

**Characterisation of the benzimidazole-binding
site on the cytoskeletal protein tubulin**

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Declaration

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

.....
(Louisa Mary MacDonald)

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This thesis is dedicated to my parents

Dr Mary MacDonald

and the memory of

Dr Robert Ewen Munn MacDonald

1930 – 2001

Abstract

The binding kinetics of several benzimidazole compounds were determined with recombinant tubulin monomers and heterodimers from benzimidazole-sensitive and -insensitive organisms. This study utilised the naturally occurring high efficacy of the benzimidazoles for the parasitic protozoa *Giardia duodenalis* and *Encephalitozoon intestinalis*. The benzimidazoles are not active against the protozoan *Cryptosporidium parvum* or mammalian hosts, including humans. The affinity of several benzimidazole derivatives for monomeric and heterodimeric β -tubulin was clearly demonstrated, thus supporting previous studies of drug-resistant nematode and fungal populations. A homology model of protozoan $\alpha\beta$ -tubulin, produced using the three-dimensional structure of mammalian $\alpha\beta$ -tubulin, identified a strongly hydrophobic domain only on the β -tubulin protein of sensitive protozoa. This domain is proposed to be the benzimidazole-binding domain and the amino acid residues within it include three key residues which are substituted between benzimidazole-sensitive and -insensitive organisms. These residues are Ile-189, Val-199, and Phe-200 that all have non-polar, hydrophobic side groups and are proposed to bind with the R₅ side chain of several benzimidazole derivatives. In addition to this, the benzimidazole derivatives were able to bind irreversibly with assembling microtubules from sensitive parasites. The incorporation of benzimidazole-bound $\alpha\beta$ -heterodimers into assembling microtubules was shown to arrest polymerisation *in vitro* although the addition of benzimidazole compounds to assembled microtubules did not result in depolymerisation. Taken together, these results suggest that the mechanism of action of these compounds is through disruption of the dynamic equilibrium that balances the cycle of microtubule polymerisation and disintegration within these protozoa. Further, this effect is brought about by preferential binding of the benzimidazoles to a hydrophobic region on the β -tubulin protein.

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Abbreviations

2-D	two-dimensional
3-D	three-dimensional
A	adenine
ABZ	albendazole; methyl [5-(proylthio)-1 <i>H</i> -benzimidazole-2-yl] carbamate
AGE	agarose gel electrophoresis
ATP	adenosine triphosphate
BIA	biomolecular interaction assay
C	cytosine
CCT	chaperonin containing T-complex polypeptide-1
c-cpn	cytosolic chaperonin
CMD	carboxymethyl dextran
CTAB	cetyl trimethyl ammonium bromide
Da	Dalton
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
E-site	exchangeable site (α -tubulin)
EDC	<i>N</i> -ethyl- <i>N'</i> -(3-diethyl-aminopropyl)-carbodiimide
EDTA	ethylene diamine tetraacetic acid
EGTA	ethylene glycol-bis-(β -aminoethyl ether)- <i>N</i> , <i>N</i> , <i>N'</i> , <i>N'</i> -tetraacetic acid
FBZ	fenbendazole
FC	flow cell
Fts	filament temperature sensitivity
G	guanine
GDP	guanosine 5'-diphosphate
GST	glutathione S-transferase
GTP	guanosine 5'-triphosphate

HBS	HEPES buffered saline
HEPES	4-(2-hydroxyethyl)-1-piperazine ethanesulphonic acid
HIV	human immunodeficiency virus
IPTG	isopropyl- β - <i>D</i> -thiogalactopyranoside
kDa	kiloDalton
k_m	mass transfer coefficient
k_{on}	association rate (on-rate)
k_{off}	dissociation rate (off-rate)
K_a	equilibrium association constant
K_d	equilibrium dissociation constant
LB	Luria-Bertani (broth)
M-loop	microtubule loop
MAP	microtubule-associated protein
MBP	maltose-binding protein
MBS	MES buffered saline
MBZ	mebendazole; methyl 5-benzoyl-2-benzimidazolecarbamate
MES	2-(<i>N</i> -morpholino) ethanesulphonic acid
MTOC	microtubule-organising centre
N-site	nonexchangeable site (β -tubulin)
NH	nonpolar hydrophobic (amino acid)
NHS	<i>N</i> -hydroxysuccinimide
Ni-NTA	nickel nitriloacetic acid
OBZ	oxibendazole; methyl 5- <i>N</i> -propoxy-2-benzimidazolecarbamate
OD	optical density
PBZ	parbendazole; methyl 5-butyl-2-benzimidazolecarbamate
PCR	polymerase chain reaction
PDB	Protein Data Bank
PEG	polyethylene glycol

pI	isoelectric point
Pipes	piperazine- <i>N,N'</i> -bis(2-ethanesulphonic acid)
PMSF	phenylmethylsulphonyl fluoride
poly-His	poly-histidine
PU	polar uncharged (amino acid)
PVDF	polyvinylidene difluoride
RNA	ribonucleic acid
RT-PCR	reverse-transcriptase polymerase chain reaction
RU	resonance units
SC	sensor chip
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SPR	surface plasmon resonance
T	thymine
TBZ	thiabendazole; 2-(4-thiazolyl)-1 <i>H</i> -benzimidazole
TCP-1	T-complex polypeptide-1
TRiC	T-complex polypeptide-1 ring complex
tRNA	transfer ribonucleic acid
TRX	thioredoxin
U	uracil
w/w	weight to weight ratio
w/v	weight to volume ratio