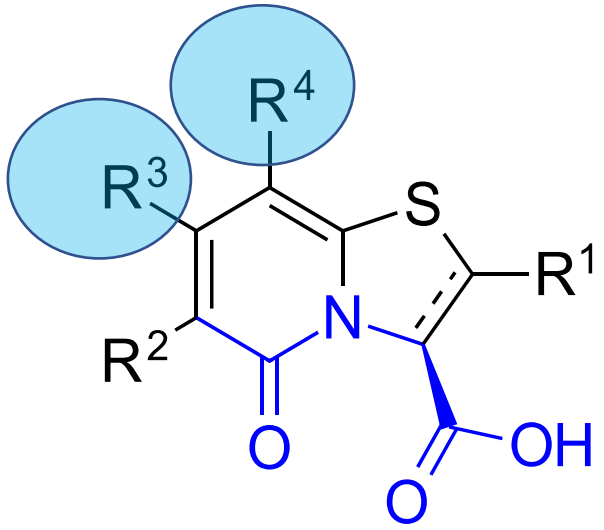


A non-classical hydrogen bonding between the dimethylamino group of the compounds with the aromatic amino acids allow a strong interaction

Kulén *et al.*, J Med Chem, 2018

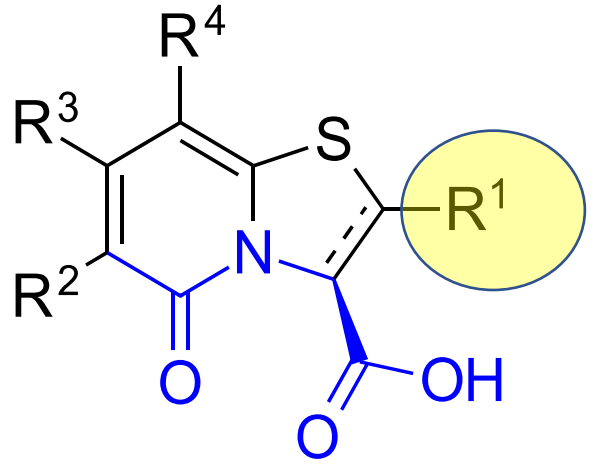
What about substituting other parts of the 2-pyridones?

