A small molecule candidate for antibiotic co-therapy in the fight against persistence

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CENTRE of MICROBIAL and PLANT GENETICS

Introduction

Background

Pseudomonas aeruginosa, one of the notorious ESKAPE pathogens, is best known for the life-threatening infections it causes in cystic fibrosis patients and its rapidly increasing antibiotic resistance. Infections caused by multidrug-resistant pathogens are increasingly difficult to treat and, predicted to cause 10 million deaths each year by 2050, pose a serious public health threat.[1] Contributing to treatment failure is the presence of a small fraction of persister cells, which transiently tolerate treatment with high doses of antibiotics. The development of novel therapies, which also eliminate persister cells, could greatly improve patient outcome.[2]

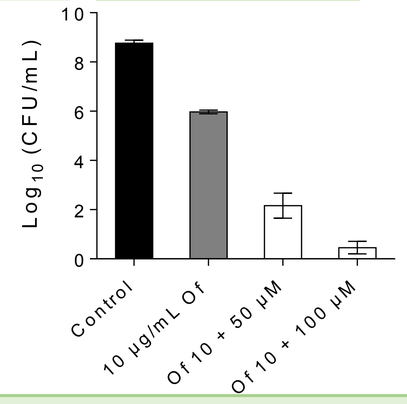
The quest for novel anti-persister therapies

We recently identified a new antibacterial compound SPI009 that, in combination with different classes of conventional antibiotics, poses an excellent candidate for novel anti-persister co-therapies in the fight against *P. aeruginosa* and other relevant pathogens.

Identification of SPI009 and initial research

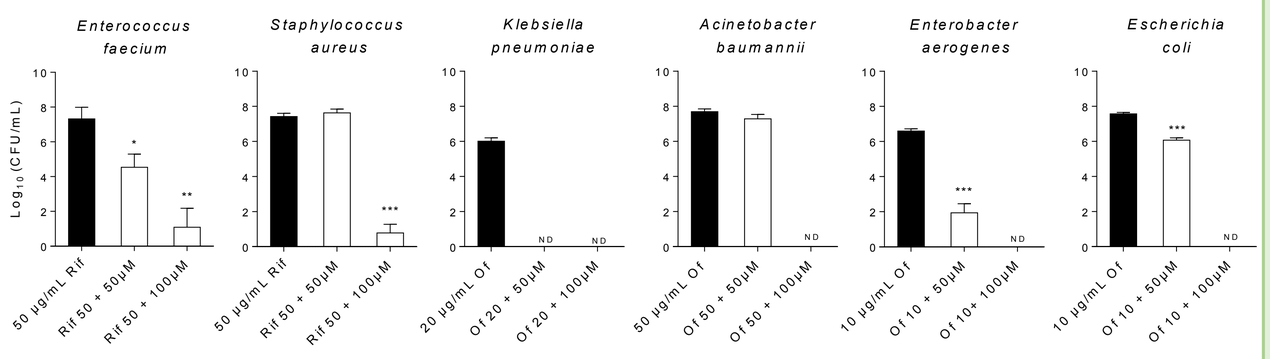
Screening of 23909 compounds → Selection of 3 compound families → Testing of 48 chemical analogues → **SPI009**

- ✓ SPI009 **significantly reduces the persister fraction** of *P. aeruginosa* in combination with ofloxacin
- ✓ SPI009 reduces the persister fraction in combination with **different classes of antibiotics**
- ✓ SPI009 **directly kills persister cells**
- ✓ The combination therapy with SPI009 can **completely eradicate** bacterial populations

