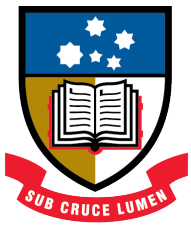
PEPTIDOMIMETIC PROTEASE INHIBITORS: ACTIVITY AND MECHANISM OF INHIBITION

A thesis submitted for the
degree of Doctor of Philosophy

Xiaozhou Zhang, B.Sc. (Hons.)



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Abstract

The study of protein mechanism and function is central to the development of biosensing tools and therapeutics for the treatment of diseases. This thesis describes an NMR and X-ray crystallography-based characterisation of the mechanism by which a macrocyclic peptidomimetic, the backbone of which is constrained into a β-strand conformation, inhibits α-chymotrypsin. This allowed the development of new peptidomimetic inhibitors that target the 26S proteasome and also inhibitors the activity of which can be modulated photochemically. This then provides a basis for biosensing and therapeutic applications.

Chapter one introduces the structures and mechanism of serine, cysteine and threonine proteases, and discusses how theses proteases universally bind ligands in an extended β-strand conformation. In addition, this chapter details the strengths and limitations of current peptidomimetic inhibitors of α-chymotrypsin, calpains and the 26S proteasome and their implications in the treatment of human diseases.

Chapter two describes optimisation of the synthesis of two macrocyclic peptidic aldehyde inhibitors **2.12** and **2.13** that target cysteine proteases and α-chymotrypsin, respectively. This allowed the preparation of an analogue of **2.13** containing a ^{13}C label in the aldehyde, which was used to confirm the mechanism of inhibition of α-chymotrypsin by ^{13}C NMR spectroscopy. This confirmed the formation of a stable hemiacetal intermediate upon the binding

of **2.13** with α-chymotrypsin. X-ray crystallography of a complex of **2.13** bound to α-chymotrypsin revealed that the backbone adopts a stable β-strand conformation as per its design. The binding of **2.13** to α-chymotrypsin is further stabilised by the oxyanion hole near the S_1 subsite and multiple hydrogen bonding interactions.

Chapter three details the development of new acyclic proteasome inhibitors **3.05**-**3.08** containing a peptidomimetic backbone and a *C*-terminal boronate. All analogues showed selectivity for the chymotrypsin-like subunit of the 26S proteasome with IC_{50} values in the low nanomolar range. Compound **3.08**, with an IC_{50} of 13 nM, was 2-fold more active than the anti-myeloma therapeutics bortezomib and carfilzomib. This inhibitor is more cytotoxic against a range of solid tumour cells and has a larger therapeutic window compared to existing FDA approved drugs.

Chapter four presents a new approach to the regulation of the activity of α-chymotrypsin using a new spiropyran-based moiety that can be reversibly switched between an 'on' (SP isomer) and 'off' (MC isomer) state photochemically. This is demonstrated in solution and also when attached to a microstructured optical fibre (MOF), as a first step to the development of a biosensor. The most active analogue in this series displayed a K_i of 115 nM in solution. The active SP isomer of an analogue **4.07** with a *C*-terminal Weinreb amide was significantly more active than the corresponding MC isomer both in solution and on fibre.

Declaration and Published Works

I certify that this work contains no material which has been accepted for the award of any other degree or diploma in my name, 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 in my name, 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. The author acknowledges that copyright of published works contained within this thesis resides with the copyright holder(s) of those works. 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 Search and also through web search engines, unless permission has been granted by the University to restrict access for a period of time.

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Xiaozhou Zhang

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Date

Work in this thesis has appeared in the following publications:

"Photoregulation of α-Chymotrypsin Activity by Spiropyran-Based Inhibitors in Solution and Attached to an Optical Fiber", Zhang, X.; Heng, S.; Abell, A. D. *Chemistry – A European Journal* **2015**, *21*, 10703.