Virulence-blockers



Target

PrfA

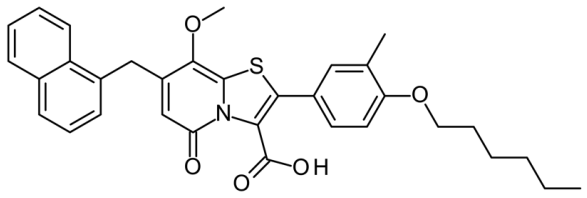


= GmPcides

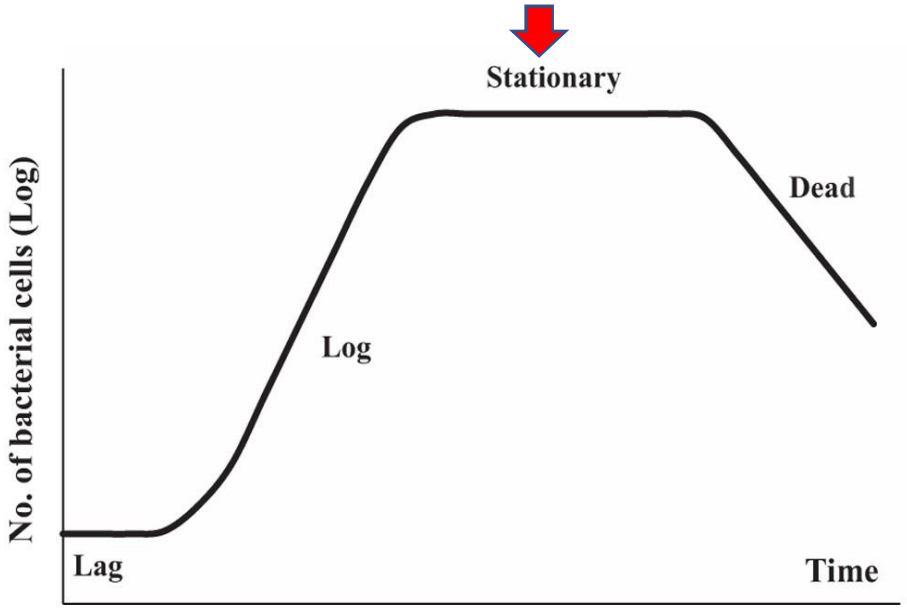
Target

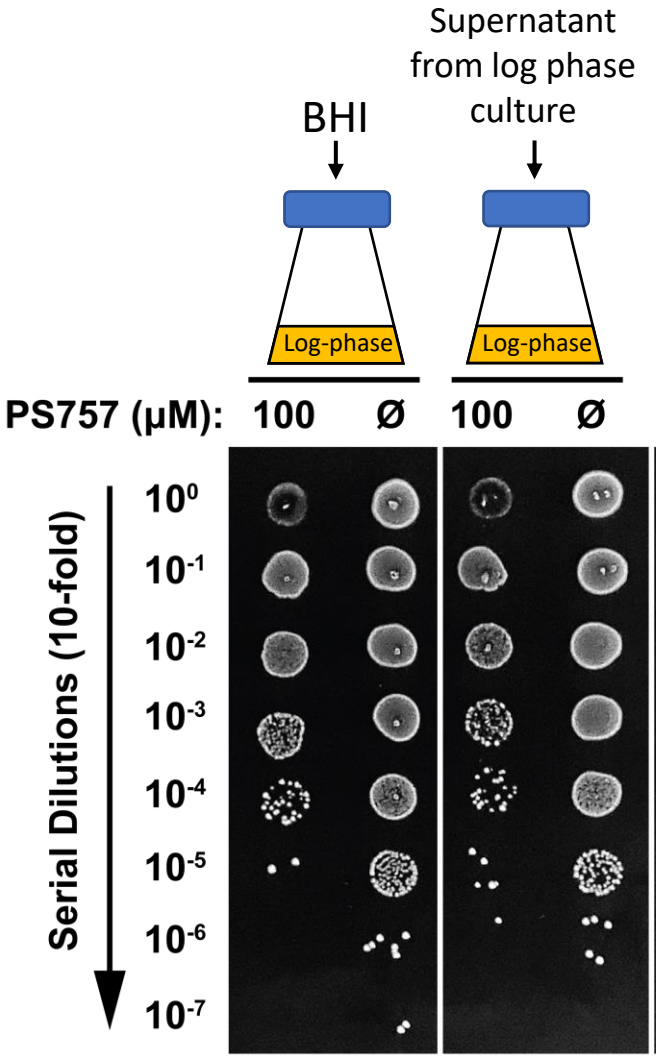
??

GmPcides are bactericidal in stationary phase (non-growing) *Enterococcus*



PS757

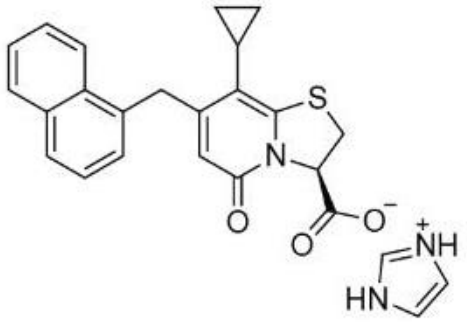




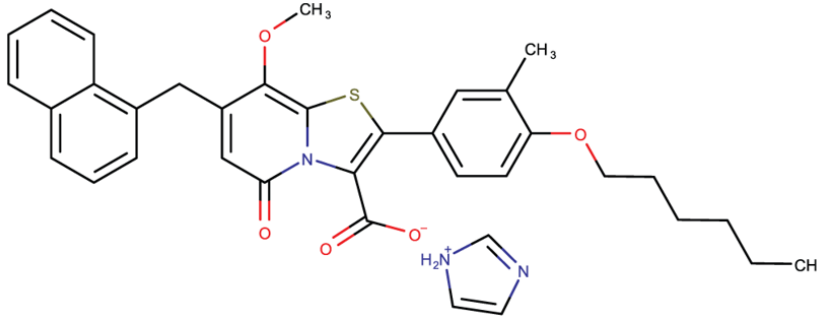
Nye *et al.*, 2022, PNAS

GmPcides block *Listeria* virulence and binds PrfA

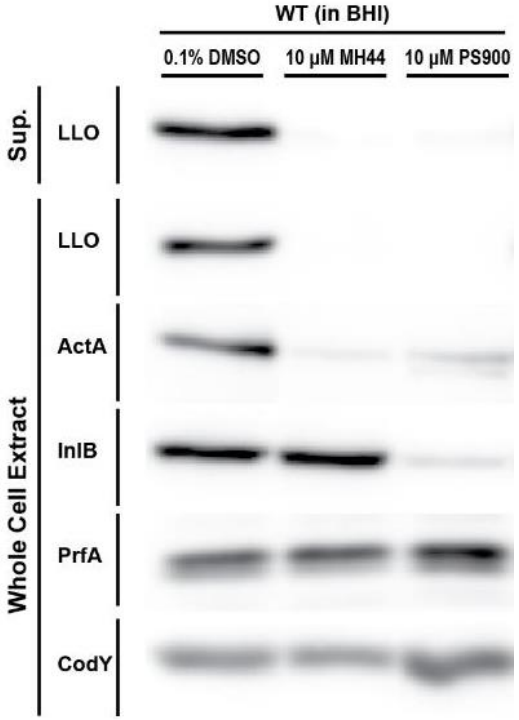
Virulence blocker (MH44)