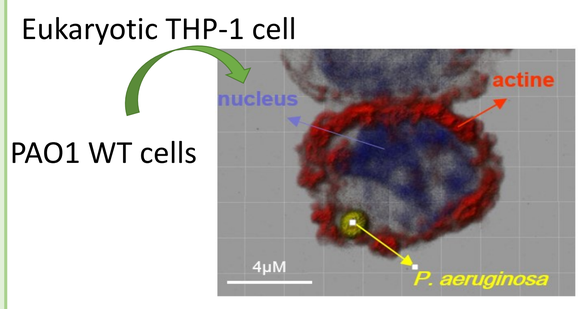


Further characterization

SPI009 shows a broad spectrum activity

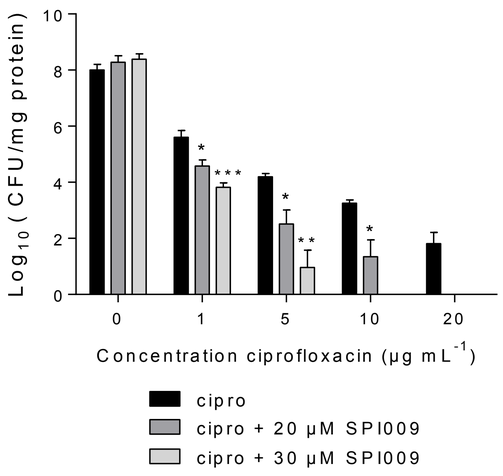


SPI009 effectively clears intracellular *P. aeruginosa* infections

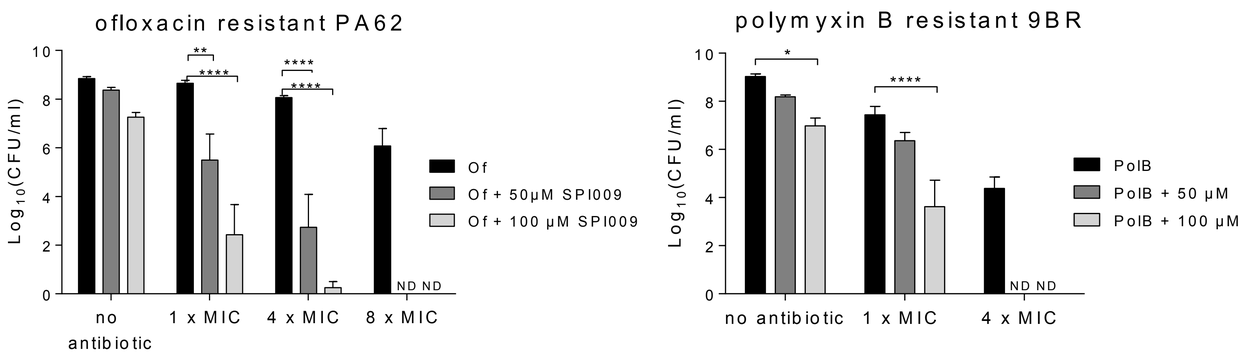


Intracellular infection model[3]

5h
ciprofloxacin
(+ SPI009)



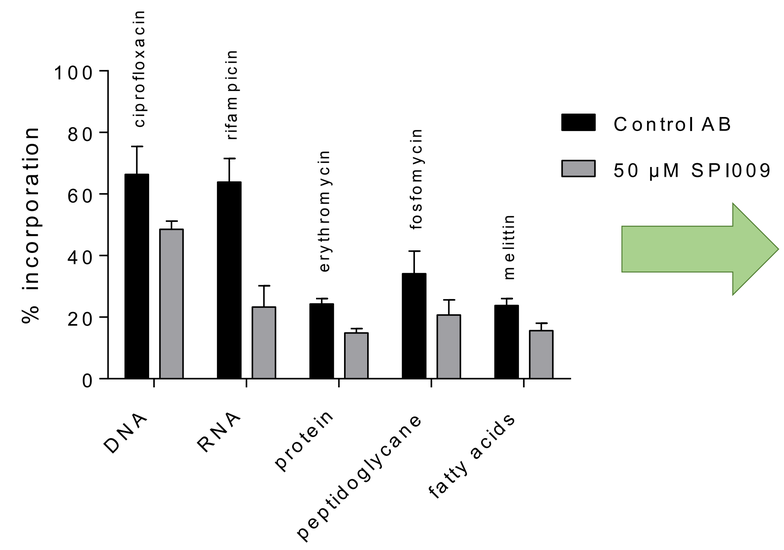
SPI009 enables treatment of resistant *P. aeruginosa* strains



