

**ISOLATION AND STRUCTURAL ELUCIDATION
OF
CYTOTOXIC AGENTS FROM MARINE
INVERTEBRATES AND PLANTS SOURCED
FROM
THE GREAT BARRIER REEF, AUSTRALIA**

Thesis submitted by

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for the degree of Doctor of Philosophy

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STATEMENT OF THE CONTRIBUTION OF OTHERS

I, the undersigned author of this work, acknowledge the contribution of others to this work. Substantial supervision was provided by Assoc. Prof. Bruce Bowden (School of Pharmacy and Molecular Sciences, JCU; primary supervision) and Dr Anna-Marie Babey (School of Biomedical Sciences, JCU). Editorial assistance in the preparation of this thesis was provided by Assoc. Prof. Bruce Bowden and Dr Anna-Marie Babey. Mr Rick Willis at the Australian Institute of Marine Sciences, Townsville, performed mass spectrometry (ESI-MS) on samples. Mr Paul Gugger from the Research School of Chemistry, ANU, Canberra, performed the optical rotation ($[\alpha]_D$) experiments.

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I, the undersigned and author of this work, declare that the research presented and reported in this thesis was conducted within the guidelines for research ethics outlined in the *James Cook University Policy on Experimentation Ethics, Standard Practices and Guidelines* (2001), and the *James Cook University Statement and Guidelines on Research Practice* (2001). Research involving the use of animals followed the *Australian Code of Practice for the Care and Use of Animals for Scientific Purposes* and the *Queensland Animal Care and Protection Act 2001*. The proposed research methodology for animal use received clearance from the James Cook University Experimentation Ethics Review Committee (approval number A618_00)

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**I DEDICATE THIS WORK TO MY MOTHER,
DR. MADHU. S. AGRAWAL**

ABSTRACT

The interest in the marine environment has been stimulated by the array of biological activities of marine natural products and hence their potential biomedical applications. The advent of high throughput screening has allowed a large number of compounds to be tested for a range of biological activities, in order to assess their potential as pharmaceuticals. This study aimed to discover drug leads from marine invertebrates collected from the Great Barrier Reef (GBR) by screening extracts for pharmacological activity.

The pharmacological target was aimed to find novel cytotoxic compounds with potential as anticancer agents. Cytotoxicity was assessed *in vitro* using the P388D1 mouse lymphoma cell line. Thus 308 samples of marine invertebrates and plants were collected from the central section of the Great Barrier Reef (GBR). Bioassay guided fractionation led to the isolation and structural elucidation of seven new and twenty known cytotoxic metabolites. Structural elucidation was done via 1D and 2D NMR spectroscopy.

Two new brominated polyether triterpenes, Armatols G (**193**) and H (**194**) were isolated from the red alga *Chondria armata*. Compounds (**193**) and (**194**) exhibited moderate cytotoxicity, with IC₅₀ values of 5.80 and 6.30 µg/ml. The relative stereochemistry throughout each molecule was determined via NOe experiments and by using Chem 3D and its MM2 energy minimization program to predict the lowest energy conformation. It is proposed that hydrolysis of the

acetate functionality and application of the Mosher's method for the resultant secondary alcohol should afford the absolute stereochemistry for compound **194**.

A new isomalabaricane triterpene, stelliferin D riboside (**195**) was isolated from the sponge *Rhabdastrella globostellata* along with the known isomalabaricanes, stelliferin A (**43**) and compound **51**. Stelliferin A (**43**) and stelliferin D riboside (**195**) exhibited IC₅₀ values of 0.16 and 1.10 µg/ml respectively.

Five furanoditerpenes were isolated from the sponge *Spongia sp.* Spongiadiol diacetate (**97**) was most cytotoxic with an IC₅₀ value of 2.10 µg/ml. This was followed by the new compound isospongiatriol (**196**) and the known compound epispongiatriol (**96**) which exhibited IC₅₀ values of 14.0 µg/ml and 16.30 µg/ml respectively. Spongiatriol triacetate (**99**) was inactive in the assay. Isospongiadiol (**109**) was not tested due to decomposition of the compound.

A new pentabrominated phenolic diphenyl ether (**197**) was isolated from the sponge *Dysidea herbacea*. Compound (**197**) exhibited a moderate IC₅₀ value of 2.20 µg/ml. All 4 positional isomers of diphenyl ethers that contain a 2,4-dibrominated B-ring and a 1-hydroxytribrominated A-ring with the ether linkage at the 2-position have now been reported from marine sponges.

A deaminated analogue of the known pyridoacridine alkaloid stellettamine (**188**) was isolated from the ascidian *Aplidium sp.* (cf *Aplidium cratiferum*). It was named nordehydrocyclodercitin (**200**). Cytotoxicity assays could not be

performed due to decomposition of the compound, but the cytotoxic activity of many pyridoacridine alkaloids is well documented

Two imidazole alkaloids, isonaamidine E (**225**) and its zinc complex, bis(isonaamidinato E)zinc (**408**) were isolated from a *Leucetta* sponge. The compound was assigned via spectroscopic methods. The electrospray results obtained for (**408**) were not readily interpreted in our hands, although molecular clusters that contained zinc and chlorine (from isotope patterns) were observed. The structure of **408** was verified by the addition of half an equivalent of ZnCl₂ to an isonaamidine E sample, which afforded a ¹H NMR spectrum that was identical to that observed for **408**.

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LIST OF ABBREVIATIONS

1D	one dimensional
2D	two dimensional
Aq	aqueous
br d	broad doublet
br s	broad singlet
C₆D₆	deuterated benzene
CDCl₃	deuterated chloroform
CH₂Cl₂	dichloromethane
COSY	Correlated Spectroscopy
d	doublet
dd	doublet of doublets
ddd	doublet of doublet of doublets
DEPT	Distortionless Enhancement by Polarisation Transfer
ESI-MS	Electrospray Ionisation Mass Spectrometry
EtOAc	ethyl acetate
EtOH	ethanol
FCS	foetal calf serum
HMBC	Heteronuclear Multiple-Bond Coherence
HSQC	Heteronuclear Single-Quantum Coherence
HPLC	High-performance Liquid Chromatography
i.d.	internal diameter
IR	Infrared
m	multiplet
MeOH	methanol
MeOH-d₄	deuterated methanol

m.p.	melting point
NCI	National Cancer Institute, Washington DC
NMR	Nuclear Magnetic Resonance
nOe	Nuclear Overhauser Effect
NOESY	Nuclear Overhauser Effect Spectroscopy
OD	optical density
PDA	photodiode-array
ROESY	Rotational Overhauser Effect Spectroscopy
S	singlet
sp.	species (singular)
spp.	species (plural)
SRB	sulforhodamine B
TCA	trichloroacetic acid
t	triplet
Tris	tris(hydroxymethyl)aminomethane
UV	Ultraviolet
Vis	Visible Light