Development of Biotin Protein Ligase Inhibitors from *Staphylococcus aureus* as New Antibiotics

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Abstract

Biotin protein ligase (BPL) catalyses the ordered reaction of biotin and ATP to give biotinyl-5'-AMP **1.03**, which then activates a number of biotin dependent enzymes that are critical to cell survival. Research undertaken in this thesis highlights strategies to selectively inhibit *Staphylococcus aureus* biotin protein ligase (*Sa*BPL) over the mammalian equivalent using 1,2,3-triazole and acylsulfonamide isosteres to replace the phosphoroanhydride linker found in biotinyl-5'-AMP **1.03**.

Chapter one describes the structure and catalytic mechanism of the target enzyme *Sa*BPL, along with an overview of chemical analogues of biotin and biotinyl-5'-AMP **1.03** as BPL inhibitors reported to date. Preliminary studies on the utility of a 1,2,3-triazole as a bioisostere of the phosphoroanhydride linker of biotinyl-5'-AMP **1.03** are also discussed.

Chapter two further examines 1,2,3-triazole analogues of lead *Sa*BPL bisubstrate inhibitors **1.22** and **1.23**. Specific chemical modifications were carried out at the ribose of biotinyl-5'-AMP **1.03**, and a new class of purine analogues was developed to mimic the adenine group as in **1.03**. *In silico* docking experiments using our x-ray structure of *Sa*BPL aided in the design of these analogues by predicting optimal binding conformations. A structure activity relationship for the ribose and adenine mimics was developed and this revealed limited improvement in potency against *Sa*BPL on modification at these two sites.

Chapter three reports the first examples of truncated 1,2,3-triazole based BPL inhibitors with a 1-benzyl substituent designed to interact with the ribose binding pocket of *Sa*BPL. *In silico* docking studies using a crystal structure of *Sa*BPL aided in the selection of benzyl groups that present in the ribose-binding pocket of *Sa*BPL. The halogenated benzyl derivatives **3.20**, **3.21**, **3.23** and **3.24** provided the most potent inhibitors of *Sa*BPL with the respective K_i value of 0.28, 0.6, 0.39 and 1.1 μM. These compounds also inhibited the growth of *S. aureus* ATCC49775 (MIC = 4 – 16 μg/ml), while possessing low cytotoxicity against HepG2 cells.

Chapter four builds upon the active 1,2,3-triazole based inhibitors of *Sa*BPL described in chapter two and three with an investigation at C5 of the triazole ring to generate 1,4,5-

trisubstituted 1,2,3-triazoles. A class of 5-iodo 1,2,3-triazoles was synthesised from 1-iodoacetylene **4.02** and azides using CuAAC. Subsequent halogen exchange reaction allowed conversion of iodide to other halogens. 5-Fluoro-1,2,3-triazole **4.07**, the lead compound from this series of inhibitors, proved to be a potent and selective inhibitor of *Sa*BPL (K_i = 0.42 $\pm$ 0.06 μM) and it significantly reduced *S. aureus* growth with no cell growth apparent at 16 μg/mL.

Chapter five investigates the use of acylsulfonamide as a bioisostere of the phosphoroanhydride linker as in biotinyl-5'-AMP **1.03**. Acylsulfonamide **5.05** was found as the most active and selective inhibitor of *Sa*BPL (K_i = 0.72 x 10^{-3} μM) and *Mtb*BPL (K_i = 0.74 10^{-3} μM) reported to date. Antibacterial studies revealed that **5.05** was active against susceptible *S. aureus* (MIC = 0.5-1.0 μg/mL), methicillin-resistant *S. aureus* ((MIC = 0.5-1.0 μg/mL) and *Mycobacterial tuberculosis* ((MIC = 51 μg/mL). Finally, the x-ray structure **5.05** bound to *Sa*BPL was solved to reveal important molecular interactions critical to the potency of **5.05** and emphasized the acylsulfonamide moiety as an effective bioisostere of phosphoroanhydride linker.

Chapter six discusses the use of *in situ* click chemistry as an alternative approach for the synthesis of 1,2,3-triazoles. The target enzyme *Sa*BPL was directly involved in the selection of its optimum triazole based inhibitor by catalysing the reaction of biotin acetylene and organic azides without copper as a catalyst. The use of high throughput LC/MS provided improved efficiency and sensitivity of detection of triazole-based inhibitors and allowed the *in situ* approach to be widely applied to BPLs from other bacteria.

Chapter seven details the experimental procedures for compounds described in chapter 2 – 6, and the chromatographic analysis of *in situ* click experiments described in chapter 6.

Declaration

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Abbreviations

AaBPL	*A. aeolicus* biotin protein ligase
ABL	ATP binding loop
AcBPL	*A. calcoaceticus* biotin protein ligase
ACC	Acetyl CoA carboxylase
ACN	Acetonitrile
AcOH	Acetic acid
AMP	Adenosine-5'-monophosphate
ATP	Adenosine-5'-triphosphate
BBL	Biotin binding loop
BCCP	Biotin carboxyl carrier protein
BOC	*tert*-Butoxycarbonyl
BPL	Biotin protein ligase
BSA	Bovine serum albumin
CaBPL	*C. albicans* biotin protein ligase
CDI	1,1' – carbonyldiimidazole
COSY	Correlation spectroscopy
^{13}C NMR	Carbon nuclear magnetic resonance
CA-MRSA	Community acquired methicillin resistant *S. aureus*
Cp*	Pentamethylcyclopentadienyl
CSI	Chlorosulfonyl isocyanate
CuAAC	Copper mediated Alkyne Azide Cycloaddition
DCC	N,N' - Dicyclohexylcarbodiimide
DCM	Dichloromethane
DMAP	Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulphoxide
DMP	2,2-Dimethoxypropane
DSC	Disuccinimidyl carbonate
EcBPL	*E. coli* biotin protein ligase
EDA	Ethylenediamine

EtOAc	Ethyl acetate
EtOH	Ethanol
FTIR	Fourier transform infrared spectroscopy
^{19}F NMR	Fluorine nuclear magnetic resonance
^{1}H NMR	Proton nuclear magnetic resonance
HA-MRSA	Hospital acquired methicillin resistant *S. aureus*
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectrometry
HsBPL	*Homo sapiens* biotin protein ligase
HSQC	Heteronuclear single quantum coherence spectroscopy
IC_{50}	Half maximum inhibitory concentration
iPrOH	isopropanol
K_i	Dissociation constant
*Kp*BPL	*K. pneumoniae* biotin protein ligase
KPhth	potassium phthalimide
Me	Methyl group
MEK	Methyl ethyl ketone (2-butanone)
MeOH	Methanol
MIC	Minimum inhibitory concentration
MRSA	Methicillin resistant *S. aureus*
MSSA	Methicillin sensitive *S. aureus*
MtbBPL	*M. tuberculosis* biotin protein ligase
NaOMe	sodium methoxide
NMO	*N*-methylmorpholin *N*-oxide
NMI	*N*-Iodomorpholine hydriodide
$Pd(OAc)_2$	Palladium(II) acetate
PhBPL	*P. horikoshii* biotin protein ligase
PPh_3	triphenylphosphine
p-TsOH	*para*-Toluenesulphonic acid
Py	Pyridine
ROESY	Rotating frame overhauser enhanced spectroscopy
RuAAC	Ruthenium mediated Alkyne Azide cycloaddition
SaBPL	*S. aureus* biotin protein ligase

SAR	Structure activity relationship
t-BuOH	*tert*-butanol
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
Ts	4-toluenesulphonyl group
TsCl	tosyl chloride