* P≤ 0.05 ** P≤ 0.01 *** P ≤ 0.001 **** P ≤ 0.0001

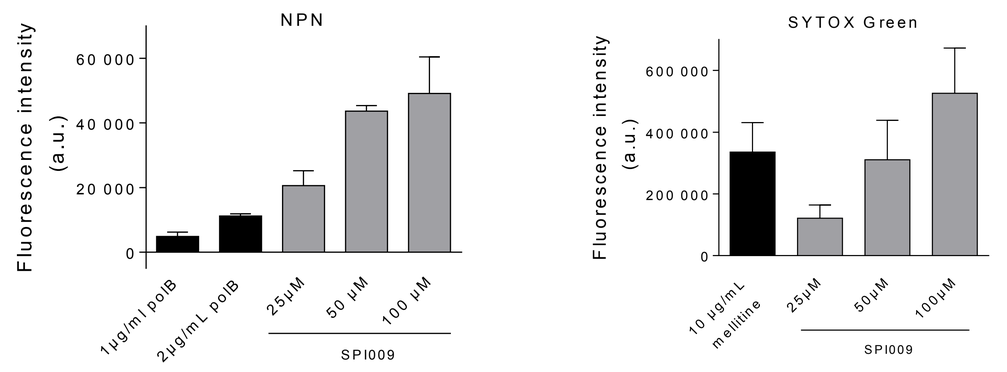
Determination of the mode of action

SPI009 inhibits all major macromolecular synthesis processes



Indication of membrane damage

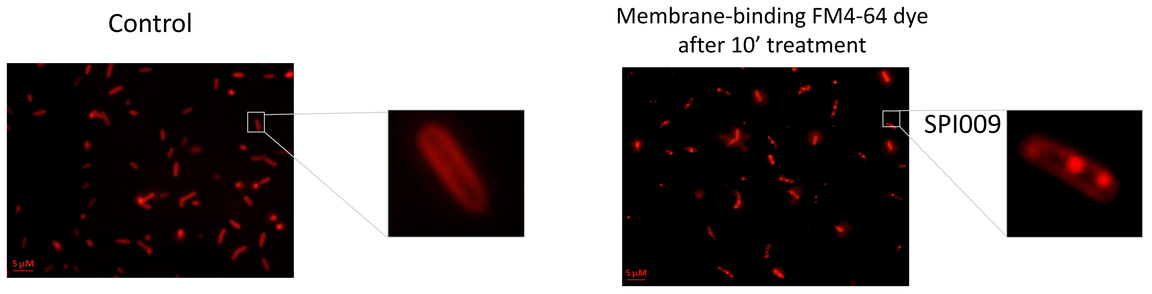
SPI009 damages outer and inner membrane



N-phenyl-1-napthylamine (hydrophobic)

Nucleic acid stain; impermeant to intact membranes

SPI009 alters the bacterial membrane



Mode Of Action?
SPI009 causes severe **membrane damage** leading to bacterial cell death and facilitating the effects of other antibiotics

Conclusion

Extensive research has shown that SPI009

- ✓ possesses a **broad spectrum activity** against different clinically relevant pathogens and clinical isolates
- ✓ allows the design of **strain-specific combination therapies**
- ✓ enables **treatment of multidrug-resistant strains**
- ✓ extensively **damages the bacterial membrane**, tackling both **normal and persister cells**
- ✓ is capable of **clearing intracellular infections** without damaging eukaryotic cells and shows **no hemolytic activity**

Crucially, SPI009 shows great clinical potential and may serve as the starting point for the development of novel antibacterial and anti-persister therapies in the fight against chronic infections. Genetic analysis of resistant mutants and assessment of antibacterial activity in different model systems will provide additional information about the mode of action and clinical applicability of our compound.

Acknowledgments and references

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