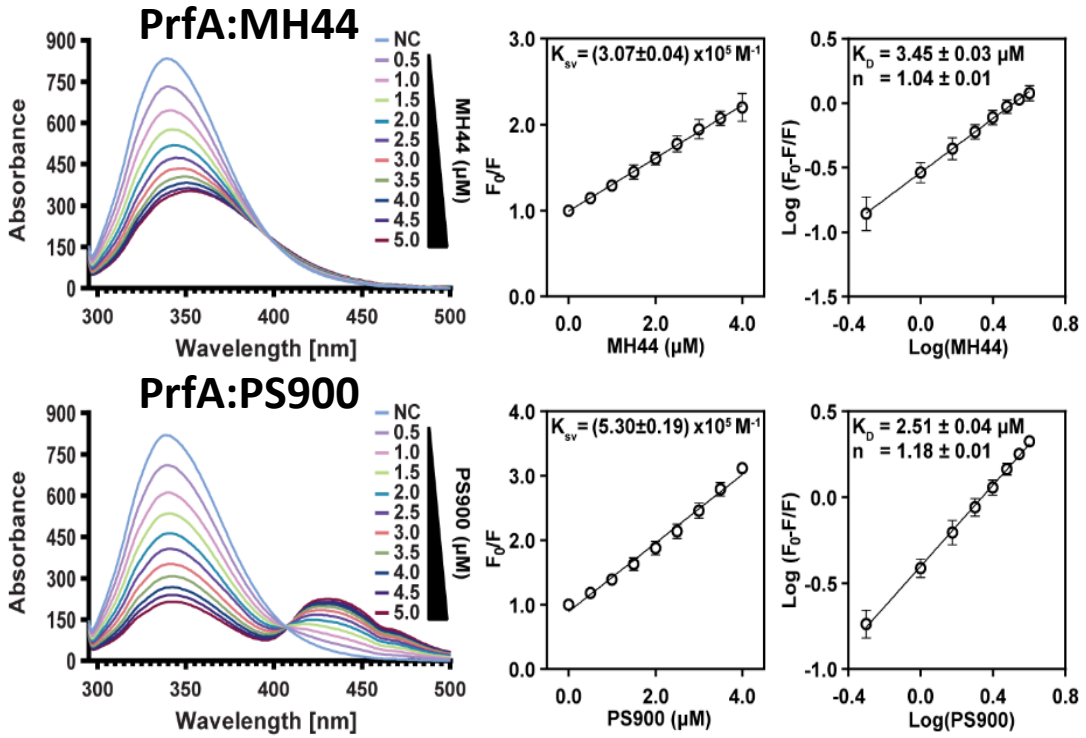
GmPcide (PS900)



