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STUDIES ON THE MODE OF ACTION OF THE ANTITUMOUR ACRIDINE 4'-(9-ACRIDINYLAMINO)METHANESULPHON-\textit{m-}ANISIDIDE (\textit{m-}AMSA)

A thesis submitted to the University of Auckland for the degree of Doctor of Philosophy

by

William R. Wilson

Department of Cell Biology
University of Auckland

March, 1978
"Too much trust should not be put in an experiment done with the object of getting information."

W.D. Bancroft (1928)
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PUBLICATIONS
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To Lynda, who tried to ignore the whole performance and almost succeeded, and to my children who waited patiently through the long days of thesis preparation, I give my love. I acknowledge that they and my close friends have shared in the costs, but not in the rewards, of experimental science.

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(1) The mode of action of a novel antitumour acridine 4′-(9-acridinylamino)-methansulphon-m-anisidine (m-AMSA) has been investigated. Two congeners of m-AMSA, 4′-(9-acridinylamino)methanesulphonanilide (AMSA) and 4′-(9-acridinylamino)methanesulphon-o-anisidine (o-AMSA) were also studied for comparative purposes. m-AMSA is being evaluated clinically at present under the auspices of the National Cancer Institute, U.S.A.

(2) Treatment of mice bearing the mast cell tumour, mastocytoma P815, with m-AMSA provided some complete cures. AMSA was almost as effective as m-AMSA but its dose potency was 6-fold lower. o-AMSA was inactive.

(3) m-AMSA was found to have a short half-life in mice. Loss of m-AMSA from plasma was due, in part, to the formation of highly fluorescent covalent protein adducts. The rate of this reaction was similar for all three AMSA congeners, and is probably not required for antitumour activity.

(4) The reaction of AMSA drugs with proteins was shown to occur by nucleophilic displacement of the methanesulphonanilide moiety. Evidence is presented that thiols are the predominant reaction centres in proteins.

(5) A tissue culture model for the antitumour action of m-AMSA was developed using mastocytoma P815 cells. Profound growth inhibition and eventual killing was demonstrated using drug concentrations and durations of exposure attainable in mice. The potencies of the three AMSA congeners paralleled their antitumour potencies in vivo, except at very high drug concentrations.

(6) The rates of biotransformation of AMSA drugs, and their extent of uptake by cells in culture, could not account for the differing potencies of the three AMSA congeners.

(7) m-AMSA prevented the progression of mastocytoma cells through the cell division cycle under conditions where net cell growth was unaffected.
Physiologically attainable drug concentrations inhibited chromosome condensation in cells which were less than 10 minutes from the $G_2$-phase/mitosis boundary at the time of drug addition. The sedimentation rate on alkaline sucrose gradients of DNA from cells treated with $m$-AMSA was lower than that from untreated cells, suggesting that this agent may cause fragmentation of DNA.

(8) A new method for the investigation of the cell cycle stage selectivity of antitumour drugs was developed. This technique demonstrated that $m$-AMSA and AMSA have significant cycle stage selectivity, the growth of cells late in cycle being most affected.

(9) Spectrophotometric determinations of binding parameters for the interaction of AMSA derivatives with native DNA indicated that $m$-AMSA bound with lower affinity than did $o$-AMSA or AMSA. This conclusion was supported by the helix stabilization caused by these drugs. However, the association constants were sufficiently high for each derivative to ensure that essentially all intracellular drug available for binding to DNA would be bound.

