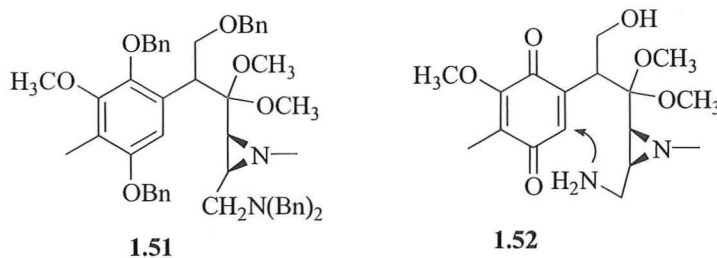


## Corrections

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

p15 Scheme 1.9



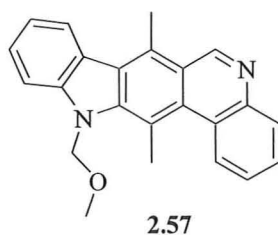
p24 line 4: Van der Waal

p38 line 5: ...which can be carried out using Friedel Crafts....

p42 line 20: *N*-sulfonylindoles **2.2** and 2.46 respectively....

p46 line 4: ...in conjunction with the acid chloride....

p51



p68 line 8: ...facilitate a population shift....

p71 line 19: ...the yield of the desired product was greatly improved

p71 line 21: ...triphenylphosphine were utilized....

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

p163 line 28: **Analytical** Anal. Calcd for C<sub>15</sub>H<sub>8</sub>N<sub>2</sub>O<sub>2</sub>: C, 72.58; H, 3.25; N, 11.28. Found: C, 71.95; H, 3.49; N, 11.25.



THE AUSTRALIAN NATIONAL UNIVERSITY

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**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**

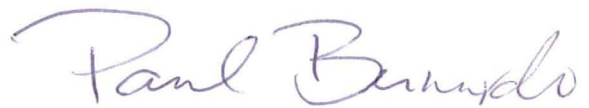
**Canberra, Australia**

**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

## Acknowledgements

I would like to thank all the wonderful people who have made this work possible and have made valuable contributions to my research. First and foremost, I would like to thank my father, Dr. Vicente S. Bernardo, for all the support he has given me these years. A doctor in the family is a powerful influence, and I would like to acknowledge the paramount role my father has in my choice of research.

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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 carbazolidiones. 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 carbazolidiones. The antiproliferative activity of the calothrixins and related carbazolidiones was measured against HeLa cervical cancer cells using the colorimetric MTT assay. The results obtained in the SAR studies of the carbazolidiones 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 carbazolidiones that are required for biological activity. In particular, the reduction potentials as well as lipophilicity of the carbazolidiones 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 Glutathione 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 14<sup>th</sup> International Conference on Organic Synthesis, Christchurch, New Zealand 2002.

# Table of Contents

|                               |      |
|-------------------------------|------|
| Title page.....               | i    |
| Author's Statement .....      | ii   |
| Acknowledgements.....         | iii  |
| Abstract .....                | v    |
| Publications and Poster ..... | vii  |
| Table of Contents.....        | viii |
| Abbreviations .....           | xii  |

## Chapter 1

|                                                                                                  |    |
|--------------------------------------------------------------------------------------------------|----|
| 1.1 Background.....                                                                              | 1  |
| 1.2 Biological Modes of Action of the Quinones.....                                              | 4  |
| 1.3 The Calothrixins and Related Alkaloids .....                                                 | 8  |
| 1.4 Literature Syntheses of Quinones and Carbazolediones.....                                    | 12 |
| 1.5 Structure-Activity Relationship (SAR) Studies.....                                           | 18 |
| 1.6 Physicochemical Parameters and Quantitative Structure-Activity Relationship<br>Studies ..... | 21 |
| 1.7 Project Aims and Objectives .....                                                            | 26 |
| 1.8 Bibliography .....                                                                           | 27 |

## Chapter 2

|                                                                             |    |
|-----------------------------------------------------------------------------|----|
| 2.1 Introduction .....                                                      | 32 |
| 2.2 Synthesis of Quinoline-3,4-anhydride .....                              | 38 |
| 2.3 Investigation of Methods for the Acylation of Indole.....               | 41 |
| 2.3.1 Friedel-Crafts Acylation of N-Protected Indoles.....                  | 42 |
| 2.3.2 Friedel-Crafts Acylation of Unprotected Indole.....                   | 43 |
| 2.3.3 Model Studies in the Metallation of the Indolic C3-Position.....      | 44 |
| 2.3.4 <i>N</i> -Metallation of Indole and Coupling to Acid Chlorides.....   | 46 |
| 2.4 <i>N</i> -Protection of the Aryl Ketone and Cyclization.....            | 46 |
| 2.5 Removal of the <i>N</i> -Methoxymethyl Group.....                       | 49 |
| 2.6 Synthesis of Calothrixin Derivatives and Carbazoledione Analogues ..... | 50 |
| 2.6.1 Synthesis of N12-Substituted Calothrixin B .....                      | 51 |

|                                                                           |    |
|---------------------------------------------------------------------------|----|
| 2.6.2 Synthesis of 9-Methoxy- <i>N</i> -methoxymethyl Calothrixin B ..... | 53 |
| 2.7 Conclusion .....                                                      | 57 |
| 2.8 Bibliography .....                                                    | 59 |

