Novel Inhibitors of Efflux Pump NorA to Target Antimicrobial Resistance (AMR)

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IMPERIAL

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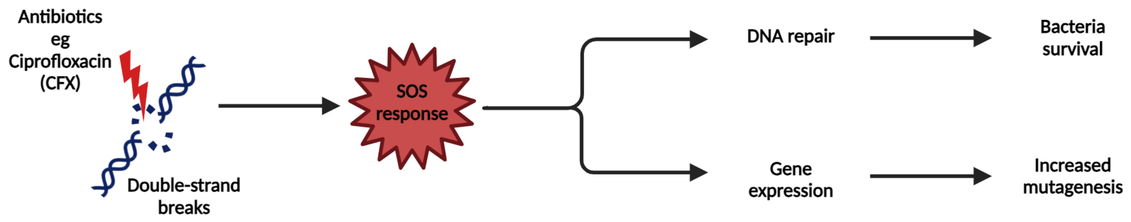
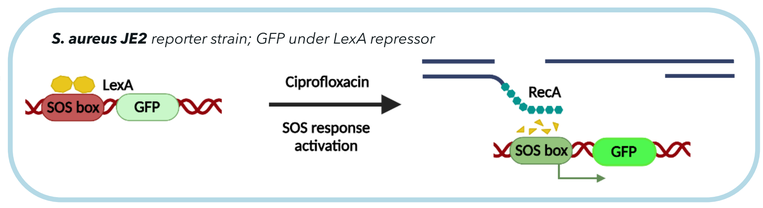
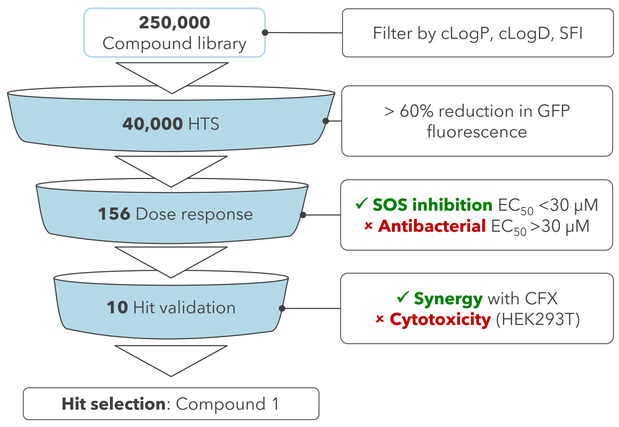
University of Dundee
Drug Discovery Unit

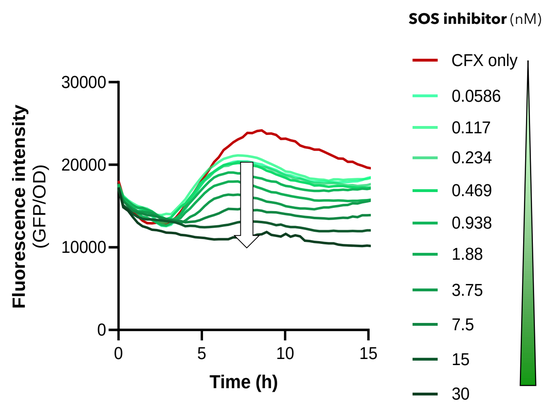
1 A High-Throughput Phenotypic Screen for Novel Inhibitors of Antibiotic Resistance



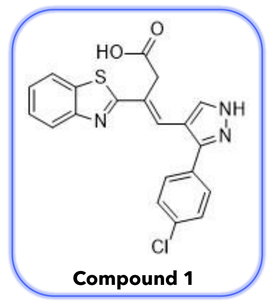
GFP reporter plasmid of SOS response used to screen **40,000 compounds in *S. aureus*** to identify inhibitors of the DNA repair pathway

