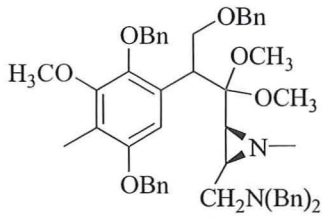
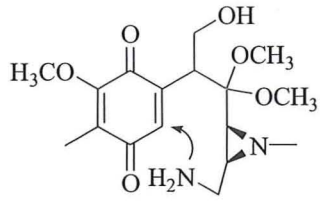
Corrections

p6 Scheme 1.3: cytosine should read cytidine, thymosine should read thymidine.

p15 Scheme 1.9



1.51



1.52

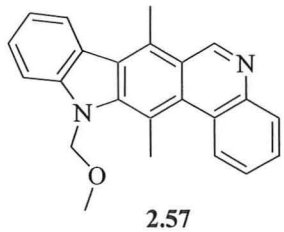
p24 line 4: Van der Waal

p38 line 5: …which can be carried <u>out</u> using Friedel Crafts….

p42 line 20: *N*-sulfonylindoles **<u>2.2</u>** and 2.46 respectively….

p46 line 4: …in <u>conjunction</u> with the acid chloride….

p51



2.57

p68 line 8: …<u>facilitate</u> a population shift….

p71 line 19: …the <u>yield</u> of the desired product was greatly improved

p71 line 21: …triphenylphosphine <u>were</u> utilized….

p74 line 4: …through a +R effect **[Figure 3.3]**.

p163 line 28: **Analytical** Anal. Calcd for <u>$C_{15}H_8N_2O_2$</u>: C, 72.58; H, 3.25; N, 11.28. Found: C, 71.95; H, 3.49; N, 11.25.

THE AUSTRALIAN NATIONAL UNIVERSITY

Synthesis and Structure-Activity Relationship Studies of the Calothrixins and Other Redox-Active Compounds

A thesis presented for the degree of

Doctor of Philosophy

of the

The Australian National University

By

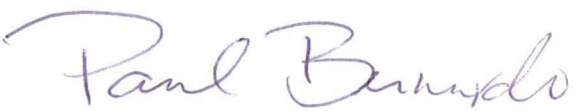
Paul Huntington Bernardo

Department of Chemistry
The Australian National University
Canberra, Australia

December 2004

Author's Statement

The work described in this thesis is original and has not been submitted for a degree or diploma at any other university. To my knowledge, it does not contain material previously published or presented by any other person except where otherwise acknowledged. The work presented is my own.



Paul H. Bernardo

10 December 2004

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Abstract

Calothrixin A and B are novel pentacyclic quinones that have been isolated from the *Calothrix* cyanobacteria. The calothrixins exhibit antiproliferative activity against chloroquine resistant strains of *Plasmodium falciparum*, the deadliest causative agent of malaria. Furthermore, these compounds have potent and selective activity against a range of human cancer cell lines. These biological properties have led to the identification of the calothrixins as lead compounds for drug development. A brief review of the calothrixins and other quinones, as well as an overview of Structure-Activity (SAR) and Quantitative SAR studies is presented in Chapter 1.

This thesis details the methodology for the synthesis of the calothrixins and a number of related carbazolediones. A *de novo* synthesis of the quinones was carried out using a combination of metallation and Friedel-Crafts acylation approaches. Calothrixin derivatives were also synthesized for Structure-Activity Relationship (SAR) studies in order to determine the pharmacophore required for biological activity. These synthetic studies are described in Chapter 2.

A combinatorial approach to the synthesis of calothrixin B was also attempted as described in Chapter 3. The strategy utilizes phenanthridine-7,10-dione as a template for further elaboration to calothrixin B. In principle, this synthetic route can be employed in the synthesis of a large number of calothrixin B analogues and/or derivatives.

Chapter 4 describes the SAR and QSAR studies of the calothrixins and related carbazolediones. The antiproliferative activity of the calothrixins and related carbazolediones was measured against HeLa cervical cancer cells using the colorimetric MTT assay. The results obtained in the SAR studies of the carbazolediones were used to elucidate the pharmacophore required for biological activity. QSAR studies were also carried out in order to determine the physicochemical properties of these carbazolediones that are required for biological activity. In particular, the reduction potentials as well as lipophilicity of the carbazolediones were measured and compared to the antiproliferative activities.

The ability of calothrixin A to undergo one-electron reduction to the semiquinone radical anion led to the hypothesis that calothrixin A may accumulate in cells. This mechanism of redox-driven accumulation of compounds was first observed in our SAR and QSAR studies of the epidithiopiperazine-2,5-diones (ETP compounds). These studies have led to the proposal that there is a relationship between the reduction potential of ETP compounds and the intracellular concentration of these toxins leading to redox-driven biological accumulation of the ETP compounds. To determine if the cellular accumulation of calothrixin A is driven by a similar process, uptake studies were carried out. This work is described in detail in Chapter 5.