### Chapter 3

|                                                                                                             |    |
|-------------------------------------------------------------------------------------------------------------|----|
| 3.1 Introduction .....                                                                                      | 61 |
| 3.2 Synthesis of the Phenanthridine-7,10-dione Template .....                                               | 66 |
| 3.3 Model Studies on the Reactivity of the Phenanthridine-7,10-dione Template<br>and Related Quinones ..... | 73 |
| 3.4 Synthesis of the 8-Bromophenanthridine-7,10-dione Template .....                                        | 80 |
| 3.5 Conclusion .....                                                                                        | 83 |
| 3.6 Bibliography .....                                                                                      | 84 |

### Chapter 4

|                                                                                                                        |     |
|------------------------------------------------------------------------------------------------------------------------|-----|
| 4.1 Introduction .....                                                                                                 | 85  |
| 4.1.1 The Role of Redox Potentials in the Biological Activity of Quinones .....                                        | 85  |
| 4.1.2 The Role of Steric Parameters in the Biological Activity of Quinones .....                                       | 86  |
| 4.1.3 The Role of Lipophilicity in the Biological Activity of Quinones .....                                           | 87  |
| 4.2 Antiproliferative Activity of the Calothrixins and Related Quinones Against<br>HeLa Cells .....                    | 89  |
| 4.2.1 Antiproliferative Properties of Calothrixin B and Related Carbazolediones .....                                  | 91  |
| 4.2.2 Antiproliferative Activity of Calothrixin B Derivatives .....                                                    | 92  |
| 4.3 Electrochemistry of the Carbazolediones and Correlation of Electrochemical<br>Results to Biological Activity ..... | 96  |
| 4.3.1 Comparison of $E_{1/2}^I$ Values of the Calothrixins and Related<br>Carbazolediones .....                        | 100 |
| 4.3.2 Comparison of $E_{1/2}^{II}$ Values of the Calothrixins and Related<br>Carbazolediones .....                     | 104 |
| 4.3.3 Comparison of $\ln K$ Values of the Calothrixins and Related<br>Carbazolediones .....                            | 105 |
| 4.3.4 Comparison of the Overall Reduction Potential of the Calothrixins<br>and Related Carbazolediones .....           | 107 |
| 4.4 Lipophilicity and Correlation of LogP Values to Biological Activity .....                                          | 109 |
| 4.5 Conclusion .....                                                                                                   | 115 |

## Chapter 5

|                                                                                            |     |
|--------------------------------------------------------------------------------------------|-----|
| 5.1 Introduction .....                                                                     | 120 |
| 5.2 Measurement of the Lipophilicities of the ETP Compounds .....                          | 123 |
| 5.3 Measurement of ETP Reduction Potentials via Thiol-Disulfide Exchange<br>Reactions..... | 125 |
| 5.4 Biological Accumulation of Calothrixin A .....                                         | 130 |
| 5.5 Conclusions .....                                                                      | 133 |
| 5.6 Bibliography .....                                                                     | 135 |

## Chapter 6

|                                                                                                               |     |
|---------------------------------------------------------------------------------------------------------------|-----|
| 6.1 General Experimental.....                                                                                 | 137 |
| 6.2 Experimental Procedures and Data for Chapter 2 .....                                                      | 139 |
| 6.2.1 Procedures for the Synthesis of Quinoline-3,4-anhydride .....                                           | 139 |
| 6.2.2 General Procedure for the Methanolysis of Anhydrides.....                                               | 140 |
| 6.2.3 Procedure for the Preparation of Indole Derivatives .....                                               | 142 |
| 6.2.4 General Procedure for the Preparation of Acid Chlorides .....                                           | 143 |
| 6.2.5 Procedures for the Coupling of Indole Derivatives to Acid Chlorides .....                               | 144 |
| 6.2.6 General Procedure for the <i>N</i> -MOM Protection of the Indole Derivatives....                        | 150 |
| 6.2.8 General Lithiation of <i>N</i> -MOM Protected Quinone Precursors .....                                  | 155 |
| 6.2.9 Procedures for the Synthesis of <i>N</i> -Unsubstituted Carbazolediones .....                           | 158 |
| 6.2.10 Synthesis of Calothrixin A .....                                                                       | 164 |
| 6.2.11 Preparation of <i>N</i> -Substituted Calothrixins from Calothrixin B.....                              | 164 |
| 6.2.12 Deuterium Labeling Studies of Metallated Indoles .....                                                 | 168 |
| 6.3 Experimental Procedures and Data for Chapter 3 .....                                                      | 170 |
| 6.3.1 Procedures for the Synthesis of 3-Bromo-2, 5-Dimethoxybenzaldehyde....                                  | 170 |
| 6.3.2 Procedures for the Synthesis of 2,5-Dimethoxybenzoic Acid and<br>3-Bromo-2,5-dimethoxybenzoic Acid..... | 171 |
| 6.3.3 Preparation of Secondary Benzamides .....                                                               | 172 |
| 6.3.4 Preparation of Tertiary Benzamides .....                                                                | 174 |
| 6.3.5 Palladium-Mediated Cyclization of Tertiary Benzamides .....                                             | 176 |
| 6.3.6 Preparation of 7,10-Dimethoxyphenanthridine .....                                                       | 177 |
| 6.3.7 Preparation of Phenanthridine-7,10-dione .....                                                          | 178 |