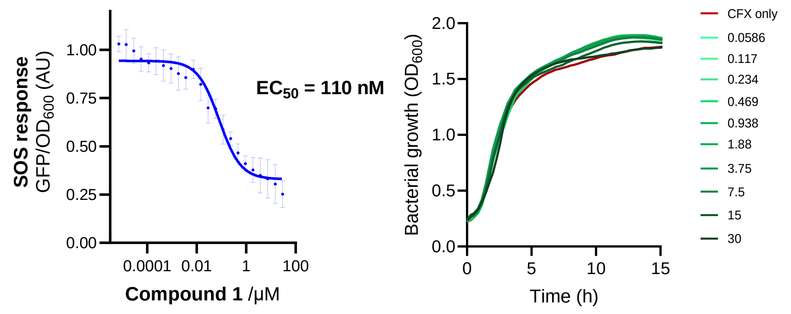




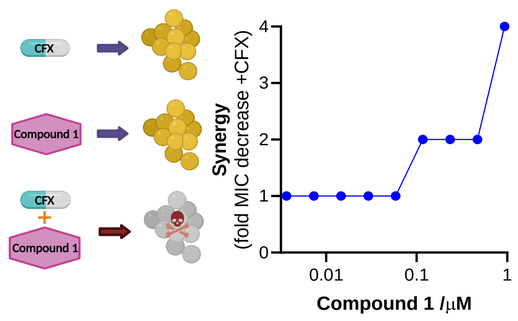


2 Compound 1 is a Nanomolar SOS Inhibitor with Low Intrinsic Toxicity

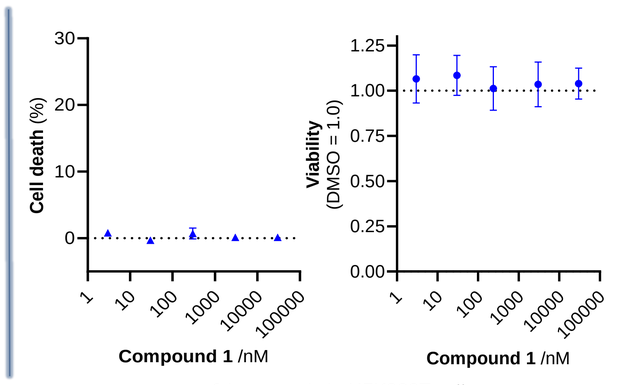




1 inhibits the SOS response (middle) with no impact on bacterial growth (right), removing selection pressure for resistance generation

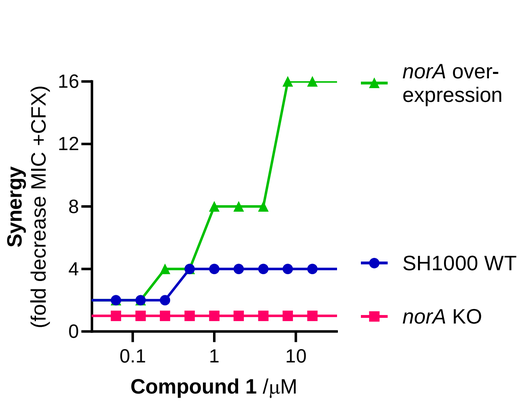


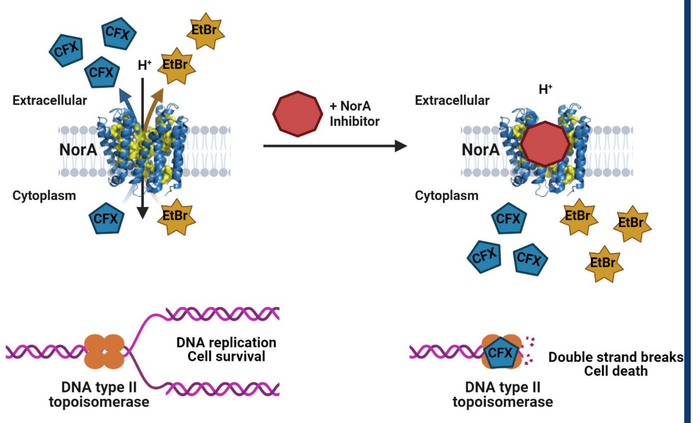
1 potentiates the antibiotic ciprofloxacin (CFX)



