Natural Products Applied in Reverse Chemical Proteomics

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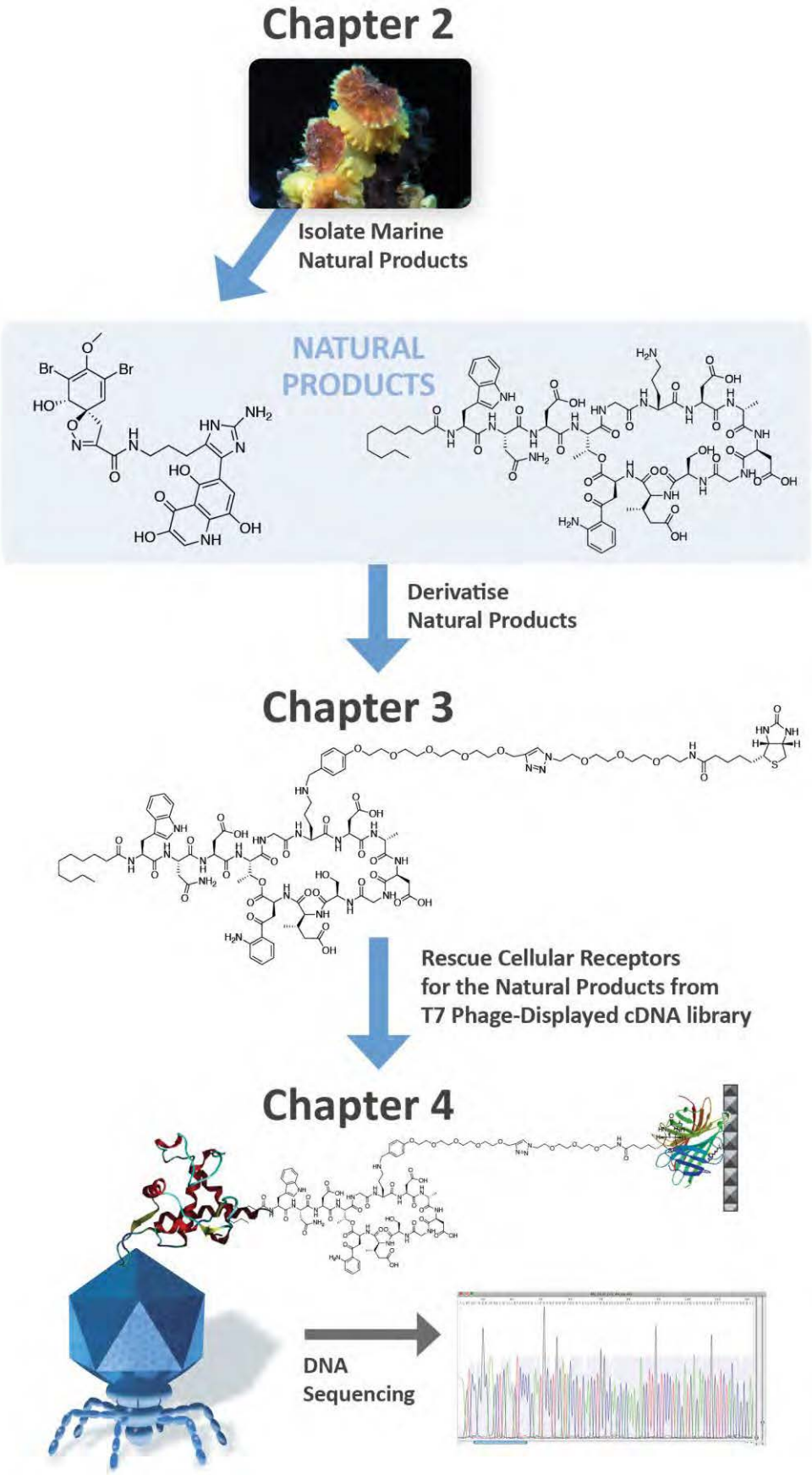
Thesis Abstract

Natural products are a rich source of structurally diverse and biologically active small molecules. They constitute a useful class of compounds as leads in rational drug design and development. However, drug discovery faces a major bottleneck due to the lack of knowledge about the active compounds' cellular targets and mode of action. For this thesis, several natural products with interesting biological activity have been applied as ligands in a technique we refer to as reverse chemical proteomics. This method rapidly generates protein-ligand pairs, which will be useful for the rational design of new and more potent therapeutics, identification of druggable targets as well as for understanding the underlying biochemical pathways of these active ligands. This thesis is divided into four main chapters. Chapter 1 introduces the techniques behind reverse chemical proteomics. Chapter 2 describes the bioassay-guided fractionation of eleven marine sponges, the isolation and characterisation of two new and seven known bromotyrosines of the highly antibacterial active extract from the marine sponge *Pseudoceratina purpurea*, as well as isolation of bromotyrosines from the opisthobranch *Tylodina corticalis*, which was collected while feeding on *P. purpurea*. In Chapter 3, the chemical derivatisation of natural products is presented alongside the synthesis and characterisation of novel, linkers and reagents required for performing reverse chemical proteomics. Chapter 4 describes the application of T7 phage display utilising the immunosuppressant natural product FK506 as a model affinity probe. Consecutively, protein binding partners for biotinylated artesunate, daptomycin and manzamine are isolated from various T7 phage-displayed human cDNA libraries. An experimental chapter and concluding remarks follow thereafter.

