University of Newcastle

Biological Characterisation of Anthranilic Acid Holoenzyme Assembly Inhibitors

A thesis submitted in fulfilment of the requirements for the degree of Doctor of Philosophy in Biological Sciences

This research was supported by an Australian Government Research Training Program (RTP) Scholarship.

Michael James Miller BBiotech(Hons)(Newcastle)
August 2022

STATEMENT OF ORIGINALITY

I hereby certify that the work embodied in the thesis is my own work, conducted under normal supervision. The thesis contains no material which has been accepted, or is being examined, for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made. I give consent to the final version of my thesis being made available worldwide when deposited in the University's Digital Repository, subject to the provisions of the Copyright Act 1968 and any approved embargo.

Michael James Miller

15 August 2022

Table of Contents

Table of Figures

Table of Tables

Abstract

The antimicrobial resistance crisis is one of the greatest threats that humankind is facing in the 21st century. Comprehensive assessments of the global burden of antimicrobial resistance (AMR) estimate that 5 million deaths were associated with bacterial AMR in 2019. AMR is not a new problem, and for the first few decades of the antibiotic era, resistance was kept in check by developing and discovering new drugs. The lack of development of novel antibiotics has multiple overlapping causes that span the spheres of economics, policy, and science. Even with all efforts to reduce and reverse the AMR crisis, new antimicrobial drugs are still needed.

A series of hybrid compounds incorporating anthranilic acid with activated 1H-indoles through a glyoxylamide linker were designed to target bacterial RNA polymerase holoenzyme formation. Biological testing of the hybrids identified a range of potent anti-transcription inhibitors with activity against a range of pathogenic bacteria with minimum inhibitory concentrations as low as 3.1 µM. A structure-activity relationship study identified the key structural components necessary to inhibit bacterial growth and transcription. Correlation of *in vitro* transcription inhibition activity with *in vivo* mechanism of action was established using fluorescence microscopy, and resistance passaging using Gram-positive bacteria showed no resistance development over 30 days. Furthermore, no toxicity was observed from the compounds in a wax moth larvae model, establishing a platform for the development of a series of new antibacterial drugs with an established mode of action. The compounds developed in this thesis are an excellent starting point for developing a series of potent new antimicrobial compounds. The future directions for this series of inhibitors are varied, but the resulting lead molecules would address underlooked clinical needs.