|                                                                                                                                           |     |
|-------------------------------------------------------------------------------------------------------------------------------------------|-----|
| 6.3.8 Preparation of Anilinophenanthridine-7,10-dione and Palladium-Catalyzed<br>Cyclization to Calothrixin B and Iso-calothrixin B ..... | 179 |
| 6.4 Experimental Procedures and Data for Chapter 4 .....                                                                                  | 181 |
| 6.4.1 Lipophilicity Measurements via High Performance Liquid<br>Chromatography.....                                                       | 181 |
| 6.4.2 Experimental Protocols for Biological Experiments .....                                                                             | 181 |
| 6.5 Experimental Procedures for Chapter 5 .....                                                                                           | 183 |
| 6.5.1 Thiol-Disulfide Exchange Studies for the ETP Compounds via HPLC .....                                                               | 183 |
| 6.5.2 Uptake Studies of Calothrixin A Measured by HPLC.....                                                                               | 186 |

## Appendices

|                                                                               |     |
|-------------------------------------------------------------------------------|-----|
| Appendix A: QSAR Output from SigmaPlot.....                                   | 189 |
| Appendix B: Selected NMR Spectra of Synthetic Intermediates and Products..... | 195 |

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## Abbreviations

|                     |                                                                  |
|---------------------|------------------------------------------------------------------|
| Å                   | angstrom units                                                   |
| Anal.               | Analysis                                                         |
| Aq.                 | aqueous                                                          |
| <i>n</i> -BuLi      | <i>normal</i> -butyl lithium                                     |
| <i>s</i> -BuLi      | <i>secondary</i> -butyl lithium                                  |
| <i>t</i> -BuLi      | <i>tertiary</i> -butyl lithium                                   |
| °C                  | degrees centigrade                                               |
| Calcd               | calculated                                                       |
| CAN                 | cerium (IV) ammonium nitrate                                     |
| <sup>13</sup> C-NMR | carbon-13 Nuclear Magnetic Resonance spectroscopy                |
| CDCl <sub>3</sub>   | deuterated chloroform                                            |
| cm <sup>-1</sup>    | wavenumber, per centimeter                                       |
| <i>m</i> -CPBA      | <i>meta</i> -chloroperbenzoic acid                               |
| CySH                | L-cysteine                                                       |
| CySSCy              | cystine                                                          |
| DCM                 | dichloromethane, CH <sub>2</sub> Cl <sub>2</sub>                 |
| DMD                 | double-mixed disulfide                                           |
| DMF                 | <i>N,N</i> -dimethylformamide                                    |
| <i>d</i> -DMSO      | deuterated dimethylsulfoxide, (CD <sub>3</sub> ) <sub>2</sub> SO |
| DMSO                | dimethyl sulfoxide                                               |
| DNA                 | deoxyribonucleic acids                                           |
| DTT                 | dithiothreitol                                                   |
| EC <sub>50</sub>    | 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)                                                          |
| <sup>1</sup> H-NMR  | proton Nuclear Magnetic Resonance spectroscopy                   |
| HMBC                | Heteronuclear Multiple Bond Correlation                          |

---

|                                  |                                                                           |
|----------------------------------|---------------------------------------------------------------------------|
| HMQC                             | Heteronuclear 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 <i>H</i> -tetrazolium bromide |
| <i>m/z</i>                       | 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( <i>o</i> -tolyl) <sub>3</sub> | tris( <i>ortho</i> -tolyl)phosphine                                       |
| PPh <sub>3</sub>                 | triphenylphosphine                                                        |
| ppm                              | part per million                                                          |
| QSAR                             | Quantitative Structure-Activity Relationship                              |
| R                                | universal gas constant                                                    |
| R <sub>f</sub>                   | retardation factor                                                        |
| RNA                              | ribonucleic acids                                                         |
| ROS                              | reactive oxygen species                                                   |
| SAR                              | Structure-Activity Relationship                                           |

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