Design and Synthesis and Testing of Conformationally Constrained Peptidomimetics

Joanna Duncan

A thesis submitted in total fulfilment of the requirements for

the degree of Doctor of Philosophy

2013

Department of Chemistry

The University of Adelaide

Abstract…….. IV

Declaration…….. VII

Acknowledgments……..VIII

Abbreviations……IX

1 Introduction…….……1

1.1 Protein-protein interactions…….……2

1.2 Mediating protein-protein interactions…….……...3

1.2.1 Peptides as drugs…….3

1.2.2 Disadvantages of peptides……...3

1.2.3 Peptidomimetics…….….5

1.3 Peptide secondary structure…….…... 6

1.4 Classes of peptidomimetic…….…. 9

1.4.1 Side chain modification……. .9

1.4.2 Strategies for restriction of φ, ψ and ω torsion angles…….. 11

1.5 A ring closing metathesis route to peptidomimetics…….18

1.5.1 A brief history of metathesis…….…18

1.5.2 Peptidomimetics by ring closing metathesis…….19

1.6 Research described in this thesis……...22

1.7 References…….23

2 Synthesis of medium sized rings comprised of β-amino acids by ring closing metathesis……. 31

2.1 Peptidomimetics from β-amino acids……... 32

2.2 Synthesis and analysis of cyclic scaffolds…… 45

2.2.1 Synthesis of building blocks 2.31 – 2.36…….. 45

2.2.2 Synthesis of cyclic scaffolds…….53

2.2.3 Synthesis and NMR analysis of tripeptides containing cyclic scaffolds……55

2.3 Conclusions……...64

2.4 References…….66

3 Synthesis of macrocyclic protease inhibitors……70

3.1 Calpain and its inhibition……..71

3.1.1 The role of calpain in pathological conditions……. 74

3.1.2 Calpain inhibitors……..76

3.2 Synthesis of the N-terminal FBS macrocycle 3.02……...84

3.3 Design, synthesis and testing of diol-containing macrocycles 3.03 and 3.04…….92

3.3.1 Computational modelling of diols 3.03 and 3.04……..94

3.3.2 Synthesis of diol-based inhibitors……...100

3.4 Design and synthesis of novel macrocycle 3.05……. 106

3.4.1 Designing a macrocycle to investigate selectivity towards calpain II, α-chymotrypsin and 20S proteasome……106

3.4.2 Synthesis of macrocycle 3.05……..109

3.5 Conclusions……112

References……...113

4 *In vitro* testing of macrocycles synthesised in Chapter 3……....119

4.1 Introduction……120

4.2 Assay of compounds 3.02, 3.03 and 3.04 against ovine calpain II…….121

4.3 Analysis of calpain II inhibition data……..126

4.4 Biological testing of 3.05……137

4.5 Conclusions……144

4.6 References……...146

5 *In vivo* testing of tritiated macrocycles……149

5.1 Introduction…….……150

5.1.1 Summary of *in vivo* trials of CAT0811……...152

5.2 Results and discussion…….……... 155

5.2.1 Synthesis and formulation of radio-labelled analogues……...155

5.2.2 *In Vivo* study methods…… .159

5.2.3 Liquid scintillation counting results……162

5.3 Conclusions…….171

5.4 References for Chapter 5…… 172

6 Experimental……... 174

6.1 General methods and experimental procedures……... 175

6.2 Experimental for Chapter 2…….178

6.3 Experimental for Chapter 3…….223

6.4 Experimental for Chapter 5…….255

A1 Liquid Scintillation Counting (LSC)……..…….....…...261

Abstract

This thesis describes the design, synthesis and testing of peptidomimetics pre-organised into bioactive conformations. **Chapter One** introduces the concept of peptidomimetics, their importance as potential pharmaceuticals. The concept of constraining a compound into a bioactive conformation (α-helix, β-turn or β-strand) by incorporation of a ring or bridge is discussed. The technique of ring closing metathesis as a strategy for cyclisation of peptidomimetics is introduced.

Chapter Two surveys β-turn mimics comprised of β-amino acids. The synthesis of novel cyclic peptidomimetics comprised of β-amino acids (cyclised by ring closing metathesis) is presented. Three of the cyclic dipeptides were predicted (through *in silico* conformational searches) to adopt a β-turn motif. Cyclic scaffolds **2.62**, **2.65** and **2.66** were each incorporated into a tri-peptide to give **2.68**, **2.69** and **2.70**. The propensity of each tri-peptide to adopt a β-turn motif was investigated by ^{1}H NMR. There is strong evidence that **2.70** has a β-turn geometry based on the presence of an intra-molecular hydrogen bond between the *i* and *i+3* residues.

Chapter Three introduces cysteine protease calpain II as the primary biological target for this thesis. Calpain is implicated in cataract formation and its inhibition is a logical approach to cataract prevention. Proteases are known to, almost universally, bind substrates and inhibitors in a β-strand conformation. Four macrocycles, designed to be preorganised in a β-strand geometry, were synthesised by ring closing metathesis (compounds **3.02** – **3.05**). Macrocycle **3.02** was made to investigate the suitability of an N-terminal 4-fluoro-benzyl-sulfonyl (FBS) in macrocyclic calpain

inhibitors. The synthesis of **3.02** was optimised to give the required compound in 33% yield compared to a reported 1% for analogue **CAT0811**. Diols **3.03** and **3.04** (as a mixture with **3.03** in an 85:15 ratio) were designed to explore possible hydrophilic interactions with the active site of calpain. Macrocycle **3.05** was designed to investigate the relative importance of having an aromatic residue at P_1 for inhibition of calpain, α-chymotrypsin and the 20S proteasome.

