Design and Synthesis of Reaction Intermediate Derivatives as Biotin Protein Ligase Inhibitors

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Table of Contents

Chapter Three

Chapter Four

Chapter Five

Chapter Six

Chapter Seven

Abstract

This thesis reports the development of selective and potent small molecule inhibitors of *Staphylococcus aureus* biotin protein ligase (SaBPL) using 1,2,3-triazole and phosphodiester linkers as bioisosteric analogues of the phosphoroanhydride linker found in the reaction intermediate biotinyl-5'-AMP **1.03**.

Chapter one describes the structure and catalytic mechanism of the essential enzyme SaBPL. An overview of reaction intermediate mimics as ligase inhibitors is discussed and the utility of 1,2,3-triazole ring as a bioisosteric analogue is outlined.

Chapter two investigates the phosphodiester reaction intermediate mimic biotinol-5'-AMP **1.05** as a potential inhibitor of SaBPL. Two different synthetic approaches towards biotinol-5'-AMP **1.05** were developed with the aim of scaling up the synthesis to enable biological characterisation and animal trials. Assay results indicated biotinol-5'-AMP **1.05** is a potent but a non-selective inhibitor of SaBPL (IC_{50} = 0.12 ± 0.01 μM).

Chapter three investigates the use of 1,2,3-triazole as a bioisostere of the phosphoroanhydride linker of the reaction intermediate biotinyl-5'-AMP **1.03**. Both 1,4-triazole **3.25** and 1,5-triazole **3.33** were synthesized from biotin alkyne **3.12** and adenosine azide **3.16** using CuAAC and RuAAC. Optimisation of both CuAAC and RuAAC in the synthesis of **3.25** and **3.33** were also investigated. 1,4-Triazole **3.25** is the first reported selective inhibitor of BPL, inhibiting SaBPL (K_i = 1.83 ± 0.33 μM).

Chapter four extends the work described in chapter three with an investigation of 1,2,3-triazole analogues based on triazole **3.25**. Structure-activity relationships were developed and a general structure for this novel class of inhibitors was obtained. Triazole **4.01**, the lead compound from this class of inhibitors, is a potent and selective inhibitor of SaBPL (K_i = 0.66 ± 0.15 μM). X-ray crystal structure of **4.01** bound to SaBPL illustrated the effective molecular recognition between the 1,2,3-triazole ring and SaBPL and emphasized the 1,2,3-triazole ring as an effective bioisostere of phosphoroanhydride linker. Additionally, a successful *in situ* click experiment was performed using a library of alkynes/azides fragments and R122G SaBPL mutant enzyme. The mutant enzyme was able

to select the appropriate fragments and selectively synthesize the potent 1,4-triazole inhibitor **4.01**.

Chapter five examines analogues of biotin alkyne **3.12**, a precursor to 1,2,3-triazole inhibitors and was found to be a potent inhibitor (SaBPL K_i = 0.30 ± 0.05 μM). Norbiotin alkyne **4.16** was found as highly effective inhibitor against SaBPL (K_i = 0.08 ± 0.01 μM) and an antibacterial agent against methicillin resistant *staphylococcus aureus* (MIC = 4 - 16 μg/ml).

Chapter six extends the work described in chapter four. Using the general structure developed in chapter four, a series of analogues with modifications to the ATP binding component were synthesized and assayed against a SaBPL. Triazole **6.10** containing the privileged scaffold, 2-benzoxazolone, was found as a potent and selective inhibitor against SaBPL (K_i = 0.09 ± 0.02 μM).

Chapter seven details the experimental procedures used to synthesize compounds described in chapter 2 – 6.

Declaration

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Date

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Abbreviations

ABL	ATP binding loop
ACC	Acetyl CoA carboxylase
AcCN	Acetonitrile
AcOH	Acetic acid
AIBN	Azobisisobutyronitrile
AMP	Adenosine-5'-monophosphate
ATP	Adenosine-5'-triphosphate
BBL	Biotin binding loop
BCCP	Biotin carboxyl carrier protein
BOC	*tert*-Butoxycarbonyl
BPL	Biotin protein ligase
CDI	1,1' – carbonyldiimidazole
COSY	Correlation spectroscopy
^{13}C NMR	Carbon nuclear magnetic resonance
Cp*	Pentamethylcyclopentadienyl
CuAAC	Copper mediated Alkyne Azide Cycloaddition
DCC	N,N' - Dicyclohexylcarbodiimide
DCM	Dichloromethane
DDQ	2,3-dichloro-5,6-dicyano-*p*-benzoquinone
DEAD	Diethyl azodicarboxylate
DEAE	Diethylaminoethyl cellulose
DIBAL-H	Diisobutylaluminium hydride
DIPEA	N,N - Diisopropylethylamine
4-DMAP	4-Dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethyl sulphoxide
DMTr	4,4'-dimethoxytrityl group
DPPA	Diphenylphosphoryl azide
EcBPL	*E. coli* biotin protein ligase
EDA	Ethylenediamine
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

EtOAc	Ethyl acetate
EtOH	Ethanol
5-ETT	5-Ethylthiotetrazole
FTIR	Fourier transform infrared spectroscopy
^{1}H NMR	Proton nuclear magnetic resonance
HPLC	High-performance liquid chromatography
HRMS	High resolution mass spectrometry
HsBPL	*Homo sapiens* biotin protein ligase
IC_{50}	Half maximum inhibitory concentration
iPrOH	isopropanol
K_i	Dissociation constant
LCMS	Liquid chromatography mass spectrometry
*m*CPBA	*meta*-Chloroperoxybenzoic acid
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*
^{31}P NMR	Phosphorous nuclear magnetic resonance
POM	pivaloyloxymethyl
p-TsOH	*para*-Toluenesulphonic acid
Py	Pyridine
RMSD	Root mean square deviation
ROESY	Rotating frame overhauser enhanced spectroscopy
RuAAC	Ruthenium mediated Alkyne Azide cycloaddition
SaBPL	*S. aureus* biotin protein ligase
SAR	Structure-activity relationship
TBAI	Tetrabutylammonium iodide
TBHP	*tert*-Butyl hydroperoxide
t-BuOH	*tert*-butanol
TEAB	Triethylamine bicarbonate buffer
TFA	Trifluoroacetic acid

THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilyl group
Ts	4-toluenesulphonyl group