Exploring Molecules to Enhance Immune Clearance of Bacteria

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Background:

Antimicrobial resistance is a global threat, with resulting deaths expected to rise to 39 million by 2050[1]. In the UK, 1 in 3 patients are on an antibiotic at any given time[2]; if new antibiotics with different MoA are not developed soon, the implications of antimicrobial resistance could be far- reaching, impacting modern medical procedures, including chemotherapy, surgery, and organ transplants.

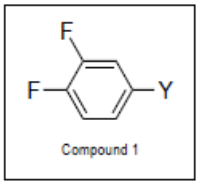
Project summary:

Oxidative stress plays a crucial role in the immune response against bacteria. Following host infection, macrophages and phagocytes generate large amounts of reactive oxygen species (ROS), e.g. hydrogen peroxide (H2O2) and hydroxyl radicals, during an oxidative burst, which causes damage to bacterial DNA and proteins, leading to bacterial cell death. However, the exact mechanism of immune clearance is not understood. The project aims to identify selectively lethal compounds that potentiate ROS, using H_2O_2 as a proxy for immune attack.

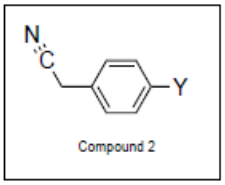
The group has identified a molecule that can sensitise bacteria to H_2O_2. However, the mechanism of how this compound exhibits this activity is not understood.

By subjecting molecules to oxidative stress, by adding H_2O_2, and examining the dose-response, we aim to uncover details about the mechanism of action and identify any promising molecules that warrant further investigation. Using SAR data, we can evaluate biological activity and identify where the compound can be functionalised to generate a chemical probe, enabling target deconvolution through activity and affinity-based protein profiling. This chemical probe can then be used to study the immune clearance of microbial infections, providing new drug targets for the development of antibiotics with novel mechanisms of action.

In addition, any data collected will be used in collaboration with the AI start-up DeepMirror. DeepMirror aims to use machine learning to advance drug discovery. The pIC50 values of compounds in the presence and absence of H_2O_2 are inputted, with the intention of new, conditionally lethal structures being predicted.



Compound 1



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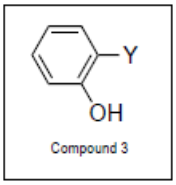
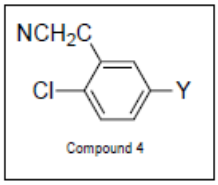
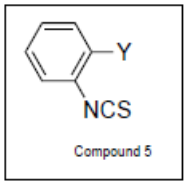


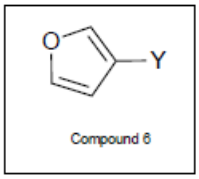
Compound 3



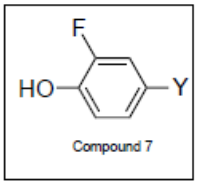
Compound 4



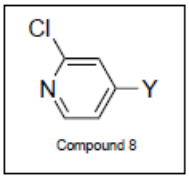
Compound 5



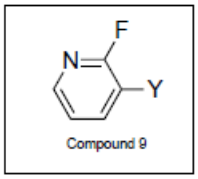
Compound 6



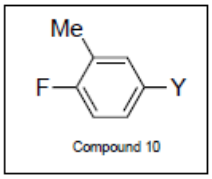
Compound 7



Compound 8



Compound 9



Compound 10

Aims

To understand the mechanism of action and effective compounds against antibiotic resistance, the aims of my project are to:

- Synthesise new chemical entities to identify any effective molecules.
- Determine the pIC50 values of synthesised molecules, both in the presence and absence of hydrogen peroxide,
- Conduct structure-activity relationship (SAR) studies to get insights on effective and ineffective functional groups to investigate the mechanism of action.
- Input this data into a machine learning program to facilitate the advancement of AI in drug discovery.

Methods and materials.

1. General synthetic procedure for Ester Hydrolysis for the preparation of compounds (Compounds 1-4, 6-10, R=OH)

A solution of the ester (Compounds 1-4, 6-10, R=OMe, 1 equiv.) and a large excess of sodium hydroxide was stirred in methanol under reflux (at 80 °C) for 3 h. TLC monitoring indicated the reaction's completion (10% MeOH in DCM). The solvent was concentrated *in vacuo*. The residue was diluted with water, and then acidified using concentrated HCl to pH 2. The solid was filtered off and washed with water to afford the respective product (Compounds 1-4, 6-10, R=OH).

2. Synthetic procedure for the preparation of compounds (Compounds 1-5, R= T)

A solution of the respectively substituted ester (compounds 1-5, R=OMe, 1 equiv.) and salt T (1 equiv.) was stirred in methanol under nitrogen for 2 h at room temperature. TLC monitored the completion of the reaction. The mixture was then concentrated using the rotary evaporator. The products (Compounds 1-5, R= T) were collected.

