
**Exploitation of the Protein Tubulin
For Controlling
African Trypanosomiasis**

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**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

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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
°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 ₂ O	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
<i>et al</i>	and other people
f	femto (10 ⁻¹⁵ x)
fmol	femtomoles
g	gram
<i>g</i>	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
μ M	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
pI	isoelectric point
PIPES	Piperazine-1,4-bis(2-ethanesulfonic acid)
pmol	picomoles
PMSF	phenylmethylsulfonyl fluoride
PVDF	polyvinylidene difluoride
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
<i>sp.</i>	Species
TEM	transmission electron microscopy
Tris-HCl	tris(hydroxymethyl)aminomethane
U	unit
V	volts
w/v	weight to volume ratio

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PRESENTATIONS

Oral Presentations

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