Listeria virulence factor expression



Fluorescence quenching spectra

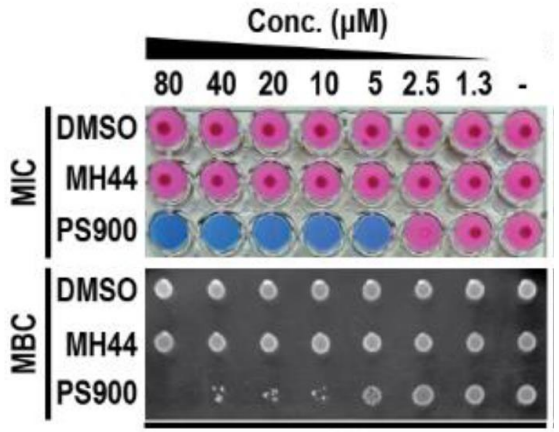


PS900 and MH44 bind PrfA equally well

Tükenmez *et al*., 2023, mBio

PS900 (but not MH44) kill *L. monocytogenes* in defined minimal media

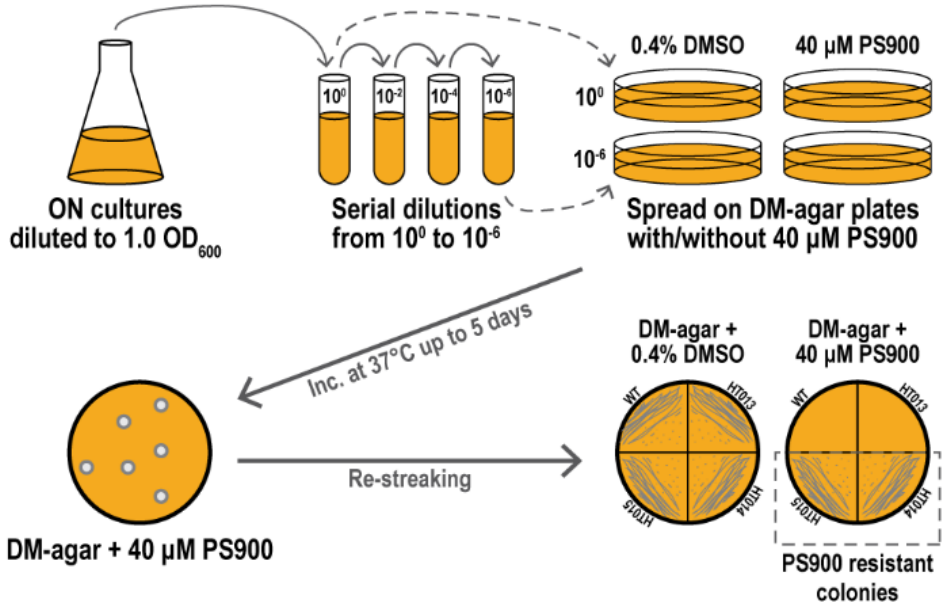
Listeria growth and survival



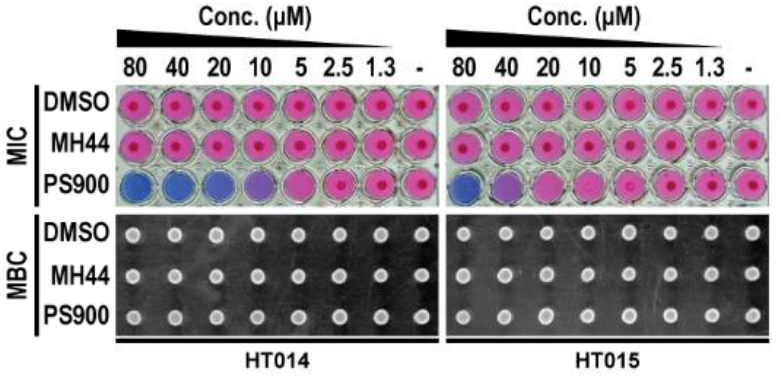
Resazurin assay

Growth

No growth

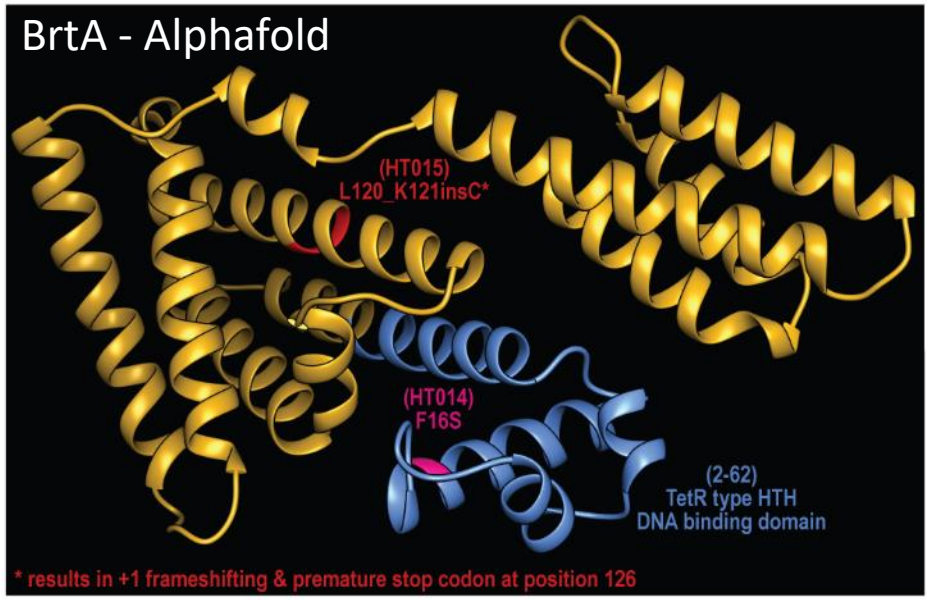


Two isolated mutants could grow at higher PS900 concentrations



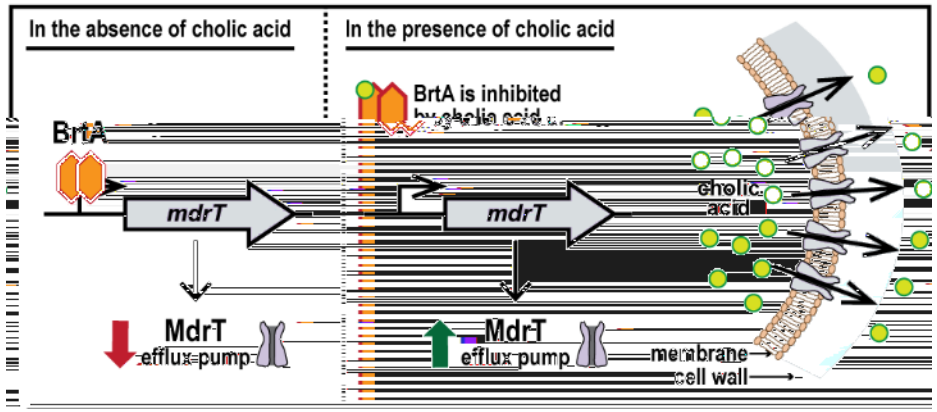