The details of the procedures for experiments carried out in Chapters 2 to 5 are given in Chapter 6, along with the relevant data used to characterize the synthetic compounds.

List of Publications and Presentations

Some of the work reported here has been published in the literature, or presented as a poster at a conference.

Publications

Bernardo, P. H., C. L. L. Chai, et al. (2002). "A simple and concise route to calothrixin B." Tetrahedron Lett. **43**: 2939-2940.

Bernardo, P. H., N. Brasch, et al. (2003). "A Novel Mechanism for the Gluatathione Dependent Reversible Uptake of a Fungal Toxin in Cells." J. Biol. Chem. **278**(47): 46549-46555.

Bernardo, P. H. and C. L. L. Chai (2003). "Friedel-Crafts Acylation and Metalation Strategies in the Synthesis of Calothrixins A and b." J. Org. Chem **68**: 8906-8909.

Bernardo, P. H., C. L. L. Chai, et al. (2004). "Synthesis, Electrochemistry, and Bioactivity of the Cyanobacterial Calothrixins and Related Quinones." J. Med. Chem. **47**: 4958-4963.

Posters

"Chemical and Biological Properties of 3,6-Epidithiopiperazine-2,5-diones." Presented at the World Chemistry Congress, Brisbane, Australia 2001.

"Synthesis of Calothrixins and Related Analogues." Presented at the 14th International Conference on Organic Synthesis, Christchurch, New Zealand 2002.

Table of Contents

Chapter 5

Chapter 6

Appendices

Abbreviations

Å	angstrom units
Anal.	Analysis
Aq.	aqueous
n-BuLi	*normal*-butyl lithium
s-BuLi	*secondary*-butyl lithium
t-BuLi	*tertiary*-butyl lithium
°C	degrees centigrade
Calcd	calculated
CAN	cerium (IV) ammonium nitrate
^{13}C-NMR	carbon-13 Nuclear Magnetic Resonance spectroscopy
$CDCl_3$	deuterated chloroform
cm^{-1}	wavenumber, per centimeter
m-CPBA	*meta*-chloroperbenzoic acid
CySH	L-cysteine
CySSCy	cystine
DCM	dichloromethane, CH_2Cl_2
DMD	double-mixed disulfide
DMF	*N,N*-dimethylformamide
d-DMSO	deuterated dimethylsulfoxide, $(CD_3)_2SO$
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acids
DTT	dithiothreitol
EC_{50}	Effective Concentration to inhibit cell proliferation by 50%
EDTA	ethylenediaminetetraacetic acid
EIMS	Electron Impact Mass Spectrometry
F	Faraday's constant
FMO	Frontier Molecular Orbital
FTIR	Fourier-Transform Infrared spectroscopy
GSH	glutathione
GSSG	oxidized glutathione
h	hour(s)
^{1}H-NMR	proton Nuclear Magnetic Resonance spectroscopy
HMBC	Heteronuclear Multiple Bond Correlation

HMQC	Heteronulcear Multiple Quantum Correlation
HOMO	Highest Occupied Molecular Orbital
HPLC	High Performance Liquid Chromatography
HRMS	High-resolution mass spectrometry
Hz	frequency in Hertz
IR	infrared spectrum
L	litres
LHMDS	lithium hexamethyldisilazide
LDA	lithium diisopropylamide
LiTMP	lithium 2,2,6,6-tetramethylpiperazide
ln	natural logarithm
log	logarithm, base-10
LUMO	Lowest Unoccupied Molecular Orbital
M	molarity, mol/L
min	minute(s)
MMD	mono-mixed disulfide
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide
m/z	mass to charge ratio
mol	moles
NaH	sodium hydride
MeMgCl	methyl magnesium chloride
MOMCl	methoxymethyl chloride
MP	melting point
OAc	acetate
PBS	phosphate-buffered saline
P(*o*-tolyl)$_3$	tris(*ortho*-tolyl)phosphine
PPh_3	triphenylphosphine
ppm	part per million
QSAR	Quantitative Structure-Activity Relationship
R	universal gas constant
R_f	retardation factor
RNA	ribonucleic acids
ROS	reactive oxygen species
SAR	Structure-Activity Relationship

T	temperature, in Kelvin (K) units for thermodynamic equations
TLC	thin-layer chromatography
THF	tetrahydrofuran
TMEDA	*N,N,N'N'*-tetramethylethylenediamine
TMS	tetramethylsilane
UV Vis	Ultraviolet and Visible wavelength spectroscopy
V	Volts