

**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 (*SaBPL*) 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 *SaBPL*, 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 *SaBPL* 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 *SaBPL* 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 *SaBPL* 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 *SaBPL*. *In silico* docking studies using a crystal structure of *SaBPL* aided in the selection of benzyl groups that present in the ribose-binding pocket of *SaBPL*. The halogenated benzyl derivatives **3.20**, **3.21**, **3.23** and **3.24** provided the most potent inhibitors of *SaBPL* with the respective  $K_i$  value of 0.28, 0.6, 0.39 and 1.1  $\mu\text{M}$ . These compounds also inhibited the growth of *S. aureus* ATCC49775 (MIC = 4 – 16  $\mu\text{g/ml}$ ), while possessing low cytotoxicity against HepG2 cells.

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

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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 SaBPL ( $K_i = 0.42 \pm 0.06 \mu\text{M}$ ) and it significantly reduced *S. aureus* growth with no cell growth apparent at 16  $\mu\text{g/mL}$ .

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 SaBPL ( $K_i = 0.72 \times 10^{-3} \mu\text{M}$ ) and MtbBPL ( $K_i = 0.74 \times 10^{-3} \mu\text{M}$ ) reported to date. Antibacterial studies revealed that **5.05** was active against susceptible *S. aureus* (MIC = 0.5-1.0  $\mu\text{g/mL}$ ), methicillin-resistant *S. aureus* (MIC = 0.5-1.0  $\mu\text{g/mL}$ ) and *Mycobacterial tuberculosis* (MIC = 51  $\mu\text{g/mL}$ ). Finally, the x-ray structure **5.05** bound to SaBPL 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 SaBPL 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.

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## Declaration

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Jiage Feng

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Date



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## Abbreviations

AaBPL	<i>A. aeolicus</i> biotin protein ligase
ABL	ATP binding loop
AcBPL	<i>A. calcoaceticus</i> 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	<i>tert</i> -Butoxycarbonyl
BPL	Biotin protein ligase
BSA	Bovine serum albumin
CaBPL	<i>C. albicans</i> biotin protein ligase
CDI	1,1' – carbonyldiimidazole
COSY	Correlation spectroscopy
<sup>13</sup> C NMR	Carbon nuclear magnetic resonance
CA-MRSA	Community acquired methicillin resistant <i>S. aureus</i>
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	<i>E. coli</i> biotin protein ligase
EDA	Ethylenediamine

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EtOAc	Ethyl acetate
EtOH	Ethanol
FTIR	Fourier transform infrared spectroscopy
$^{19}\text{F}$ NMR	Fluorine nuclear magnetic resonance
$^1\text{H}$ NMR	Proton nuclear magnetic resonance
HA-MRSA	Hospital acquired methicillin resistant <i>S. aureus</i>
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectrometry
HsBPL	<i>Homo sapiens</i> biotin protein ligase
HSQC	Heteronuclear single quantum coherence spectroscopy
IC <sub>50</sub>	Half maximum inhibitory concentration
iPrOH	isopropanol
K <sub>i</sub>	Dissociation constant
KpBPL	<i>K. pneumoniae</i> biotin protein ligase
KPhth	potassium phthalimide
Me	Methyl group
MEK	Methyl ethyl ketone (2-butanone)
MeOH	Methanol
MIC	Minimum inhibitory concentration
MRSA	Methicillin resistant <i>S. aureus</i>
MSSA	Methicillin sensitive <i>S. aureus</i>
MtbBPL	<i>M. tuberculosis</i> biotin protein ligase
NaOMe	sodium methoxide
NMO	<i>N</i> -methylmorpholin <i>N</i> -oxide
NMI	<i>N</i> -Iodomorpholine hydriodide
Pd(OAc) <sub>2</sub>	Palladium(II) acetate
PhBPL	<i>P. horikoshii</i> biotin protein ligase
PPh <sub>3</sub>	triphenylphosphine
<i>p</i> -TsOH	<i>para</i> -Toluenesulphonic acid
Py	Pyridine
ROESY	Rotating frame overhauser enhanced spectroscopy
RuAAC	Ruthenium mediated Alkyne Azide cycloaddition
SaBPL	<i>S. aureus</i> biotin protein ligase

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