Tükenmez *et al.*, 2023, mBio

HT014 and HT015 carry base-substitution mutations in the gene encoding the efflux repressor BrtA



The amino acid substitutions in BrtA are located in the DNA-binding domain (HT014) or cause a premature translational termination (HT015)

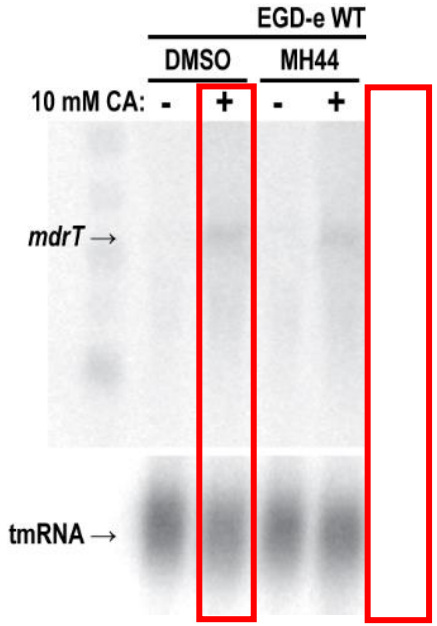
BrtA is a transcriptional repressor of *mdrT* encoding the MdrT efflux pump

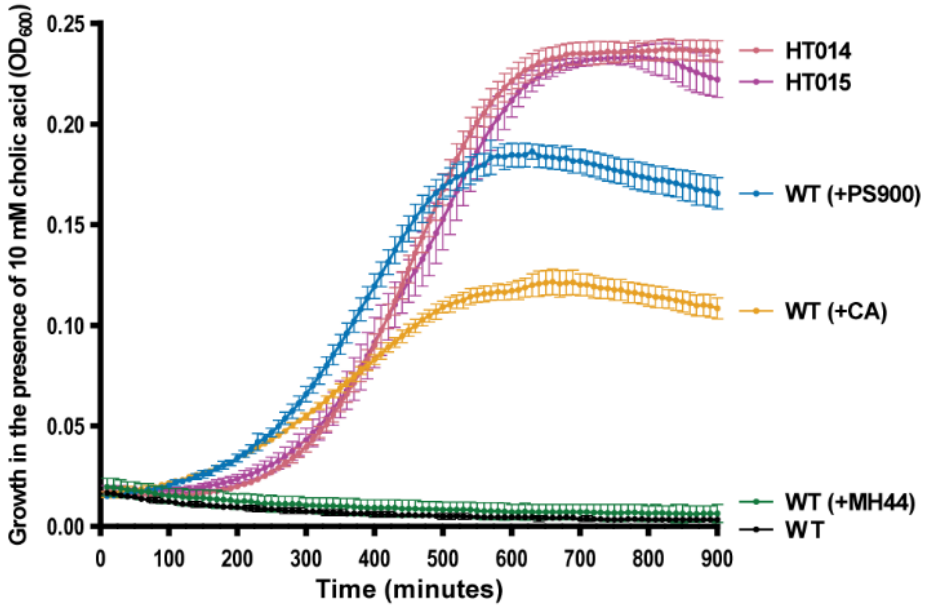


Tükenmez *et al.*, 2023, mBio

PS900 induces *mdrT* expression which allow bacterial growth at elevated cholic acid levels