3. Dose-response experiments

An overnight culture *of* Escherichia coli MG1655 was revived in Mueller-Hinton broth (MHB). The optical density was measured and diluted to a final starting seeding density of OD_{600} 0.001. Compounds tested were prepared as DMSO stocks and diluted to the appropriate concentration in MHB before being serially diluted in 96-well plates (3370, Corning Co-Star). 15 µL compound in duplicate was transferred to each row of a 384-well plate (781090, Griener), each compound in triplicate. 30 µL cells were added, and the plate was incubated at 37 ˚C for 1 h, shaking at 200 RPM with a BreathEasy Plate Seal. 15 µL MHB or MHB+ H2O2 (500 µM, final concentration) was added to alternate columns. The OD_{600} was read every 30 min in a plate reader (BMG LabTech, SpectroStar Omega) for 18 h at 37 ˚C, shaking at 200 RPM between reads. pIC50 values +&- H_2O_2 of each compound were inputted into DeepMirror.

Results

Synthesis

On completion of the reactions the compounds were purified with automated flash column chromatography and thoroughly characterised by ^{13}CNMR, ^{1}HNMR, ^{19}F NMR and HRMS. Over 6 weeks, I added 24 compounds to the groups inventory. These molecules will provide valuable information for considering future molecules to test.

Dose-response results.

Dose-response experiments of compounds were performed in the presence and absence of H_2O_2, and pIC50 values were calculated to determine which parts of the molecule are essential for bacterial lethality. I tested four compounds and generated pIC50 values to compare against the reference compound (Figure 1).

The ester functional group appears ineffective in preventing antibiotic resistance, as the change in the compounds' activities with and without added H_2O_2 is not significant for all the compounds tested. There was low SEM and very small error bars. Minor changes in pIC50 values may be due to different groups attached to the aromatic rings, but they still do not significantly affect them.

Since this was my first attempt at SAR, I compared the results with those collected previously in the group for the same molecules. On average, there was a 10.03% difference in results. This may be due to biological variability and the sensitivity of cells to H_2O_2. Furthermore, I could work on keeping my hand steady to ensure no solution is stuck to the walls of the assay plate and dedicate time to practising reverse pipetting. Due to time constraints, I did not dose- response the molecules I synthesised, but these will be tested by the group soon.

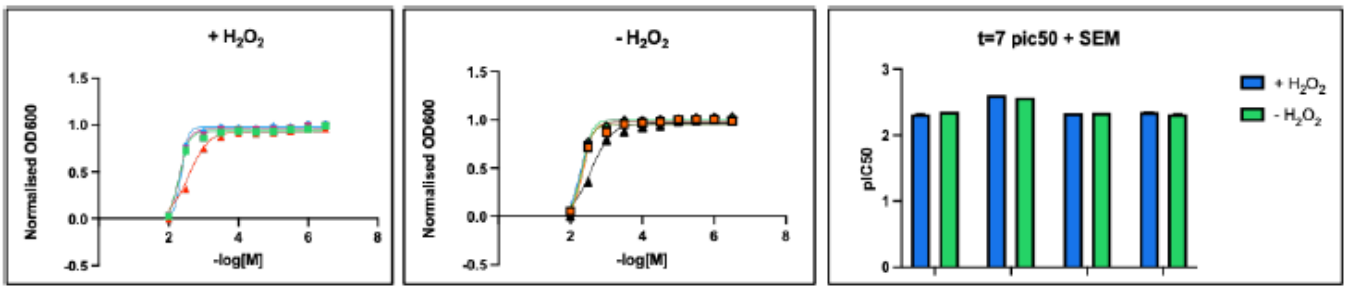


Figure 1: pIC50 and OD_{600} with and without H_2O_2 of four known compounds.

Conclusion and future directions.

In conclusion, I consider my project successful; I provided the team with a larger inventory of molecules for testing. Furthermore, I gained invaluable insights into biochemistry and expanded my skill set with previously unfamiliar techniques and equipment.

Further directions for the lab's research could include following a Topliss tree, which can be used for further molecule testing decisions. Also, the lab could test any structures generated by the DeepMirror algorithm to see if this can help advance drug discovery.

Acknowledgements:

I am immensely grateful to the Biochemical Society for funding my first ever research project, which has deepened my passion for research and motivated me to pursue further studies by applying for a PhD. They have provided me the opportunity to acquire essential research skills and techniques such as multichannel pipetting, calculation of pIC50 values, HPLC, and mass spectrometry, amongst many others that I will carry forward into my degree and future careers. I would also like to express my appreciation to the Lanyon- Hogg group for hosting me and providing unwavering support and guidance throughout.

References

1. *Nature 633, 747-748 (2024) doi: https://doi.org/10.1038/d41586-024-03033-w M.Naddaf*
2. *Clin Med (Lond). 2013 Oct; 13(5): 499–503. doi: 10.7861/clinmedicine.13-5-499 K.Hand*