

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

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



2011

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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 biotinyl-5'-AMP **1.05** as a potential inhibitor of SaBPL. Two different synthetic approaches towards biotinyl-5'-AMP **1.05** were developed with the aim of scaling up the synthesis to enable biological characterisation and animal trials. Assay results indicated biotinyl-5'-AMP **1.05** is a potent but a non-selective inhibitor of SaBPL ($IC_{50} = 0.12 \pm 0.01 \mu\text{M}$).

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 ($K_i = 1.83 \pm 0.33 \mu\text{M}$).

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 ($K_i = 0.66 \pm 0.15 \mu\text{M}$). 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 $K_i = 0.30 \pm 0.05 \mu\text{M}$). Norbiotin alkyne **4.16** was found as highly effective inhibitor against SaBPL ($K_i = 0.08 \pm 0.01 \mu\text{M}$) and an antibacterial agent against methicillin resistant *staphylococcus aureus* (MIC = 4 - 16 $\mu\text{g/ml}$).

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 ($K_i = 0.09 \pm 0.02 \mu\text{M}$).

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

Declaration

This work contains no material which has been accepted 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 published or written by another person, except where due reference has been made in the text.

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William Tieu

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Date

Acknowledgement

First and foremost I would like to thank Professor Andrew Abell for his supervision and guidance during my candidature. I am indebted to his helpful advice, extensive knowledge, positive and forward thinking attitude to research and tireless efforts in drafting and revising this thesis.

I would like to thank the collaborators in the BPL project. Belinda Ng, Tatiana Soares Da Costa and Steven Polyak for performing the BPL and antimicrobial assays and *in situ* click experiments. Min Yap, Nicole Pardini and Matthew Wilce are thanked for providing the invaluable x-ray crystallographic structures of inhibitors bound to SaBPL. Particular mention goes to Grant Booker (co-supervisor) and Steven Polyak for their support, enthusiasm and tireless efforts throughout my candidature.

I would like to thank all the past and present members of the Abell group. To the post-docs (Sabrina Heng, Daniel Pedersen, Markus Pietsch and Ondrej Zvarec), thank you for the technical advice and friendship.

Thank you to my supportive parents, Hoa and Hue, for always being there for me and understanding the life of a PhD student. Thank you to my sister Joanna for the moral support and encouragement.

Unreservedly, I must thank my girlfriend Thao. She has always believed in me and has the magical ability to make me happy whenever I'm with her (especially when my experiments are not working).

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	<i>tert</i> -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- <i>p</i> -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	<i>E. coli</i> 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	<i>Homo sapiens</i> biotin protein ligase
IC ₅₀	Half maximum inhibitory concentration
iPrOH	isopropanol
K _i	Dissociation constant
LCMS	Liquid chromatography mass spectrometry
<i>m</i> CPBA	<i>meta</i> -Chloroperoxybenzoic acid
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>
³¹ P NMR	Phosphorous nuclear magnetic resonance
POM	pivaloyloxymethyl
<i>p</i> -TsOH	<i>para</i> -Toluenesulphonic acid
Py	Pyridine
RMSD	Root mean square deviation
ROESY	Rotating frame overhauser enhanced spectroscopy
RuAAC	Ruthenium mediated Alkyne Azide cycloaddition
SaBPL	<i>S. aureus</i> biotin protein ligase
SAR	Structure-activity relationship
TBAI	Tetrabutylammonium iodide
TBHP	<i>tert</i> -Butyl hydroperoxide
<i>t</i> -BuOH	<i>tert</i> -butanol
TEAB	Triethylamine bicarbonate buffer
TFA	Trifluoroacetic acid

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