1 is not toxic in HEK293T cells

3 Efflux Pump NorA is the Target of Compound 1





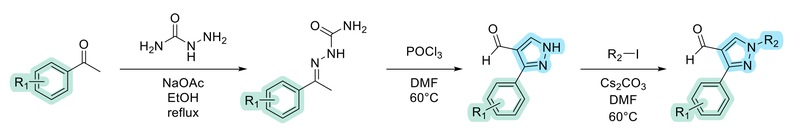
Compound 1 loses activity in *norA* knockout mutant generated by transposon mutagenesis (left)

NorA is a multidrug efflux pump in *S. aureus,* uses proton gradient to drive **efflux of antibiotics** (e.g. ciprofloxacin, CFX) or dyes (ethidium bromide, EtBr)

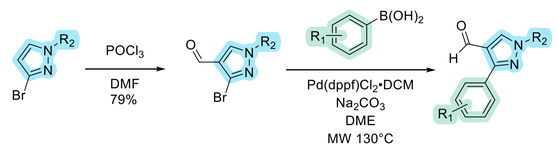
NorA inhibition can **block efflux** and increase antibiotic efficacy in antibiotic resistance bacteria

4 Hit Expansion using Key Knoevenagel Condensation

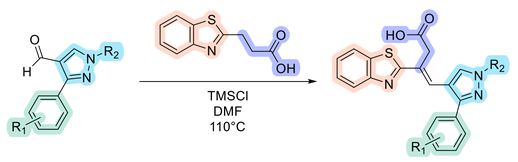
Vilsmeier-Haack Aryl-Pyrazole Formation



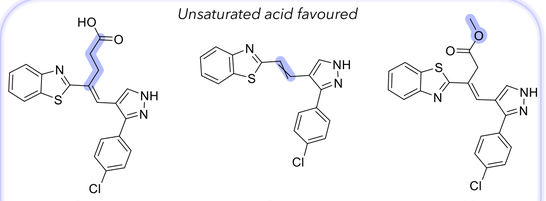
Suzuki-Miyuara Aryl-Pyrazole Formation



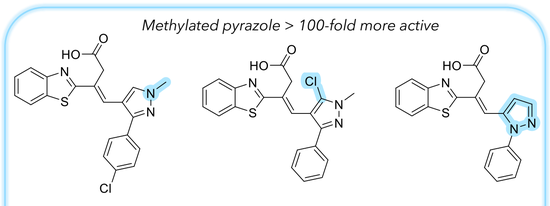
Knoevenagel Condensation Reaction



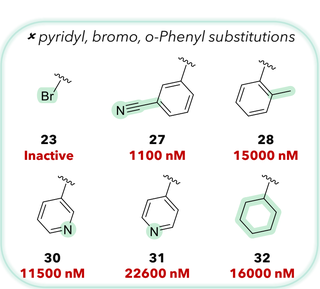
Unsaturated acid favoured

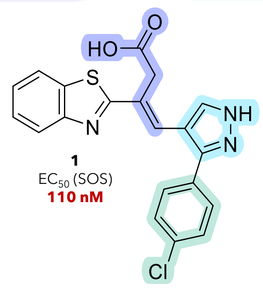


Methylated pyrazole > 100-fold more active

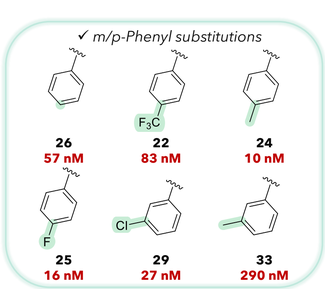


× pyridyl, bromo, o-Phenyl substitutions

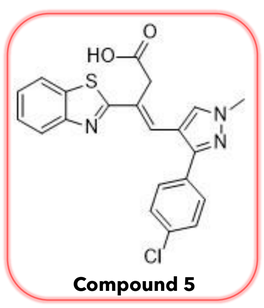


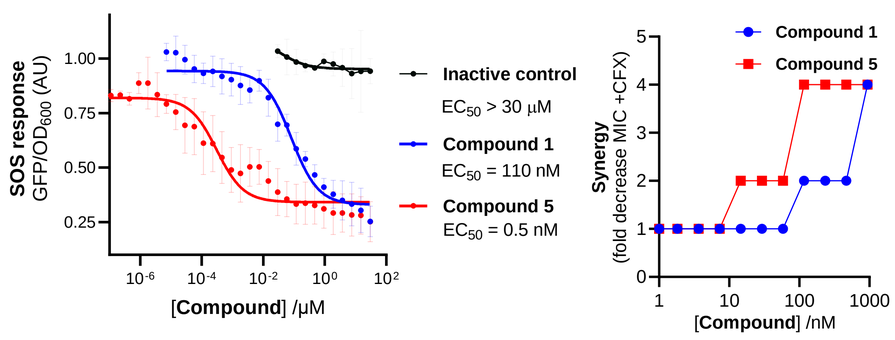


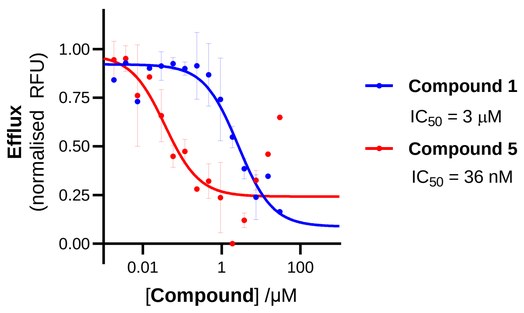
✓ m/p-Phenyl substitutions

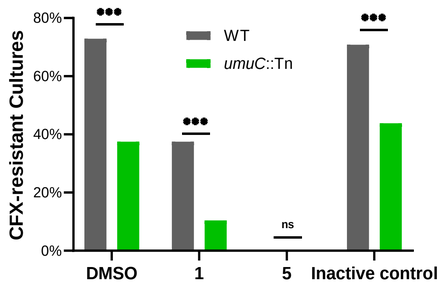


5 SAR Studies Identified Potent Analogue 5







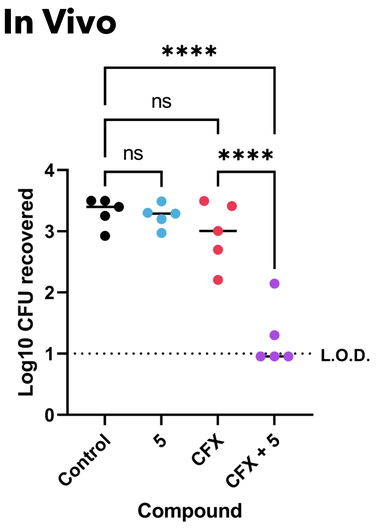


5 inhibits the SOS response at 500 pM (top, middle), potentiates the activity (top, right) of CFX and inhibits NorA efflux activity (bottom, left), with **a 400-fold improvement** over literature reference NorA inhibitor reserpine (IC_{50} = 12 μM)

