# Exploitation of the Protein Tubulin For Controlling African Trypanosomiasis

**Natalie Lydia Giles (BSc.)**

**This thesis is presented for the Degree of Doctor of Philosophy of the**

**Division of Health Sciences, Murdoch University, July 2005.**

I declare that this thesis is my own account of my research and contains as its main content work which has not been submitted for a degree at any tertiary education institution.

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Natalie Lydia Giles

## ACKNOWLEDGEMENTS

To my supervisors, Dr Simon Reid and Dr Anthony Armson. Firstly, thank you for the opportunity to do my PhD. Secondly, thank you for your enthusiasm, ideas, and support. In particular, thank you Simon for your technical assistance and critical feedback in the completion of this thesis. I am indebted to you.

To Dr Vanessa Cull, thank you for encouraging me to do a PhD. If you didn't think I could do it, I wouldn't be at this point now.

Thank you to the Parasitology group. I consider myself very lucky to have been part of such a fabulous group of individuals. In particular, thank you to Carolyn, Ryan, Michael, Linda, Marion, Bong, Tanya, Louise and "wanna-be Parasites" Emmalene, Kim, Meredith and Susannah who have all been with me since day one. I would not have made it through each day without the comic relief, fascinating lunchtime conversations about TV and Hollywood gossip and most importantly, the friendship you all provided me with. To ex-parasites Andrew and Louisa for staying in touch with me after you moved on and for assisting me with every stupid question about drug-binding kinetics I asked. Thank you also to Professor Jim Reynoldson, for your wisdom in drug studies.

A big thank you to Dr Derrick Robinson, for your expertise in electron microscopy and immunofluorescence.

To my cheer squad Mum, Ivana and Mariana. Thank you for your never-ending love and support and for pretending to know what I was on about when I complained about my project.

To my husband, Murray. Thank you for your infinite support, encouragement and belief in me. I could never have done this without you.

## ABSTRACT

This thesis presents the results of an investigation into the structural protein, tubulin, as a potential target for anti-trypanosomatid drug discovery and vaccine development. Recombinant α- and β- tubulin proteins from *Trypanosoma brucei rhodesiense* were expressed as soluble fusion proteins in an *E. coli* expression system. The recombinant α- and β- tubulins were used to determine the nature of binding of novel trifluralin analogues EPL-AJ 1003, 1007, 1008, 1016 and 1017. Native tubulin from rats was used to determine the extent of binding to mammalian tubulin. The results of this study clearly demonstrate two important aspects of the binding of trifluralins to tubulin. Firstly, they have specific affinity for trypanosomal tubulin compared with mammalian regardless of the chemical composition of the trifluralin analogue tested. Secondly, they have a demonstrably stronger affinity for α-tubulin compared with β-tubulin. In addition, compounds 1007, 1008, 1016 and 1017 have strong binding affinities for α-tubulin, with limited binding affinity for mammalian tubulin, which indicates that these compounds selectively bind to trypanosomal tubulin.

The morphology of bloodstream forms of *T. b. rhodesiense* exposed to trifluralin analogues was studied using electron microscopy and immunofluorescence to determine the ultrastructural changes these compounds induce as a result of binding to tubulin. All compounds tested induced severe irreparable damage in *T. b. rhodesiense*, including perturbation of subpellicular microtubules, extensive cytoplasmic swellings, axoneme and paraflagellar rod malformation, disconfiguration around the flagellar pocket and membrane disintegration. These results suggest that the mechanism of action of these trifluralin analogues is through the disruption of polymerization of tubulin into microtubules as a result of binding to α-tubulin.

The potential for recombinant trypanosomal tubulins to be used as vaccine candidates was assessed by monitoring parasitaemia and length of survival of mice immunised with the proteins and challenged with a lethal infection of *T. b. rhodesiense*. Although all the mice vaccinated with recombinant tubulin developed a patent parasitaemia and did not survive, they were partially protected because their patency period and length of survival were significantly greater than the control groups. Furthermore, plasma collected from mice immunised with recombinant trypanosomal tubulin contained antibodies that recognized tubulin in a soluble extraction from *T. b. rhodesiense*. The results of this thesis confirm the potential for the structural protein, tubulin, to be used as a target for anti-trypanosomatid drug discovery and vaccine development.

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## ABBREVIATIONS

| | |
|---|---|
| < | less than |
| > | greater than |
| % | percent |
| $^{o}C$ | degrees celcius |
| ABZ | albendazole |
| ATP | adenosine triphosphate |
| b.w. | body weight |
| BZ | benzimidazole |
| $CaCl_2$ | calcium chloride |
| CNS | central nervous system |
| CSF | cerebrospinal fluid |
| DAPI | 4'6-diamidino-2-phenylindole |
| DB | depolymerisation buffer |
| $ddH_2O$ | double deionised water |
| DMSO | dimethyl sulphoxide |
| DNA | deoxyribonucleic acid |
| dNTP | deoxynucleotide triphosphates |
| EDTA | ethylenediaminetetraacetic acid |
| EGTA | ethylene glycol-bis(β-aminoethyl ether) N,N,N',N'-tetraacetic acid |
| EM | electron microscopy |
| *et al* | and other people |
| f | femto ($10^{-15}$x) |
| fmol | femtomoles |
| g | gram |
| *g* | unit of gravitational field |
| GST | glutathione s-transferase |
| GTP | guanosine triphosphate |
| HCl | hydrochloric acid |
| hr | hour |
| $IC_{50}$ | concentration required to cause 50% inhibition |
| $IC_{70}$ | concentration required to cause 70% inhibition |
| IgG | immunoglobulin G |
| i.m. | intra-muscular |
| i.p. | intra-peritoneal |
| IPTG | isopropyl-beta-D-thiogalactopyranoside |