"Macrocyclic Protease Inhibitors with Reduced Peptide Character", Chua, K. C. H.; Pietsch, M.; Zhang, X.; Hautmann, S.; Chan, H. Y.; Bruning, J. B.; Gütschow, M.; Abell, A. D. *Angew. Chem. Int. Ed.* **2014**, *53*, 7828.

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Abbreviations

AAF-AMC	Ala-Ala-Phe-7-amido-4-methylcoumarin
Ac-nLPnLD-AMC	*N*-acetal-Nle-Pro-Nle-Asp-7-amino-4-methylcoumarin
ACN	acetonitrile
Ala	alanine
AMC	7-amino-4-methylcoumarin
Asn	asparagine
Asp	aspartic acid
ATP	adenosine triphosphate
bCT	bovine α-chymotrypsin
Boc	*tert*-butyloxycarbonyl
Boc-LSTR-AMC	*N*-Boc-Leu-Ser-Thr-Arg-7-amino-4-methylcoumarin
Boc_2O	di-*tert*-butyl dicarbonate
BOP	(benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate
br s	broad singlet (in NMR)
Bz-VGR-AMC	*N*-benzyl-Val-Gly-Arg-7-amino-4-methylcoumarin
C-L	caspase-like activity (of the proteasome)
cat.	catalytic amount
Cbz	carboxybenzyl
CDK	cyclin-dependent kinase
CT-L	chymotrypsin-like activity (of the proteasome)
Cys	cysteine

d	doublet (in NMR)
DCM	dichloromethane
dd	doublet of doublet (in NMR)
ddd	doublet of doublet of doublet
DIC	*N,N′*-diisopropylcarbodiimide
DIPEA	N,N-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
dt	doublet of triplet (in NMR)
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
equiv	equivalents
ESI	electrospray ionisation (in HRMS)
FDA	United States Food and Drug Administration
FTIR	fourier transform infrared spectroscopy
G1	growth-1 phase
G2	pre-mitotic phase
Glu	glutamic acid
Gly	glycine
HATU	1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
His	histidine
HIV	human immunodeficiency virus
HIVPR	the human immunodeficiency virus protease

HMDS	bis(trimethylsilyl)amine
HOBt	hydroxybenzotriazole
HRMS	high-resolution mass spectrometry
Ile	isoleucine
LDA	lithium diisopropylamide
Leu	leucine
LiHMDS	lithium bis(trimethylsilyl)amide
M	mitosis phase
m	multiplet (in NMR)
MC	merocyanine
Met	methionine
min	minute
MMF	multi-mode fibre
MOF	microstructured optical fibre
n-Buli	n-butyllithium
NMR	nuclear magnetic resonance
o-CAPN2	ovine calpain 2 (m-calpain)
PDB	protein data bank
PG	protecting group
Phe	phenylalanine
PMP-C	pars intercerebralis major peptide-C
q	quartet (in NMR)
quant	quantitative (yield)
r.t.	room temperature
RCM	ring-closing metathesis

RMS	root mean square (in X-ray crystallography)
RP-HPLC	reverse phase high-performance liquid chromatography
S	synthesis phase
s	singlet (in NMR)
sat.	saturated
SEM	scanning electron microscopy
Ser	serine
SP	spiropyran
Suc-LLVY-AMC	*N*-succinyl-Leu-Leu-Val-Tyr-7-amino-4-methylcoumarin
t	triplet (in NMR)
T-L	trypsin-like activity (of the proteasome)
TBAI	tetrabutylammonium iodide
TES	*N*-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid
TFA	trifluoroacetic acid
THF	tetrahydrofuran
Thr	threonine
TLC	thin layer chromatography
TLCK	tosyllysine chloromethyl ketone
TMS	trimethylsilane
Tris	tris(hydroxymethyl)aminomethane hydrochloride
Trp	tryptophan
TTL	transistor-transistor logic
Tyr	tyrosine
UV	ultraviolet
Vis	visible light