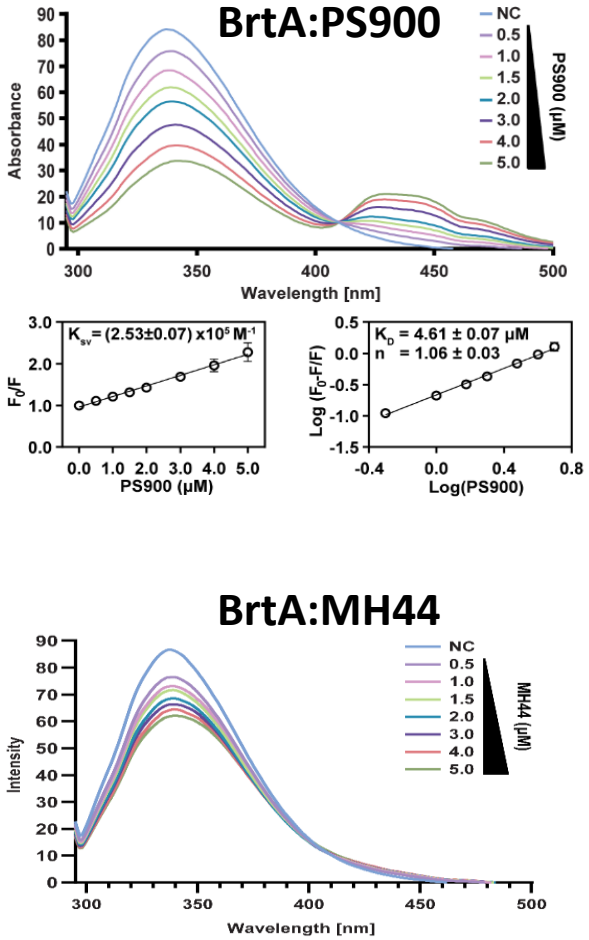
BHI

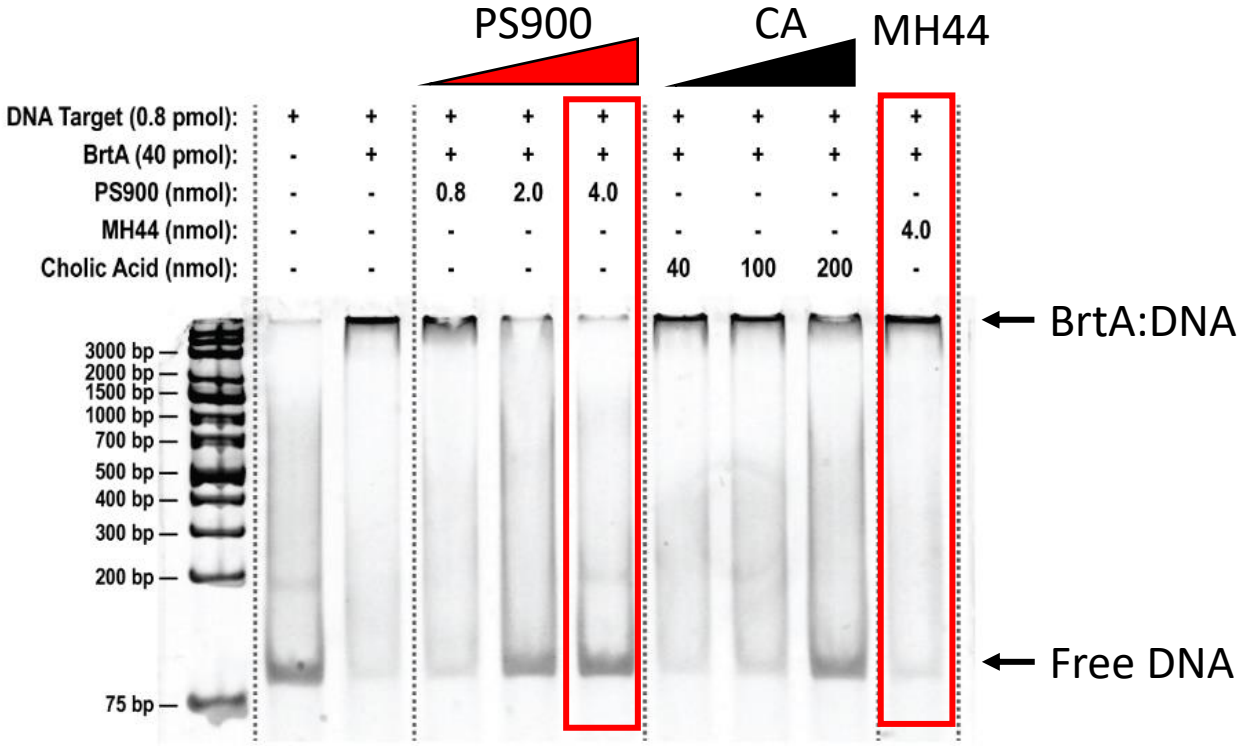




Tükenmez *et al.*, 2023, mBio

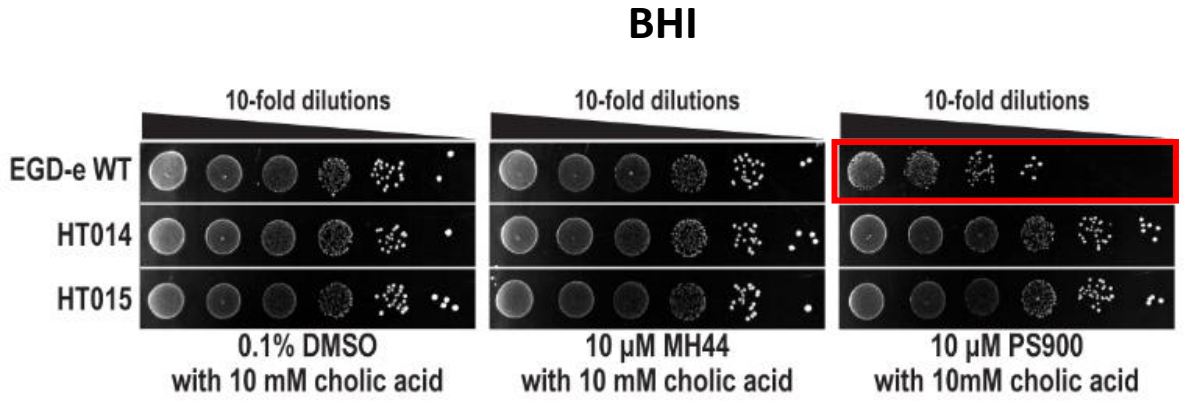
PS900, but not MH44, can interact with BrtA and displace it from the *mdrT* promoter

