Development of molecules that slow and reverse antibiotic resistance in MRSA 'superbugs'

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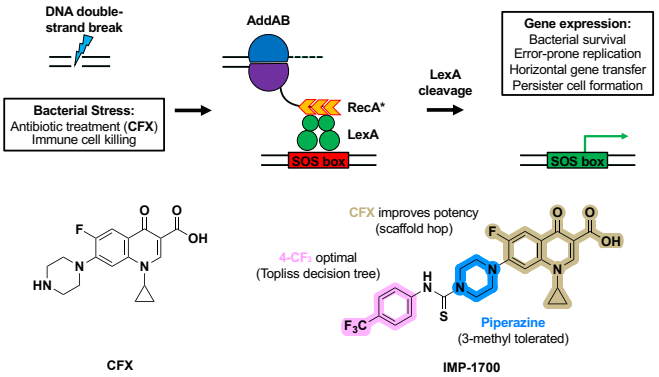
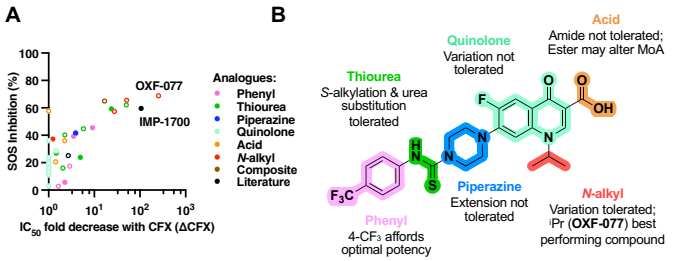
1. Introduction

- **Antimicrobial resistance (AMR)** has the potential to make life-saving medical advances redundant;[1] predicted to cause 39 million deaths between 2025 – 2050, and be associated with 169 million deaths, more than HIV/AIDS and malaria combined.[2] AMR will **disproportionately affect low to middle income countries**, and is predicted to cost the global economy 100 trillion USD by 2050.[1]
- Novel compounds that target new **mechanisms of action** (MoA) are required to reduce AMR. One such target is the **SOS response**, which **activates virulence, persistence, and resistance mechanisms**.[3]
- **IMP-1700** inhibits the SOS response and potentiates ciprofloxacin (**CFX**) in methicillin-resistant *Staphylococcus aureus* (MRSA),[4] a 'superbug'. The further development of **IMP-1700** is hindered by the limited understanding of the **structure-activity relationship** (SAR) and **unknown MoA** of this series.



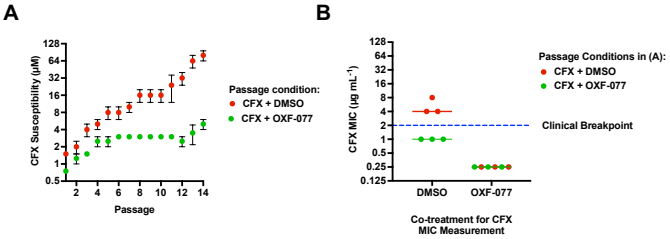
2. IMP-1700 structure activity relationship

37 analogues of **IMP-1700** were synthesised and SOS response inhibition and **CFX** synergy tested, **uncovering their SAR** (**A**).[5] **OXF-077** (**B**) showed an improvement both assays compared to **IMP-1700**.[5]



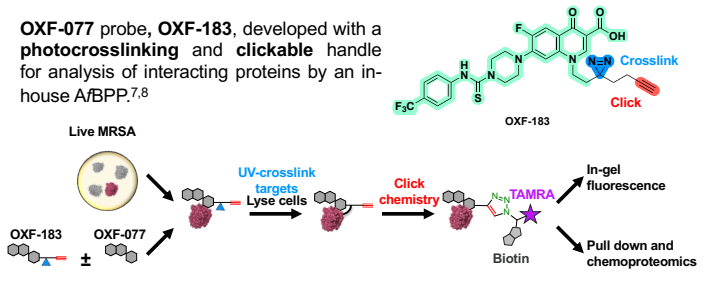
3. OXF-077 effect on resistance evolution

OXF-077 significantly slowed the resistance evolution during serial passage of non-CFX resistant *S. aureus* (MSSA) (**A**), and kept the **MIC below** the **clinical breakpoint for CFX** in *S. aureus*[6] after 14 passages for treated cells, and **reversed resistance** for untreated cells (**B**).[5]



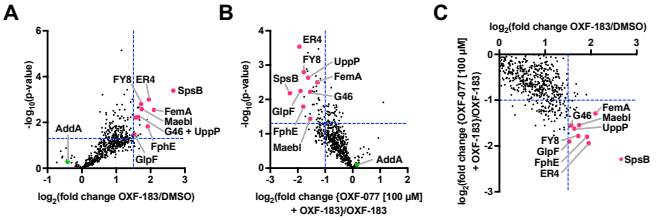
4. Affinity-based protein profiling (A*f*BPP)

OXF-077 probe, **OXF-183**, developed with a **photocrosslinking** and **clickable** handle for analysis of interacting proteins by an in-house A*f*BPP.[7,8]



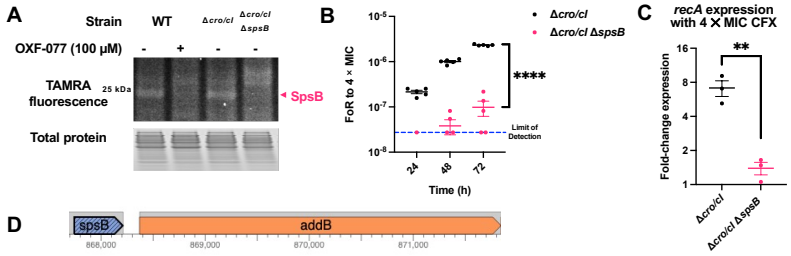
5. OXF-077 target identification

Chemoproteomic A*f*BPP with **OXF-183** identified proteins enriched upon probe treatment (**A**) and competed with subsequent addition of **OXF-077** (**B**). **SpsB was identified as the most enriched and competed protein** (**C**).[8]



6. Validation of SpsB as a target of OXF-077

- Δ*spsB* knockout strain in the Δ*cro/cI* background was **used to validate SpsB**.[8]
- SpsB validated as a binding partner of **OXF-183** (**A**), linked to **CFX** frequency of resistance (FoR) (**B**), shown to affect *recA* expression upon **CFX** treatment (**C**).[8]
- **These data validate SpsB as a target of OXF-077 that modulates the SOS response.**[8]
- *spsB* is the adjacent gene to *addB*, the DNA double stranded break repair protein (**D**).



7. Conclusions

- The SAR of **IMP-1700** was explored, and **OXF-077** delivered as the most potent SOS response and DNA repair inhibitor, which **slows and reverses CFX resistance** development.
- A*f*BPP revealed **SpsB** as a target of **OXF-077** that **modulates the SOS response** and resistance development.
- **This work delivers novel tools and strategies to combat AMR,** one of the greatest chronic threats to human health.

8. References and funding

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