Chapter Four reports the *in vitro* testing of macrocycles **3.02**, **3.03** and **3.04** against calpain II and discusses these results in the context of the SAR study completed by the Abell group to identify the criteria for the most potent macrocyclic calpain inhibitor. **CAT0811** was confirmed as the most potent macrocyclic calpain II inhibitor to date with an IC_{50} of 0.03 μM. Macrocycle **3.02** had an IC_{50} of 0.045 μM against calpain II, confirming the suitability of FBS as an N-terminus in these macrocycles. Macrocycle **3.03** had an IC_{50} of 3.7 μM against calpain II, suggesting a diol substituent is not tolerated by the enzyme at P_1. Diol **3.04** (as a mixture with **3.03** in an 85:15 ratio) was essentially inactive against calpain II (IC50 > 50 μM), presumably as **3.04** has a low propensity to adopt a β-strand conformation. Macrocycle **3.05** had an IC_{50} of 0.15 μM against calpain II, a Ki of 686 μM against α-chymotrypsin and an IC_{50} of 1.46 μM against the chymotrypsin-like sub-site of the 20S proteasome. **CAT0811** was inactive against α-chymotrypsin and had an IC_{50} of 1.51 μM against the chymotrypsin-like sub-site of the 20S proteasome. While modification to the P_1 and P_3 positions moderately influenced the selectivity of the macrocycles (comparing **3.05** with **CAT0811**), a much more dramatic affect was gain by modification of the P_2 residue (as in **4.02**).

Chapter Five reports the synthesis and *in vivo* testing of tritiated analogues of **CAT0811** and **4.04** by reduction of **CAT0811** with $NaBT_4$ to give macrocycle **5.02**, and subsequent oxidation of **5.02** to give **5.03**. Compounds **5.02** and **5.03** were separately formulated and administered to sheep from the cataract flock. Liquid scintillation counting was used to get a preliminary outlook on the absorption, distribution and excretion of the macrocycles and to investigate the phenomenon of les crossover of the inhibitors. Previous *in vivo* trials of **CAT0811** have reported that when the formulated inhibitor is administered to the left lens, both lenses are equally observed to have slowing of cataract progression ($p < 0.05$). Levels of tritium in the treated and untreated lenses were measured. Equal amounts of **5.02** were found in both lenses 48 h after application. This supports our hypothesis that lens crossover of the macrocycles is occurring.

Declaration

I certify that 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 previously published or written by another person, except where due reference has been made in the text. In addition, I certify that no part of this work will, in the future, be used in a submission for any other degree or diploma in any university or other tertiary institution without the prior approval of the University of Adelaide and where applicable, any partner institution responsible for the joint-award of this degree.

I give consent to this copy of my thesis, when deposited in the University Library, being made available for loan and photocopying, subject to the provisions of the Copyright Act 1968.

I also give permission for the digital version of my thesis to be made available on the web, via the University's digital research repository, the Library catalogue and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

Joanna Duncan Date:

Acknowledgements

Primarily, thank you to Professor Andrew Abell for his support, guidance and supervision throughout this PhD. I am especially grateful for the time he has spent guiding me in the writing of this thesis.

I would like to thank the post doctoral fellows and senior PhD students who have provided invaluable assistance during my studies, especially Dr Matthew Jones, Dr Steve Aiken, Dr Stephen McNabb, Dr Markus Pietsch, Dr Seth Jones, Dr Scott Walker, Dr William Tieu and Dr Ashok Pehere.

I would also like to acknowledge the people who helped me in this research, especially, Dr Matthew Jones, Dr Markus Pietsch and Dr Ondrej Zvarec for performing the calpain assays, Dr Paul Nielson and Limei Sieu for performing the 20S proteasome assays, Dr Stephen McNabb and Dr Blair Stuart for performing the molecular modelling studies, Michelle Zhang for help with the chymotrypsin assay and Jade Cottam for helping with the HPLC. I would also like to say a big thank you to all the technical staff at the University of Canterbury and the University of Adelaide for their help over the years.

Finally I would like to thank my family and friends for their support and love over the course of these studies – I could not have done this without you.

Abbreviations

aq	aqueous
Boc	*tert*-butoxycarbonyl
br	broad (spectroscopic)
calc	calculated
Cbz	benzyloxycarbonyl
conc	concentrated
Cy	cyclohexyl
DCM	dichloromethane
d.e.	diasteromeric excess
DIPEA	N,N-diisopropylethylamine
DMB	2,4-dimethoxy benzaldehyde
DMF	dimethylformamide
DMSO	dimethyl sulphoxide
EM	effective molarity
eq	equivalent
ESI	electrospray ionisation
Et	ethyl
FDA	Food and Drug Administration (US)
FTIR	Fourier transform infrared
GABA	gamma-aminobutyric acid
h	hour(s)
HATU	2-(7-aza-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

HIV	Human Immunodeficiency Virus
HPLC	high-performance liquid chromatography
HRMS	high-resolution mass spectrometry
IR	infrared
LDA	lithium diisopropylamide
LiHMDS	lithium hexamethyl-disilyl-amide
lit.	literature value
Me	methyl
MS	mass spectrometry
m/*z*	mass-to-charge ratio
NaHMDS	Sodium-hexamethyl-disilyl-amide
NMR	nuclear magnetic resonance
PDB	Protein Data Bank
Ph	phenyl
ppm	part(s) per million
Py	pyridine
quant	quantitative
RCM	ring closing metathesis
rt	room temperature
SAR	structure activity relationship
spec	spectrometry
TCE	1,1,2-trichloroethane
temp	temperature
TFA	trifluoroacetic acid
THF	tetrahydrofuran

TLC	thin layer chromatography
UV	ultraviolet
v/v	volume per unit volume
w/w	weight per unit weight