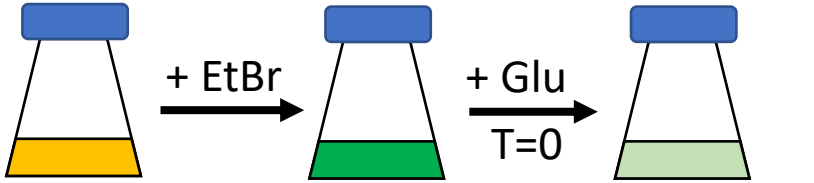




Tükenmez *et al.*, 2023, mBio

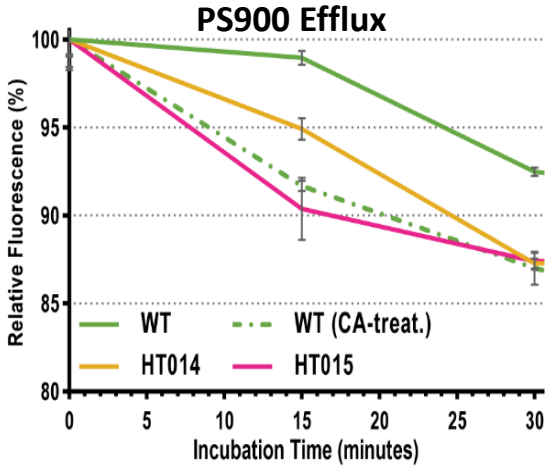
Simultaneous addition of PS900 and MH44 is toxic for the bacterium, possibly because efflux is impaired