Graphical Abstract



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Abbreviations

aa	amino acids
amu	atomic mass unit
ATP	adenosine triphosphate
AUC	area under curve
BES	*N,N*-Bis(2-hydroxyethyl)-2-aminoethanesulfonic acid
Boc	*tert*-butoxycarbonyl
bp	base pairs
br	broad (IR, NMR)
BuOH	1-butanol
$CDCl_3$	deuterated chloroform
$CHCl_3$	chloroform
cDNA	complementary DNA
CDS	coding sequence
COSY	correlated spectroscopy
d	doublet (NMR)
DCC	1,3-dicyclohexylcarbodiimide
DCM	dichloromethane
DHA	dihydroartemisinin
DMAP	4-dimethylaminopyridine
DMSO	dimethylsulfoxide
DMSO-d_6	deuterated dimethylsulfoxide
DNA	deoxyribonucleic acid
dNTP	deoxyribonucleotide triphosphate
DSC	*N,N'*-disuccinimidyl carbonate
dsDNA	double-stranded DNA
E. coli	*Escherichia coli*
EDC	1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride
EDTA	ethylenediaminetetraacetic acid
ESI	electrospray ionisation
FDA	US Food and Drug Administration
FKBP	FK506 binding protein
FTIR	Fourier transform infrared
gp	gene product
Grubbs' catalyst	benzylidine-bis(tricyclohexylphosphine) dichlororuthenium

HMBC	heteronuclear multiple bond coherence
HOBt	hydroxybenzotriazole
HPLC	high performance liquid chromatography
HRESI	high resolution electrospray ionisation (mass spectrometry)
HSQC	heteronuclear single quantum coherence
Hünig's base	*N,N*-diisopropylethylamine
IL	interleukin
IPTG	isopropyl-β-D-thiogalactopyranoside
IR	infrared
kb	kilobase pairs
LB	Luria Media
LC-MS	liquid chromatography - mass spectrometry
LR-MS	low resolution - mass spectrometry
m	multiplet (NMR), medium (IR)
MCS	multiple cloning site
MeCN	acetonitrile
MeOH	methanol
MOA	mode of action
mRNA	messenger RNA
MS	mass spectrometry
MTT	Thiazolyl Blue Tetrazolium Bromide [or (3-(4,5 Dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide]
$NaBH(OAc)_3$	sodium(triacetoxy)borohydride
NHS	*N*-hydroxysuccinimide
NMR	nuclear magnetic resonance
ODn	optical density at 'n' nanometres
PBS	phosphate buffered saline
PCR	polymerase chain reaction
PE	petroleum ether
PEG	poly(ethylene glycol)
PEG-n	poly(ethylene glycol) with average molecular weight of 'n'
P. aeruginosa	*Pseudomonas aeruginosa*
PWB	phage wash buffer (PBS + 0.05% Tween-20)
q	quartet (NMR)
RNA	ribonucleic acid
RP-HPLC	reverse phase – high performance liquid chromatography

rpm	revolutions per minute
RPS19	ribosomal protein S19
rRNA	ribosomal RNA
s	singlet (NMR), sharp (IR)
SAR	structure-activity relationship
S. aureus	*Staphylococcus aureus*
SCUBA	self contained underwater breathing apparatus
SDS	sodium dodecyl sulfate
sp.	species
spp	species (pl.)
ssDNA	single-stranded DNA
t	triplet (NMR)
Taq	*Thermus aquaticus*
TEA	triethylamine
TEG	tetra(ethylene glycol)
TFA	trifluoroacetic acid
THF	tetrahydrofuran
TLC	thin layer chromatography
TNF	tumour necrosis factor
TOCSY	totally correlated spectroscopy
Tris	tris(hydroxymethyl)aminomethane
UV	ultraviolet
UV-Vis	ultraviolet-visible
WHO	World Health Organisation
w	weak (IR, NMR)
λ_{max}	wavelength of maximal UV absorption
ν_{max}	frequency of maximal IR absorption

Declaration

I certify that the work in this thesis entitled “Natural Products Applied in Reverse Chemical Proteomics” has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree to any other university or institution other than Macquarie University.

I also certify that the thesis is an original piece of research and it has been written by me. Any help and assistance that I have received in my research work and the preparation of the thesis itself have been appropriately acknowledged.

In addition, I certify that all information sources and literature used are indicated in the thesis.

The research presented in this thesis was approved by Macquarie University Biosafety Review Committee, reference number: 5201001557 NLRD on the 8.12.2010.

Michael Gotsbacher
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