| | |
|---|---|
| i.v. | intra-venous |
| $K_a$ | affinity constant |
| $k_{off}$ | dissociation constant |
| $k_{on}$ | association rate constant |
| KCl | potassium chloride |
| kDa | kilodalton |
| $KH_2PO_4$ | potassium dihydrogen orthophosphate |
| L | litre |
| LB | Luria-Bertani (broth) |
| μ(prefix) | micro ($10^{-6}$x) |
| m(prefix) | milli ($10^{-3}$x) |
| M | moles |
| μM | micromoles |
| mM | millimoles |
| MAP | microtubule-associated protein |
| MBP | maltose binding protein |
| MBS | MES buffered saline |
| MES | 2-[N-morpho-lino]ethanesulphonic acid |
| $MgCl_2$ | magnesium chloride |
| min | minute |
| M.W | molecular weight |
| n | nano ($10^{-9}$x) |
| NaCl | sodium chloride |
| $Na_2HPO_4$ | di-sodium hydrogen orthophosphate |
| $Na_2PO_4$ | sodium phosphate |
| OD | optical density |
| p | pico ($10^{-12}$x) |
| PBS | phosphate buffered saline |
| PCR | polymerase chain reaction |
| pH | minus log of the hydrogen ion concentration |
| p*I* | isolectric point |
| PIPES | Piperazine-1,4-bis(2-ethanesulfonic acid) |
| pmol | picomoles |
| PMSF | phenylmethylsulfonyl fluoride |
| PVDF | polyvinylidene diflouride |
| s.c. | subcutaneous |
| SD | standard deviation |

| | |
|---|---|
| SDS | sodium dodecyl sulphate |
| SDS-PAGE | sodium dodecyl sulphate-polyacrylamide gel electrophoresis |
| sec | second |
| SEM | standard error of the mean |
| *sp*. | Species |
| TEM | transmission electron microscopy |
| Tris-HCl | tris(hydroxymethyl)aminomethane |
| U | unit |
| V | volts |
| w/v | weight to volume ratio |

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### Chapter 1



### Chapter 2

**Chapter 3**

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## PRESENTATIONS

### Oral Presentations

"Exploitation of the Protein Tubulin for Controlling Human African Trypanosomiasis."
Inaugural Australian Society for Medical Research (ASMR) Symposium for Medical Research Week.
Queen Elizabeth II Medical Centre, Western Australia
June, 2005

"Exploitation of the Protein Tubulin for Controlling Trypanosomiasis."
Postgraduate Seminar Programme
Division of Health Sciences, Murdoch University
May, 2005

"Characterisation of Dinitroaniline Binding to Tubulin from *Trypanosoma brucei*."
46$^{th}$ Annual Scientific Meeting of the Australian Society for Parasitology Inc.
Fremantle, Western Australia
September, 2004

"Expression of Recombinant Tubulin from *Trypanosoma brucei* Using Fusion Partners."
Inaugural Australian Society for Medical Research (ASMR) Symposium for Medical Research Week.
Queen Elizabeth II Medical Centre, Western Australia
June, 2003

"Exploitation of a Novel Drug Target for the Treatment of Trypanosomiasis."
Postgraduate Seminar Programme
Division of Health Sciences, Murdoch University
November, 2002

**Poster Presentations**

"The Binding of New Trifluralin Analogues to Trypanosomal Tubulin."
Awarded "Best Poster in Drug Discovery or Diagnostics"
Postgraduate Poster Day
Division of Health Science, Murdoch University
November, 2004

"Characterisation of Dinitroaniline Binding to Tubulin from *Trypanosoma brucei.*"
IX European Multicolloquium of Parasitology (EMOP IX)
Valencia, Spain
July, 2004

"Expression of *Trypanosoma brucei rhodesiense* α-tubulin as a Soluble Protein in *Escherichia coli*."
Postgraduate Poster Day
Division of Health Sciences. Murdoch University
November, 2003
and
45th Annual Scientific Meeting of the Australian Society for Parasitology Inc.
Darwin, Northern Territory
July, 2003

"Exploitation of a Novel Drug Target for the Treatment of Trypanosomiasis."
Awarded "Best First Year Introductory Poster" and "The Dean's Prize for Overall Best Poster"
Postgraduate Poster Day
Division of Veterinary and Biomedical Sciences. Murdoch University
November, 2002