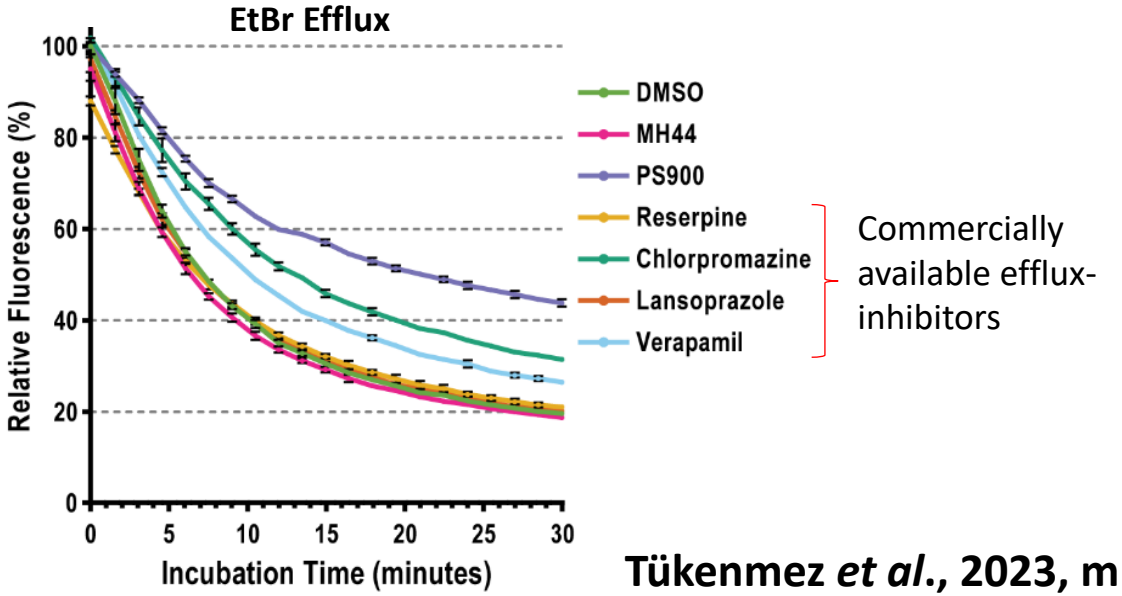




Fluorescence when EtBr bound to DNA

Reduced fluorescence when EtBr is effluxed

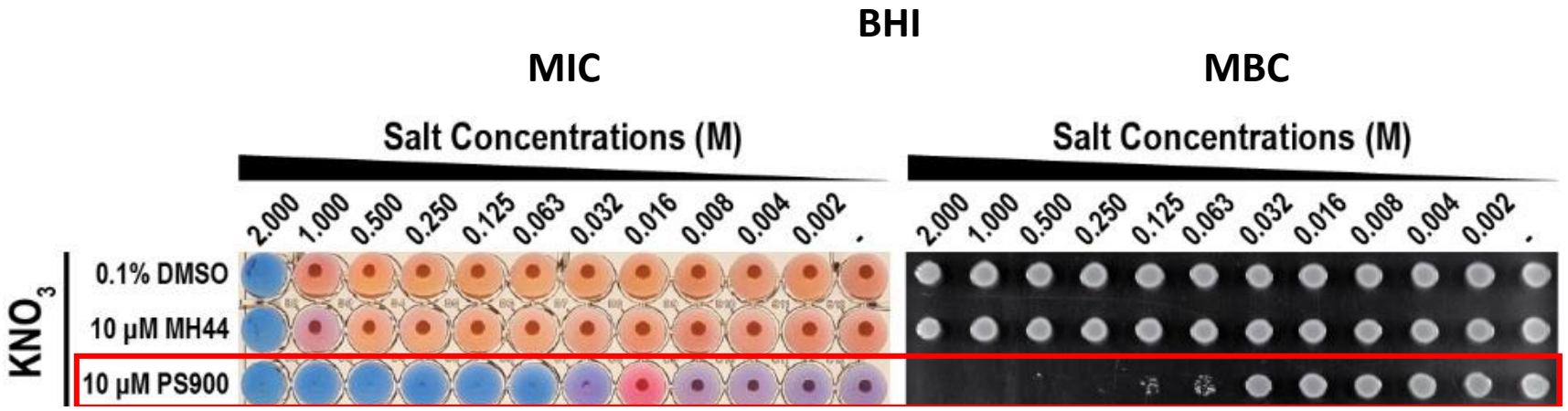




Commercially available efflux-inhibitors

Tükenmez *et al.*, 2023, mBio

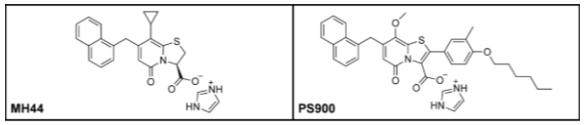
PS900 potentiates sensitivity to osmotic salt stress, particularly Potassium Nitrate



Tükenmez *et al.*, 2023, mBio

Summary:

- Virulence-blocking 2-pyridones bind PrfA and block its DNA binding capacity
- *Listeria* virulence can be inhibited by both MH44 and PS900 through PrfA-binding without affecting bacterial growth in BHI



- PS900 can block growth and kill *Listeria* in defined media
- PS900 binds and inactivates the transcriptional repressor BrtA, thereby inducing expression of the efflux pump MdrT
- PS900 sensitizes *Listeria* to osmotic salt stress

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Group of Prof Scott Hultgren

Group of Prof Mike Caparon

European Research Council

MiMS

EMBL

Vetenskapsrådet

Knut och Alice Wallenbergs Stiftelse

QureTechBio
Defeats bacterial virulence

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