5 blocks emergence of resistance to CFX in WT and SOS response-deficient strains (bottom, right)

6 Compound 5 is Active In Vivo

Compound 5 PK parameters	
In vitro	
Clearance in MLM (ml/min/kg)	10.5
Solubility (μM)	80
Plasma protein binding (Fu)	0.0033
MDCK-MDR1 Permeability (Papp, nm/sec)	133
HEK293T Cytotoxicity (CC_{50})	> 30 μM
***In vivo* (Single i.p, 10 mg/kg)**	
Time for free drug concentration in blood ≥ synergy concentration with CFX	30 - 60 mins



Compound 5 potentiates CFX *in vivo* and has suitable PK properties for use *in vitro* and *in vivo*

Conclusions & Future Work

A novel HTS identified **a new chemical series which inhibits NorA and potentiates ciprofloxacin activity in *S. aureus*** with low nanomolar potency

NorA inhibition also **prevents the emergence of further resistant mutations**

Compound 5 represents the **first fully validated nanomolar inhibitor of NorA** which is **potent *in vivo***

Patent Filed: NorA Inhibitors, Patent Application Number: (GB2319181.0) GB Intellectual Property Office.

Acknowledgements

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Dundee Drug Discovery Unit for advice, including Sandra O'Neill for her help establishing the HTS
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Rosetrees Supports the best medical research
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