(10) The ratio of the association constant for native DNA to that for a single-stranded viral RNA was higher for $m$-AMSA than for AMSA. This selectivity, if operative in vivo, could account for the high dose potency of $m$-AMSA.
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<th>Symbol</th>
<th>Definition</th>
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<tr>
<td>A</td>
<td>amplification factor (Coulter Counter)</td>
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<tr>
<td>9-AA</td>
<td>9-aminoacridine</td>
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<td>[acridinyl-G-³H]AMS A</td>
<td>AMS A randomly labelled (³H) in the acridine ring</td>
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<td>[acridinyl-G-³H]ø-AMS A</td>
<td>ø-AMS A randomly labelled (³H) in acridine ring</td>
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<td>Ara-C</td>
<td>1-β-D-arabinofuranosylcytosine</td>
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<tr>
<td>Aₓ</td>
<td>absorbance at wavelength x(nm)</td>
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<td>BCNU</td>
<td>1,3-bis(2-chloroethyl)-1-nitrosourea</td>
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<td>bovine serum albumin</td>
</tr>
<tr>
<td>C_b</td>
<td>concentration of bound ligand</td>
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<tr>
<td>C_f</td>
<td>concentration of free ligand</td>
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<td>Ci</td>
<td>curie</td>
</tr>
<tr>
<td>CM-protein</td>
<td>carboxymethylated protein</td>
</tr>
<tr>
<td>cpm</td>
<td>counts per minute</td>
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<tr>
<td>[¹⁴C]TDR</td>
<td>[2-¹⁴C]thymidine</td>
</tr>
<tr>
<td>CT DNA</td>
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</tr>
<tr>
<td>DNA</td>
<td>deoxyribonucleic acid</td>
</tr>
<tr>
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<td>disintegrations per minute</td>
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<td>dithiothreitol</td>
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<td>fl</td>
<td>femtolitre</td>
</tr>
<tr>
<td>fu</td>
<td>unit of fluorescence intensity</td>
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<tr>
<td>g</td>
<td>gram, or gravitational acceleration</td>
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<td>h</td>
<td>hour</td>
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<td>HEPES</td>
<td>N-2-hydroxyethylpiperazine-N'-2-ethanesulphonic acid</td>
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<td>i.c</td>
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<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>i.v.</td>
<td>intravenous</td>
</tr>
<tr>
<td>k</td>
<td>calibration factor (Coulter Counter)</td>
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<tr>
<td>$K_e$</td>
<td>association constant for a single lattice residue</td>
</tr>
<tr>
<td>$K_s$</td>
<td>association constant for a site defined by the saturation binding ratio</td>
</tr>
<tr>
<td>kg</td>
<td>kilogram</td>
</tr>
<tr>
<td>$LD_{10}$</td>
<td>dose of drug which is lethal to 10% of the test organisms</td>
</tr>
<tr>
<td>LDS</td>
<td>lithium dodecyl sulphate</td>
</tr>
<tr>
<td>LFER</td>
<td>linear free energy relationship</td>
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<td>m</td>
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<td>$M$</td>
<td>molar</td>
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<td>$m$-A$	ext{MSA}$</td>
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<td>millicurie</td>
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<td>n</td>
<td>nucleic acid binding site size, in nucleotides</td>
</tr>
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<td>n'</td>
<td>nucleic acid binding site size, in base pairs</td>
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<tr>
<td>N</td>
<td>nucleic acid concentration in nucleotide residues (unless otherwise defined)</td>
</tr>
<tr>
<td>$N_i$</td>
<td>number of pulses in the $i$th channel</td>
</tr>
<tr>
<td>nm</td>
<td>nanometre</td>
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<td>nanomolar</td>
</tr>
<tr>
<td>mmol</td>
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<tr>
<td>NNCS</td>
<td>neonatal calf serum</td>
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<td>o-$A$MSA</td>
<td>4'-((9-acridinylamino)methanesulphon-o-anisidide</td>
</tr>
<tr>
<td>P</td>
<td>partition coefficient</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate-buffered saline</td>
</tr>
<tr>
<td>PF</td>
<td>proflavine</td>
</tr>
<tr>
<td>pg</td>
<td>picogram</td>
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</table>
[phenyl-3H]AMSA

AMS randomly labelled (3H) in the phenyl ring

pmol

picomole

POPOP

1,4-bis[2-(5-phenyloxazoly)]-benzene; phenyl-
oxazolylphenyl-oxazolylphenyl

PPO

2,5-diphenyloxazole

g.d.
daily administration

QSAR

quantitative structure-activity relationship

r

radius

r

binding ratio (unless otherwise defined)

Rf

chromatographic mobility

RNA

ribonucleic acid

rpm

revolutions per minute

s

second

SBSS

Shortman's balanced salt solution

s.c.

subcutaneous

S.D.

standard deviation

Sf

ccentration of free (unoccupied) ligand
binding sites

SHE

buffer (saline-HEPES-EDTA)

t

elapsed time

T

sedimentation time

T1/2

half-life (unless otherwise defined)

Tg

cell generation time

TCA

Trichloroacetic acid

TdR

thymidine

TLC

thin layer chromatography

Tris

tris(hydroxymethyl)aminomethane

TYMV

turnip yellow mosaic virus

uv

ultraviolet

V

mean cell volume

Vm

volume of medium in wet pellet

Vc

volume of cells in wet pellet

W/V

weight per volume

V/V

volume per volume

α

number of ligand binding sites per lattice
residue

δ

channel number

e

molar extinction coefficient
Δε  differential molar extinction coefficient
η  absolute viscosity
λ  wavelength
λ_i  wavelength of an isobestic point
λ_m  wavelength at maximum of difference spectrum
μ  ionic strength
μCi  microcurie
μg  microgram
μl  microlitre
μM  micromolar
μmol  micromole
ν  binding ratio (mol ligand/mol base pairs)
ν_L  binding ratio (mol ligand/mol lattice residues)
ρ  absolute density of cells
ρ_o  absolute density of solution
ρ_w  absolute density of water
σ_m  Hammett substituent constant (meta)
σ_p  Hammett substituent constant (para)
ϕ  cell volume fraction
ω  mitotic index