ANTITUMOUR METALLOCENES

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Doctor of Philosophy

by

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Preface

This thesis is a summary of the research carried out in the School of Chemistry at the University of Sydney under the supervision of Associate Professor Margaret M. Harding between February 1997 and October 2000. Except where reference is made in the text, this thesis contains no material previously published or extracted in whole or in part from a thesis presented by me for another degree or diploma. No other person's work has been used without due acknowledgement in the main text of the thesis.

Sections of this work have been published elsewhere:

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"Water Soluble, Hydrolytically Stable Derivatives of the Antitumour Drug Titanocene Dichloride and Binding Studies with Nucleotides", Mokdsi, G.; Harding, M. M.
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Metal-Based Drugs **1998**, *5*, 207-215.

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Abstract

This thesis reports a study of the chemical stability and coordination chemistry of several antitumour metallocenes Cp_2MCl_2 (Cp = η^5-C_5H_5; M = Ti **1**, V **2**, Nb **3**, Mo **4)**, as well as derivatives of Cp_2TiCl_2 **1**, with nucleic acids, nucleic acid constituents and proteins. These studies were carried out in order to identify the biologically active species and more fully understand the molecular level mechanism of action of the antitumour metallocenes, in particular Cp_2TiCl_2 **1**, which is currently undergoing phase II clinical trials.

The interactions of Cp_2MoCl_2 **4** with four oligonucleotides were studied by ^1H and ^{31}P NMR spectroscopy. In 50 mM salt solutions of Cp_2MoCl_2 **4**, hydrolysis of the halide ligands occurred to give a solution with pD ~2, containing a species in which both Cp rings remain metal bound for 24 h. At pD ~7, partial hydrolysis of the Cp rings (~30%) occurred after 24 h. Addition of an aqueous solution of Cp_2MoCl_2 **4** in 50 mM salt to the self-complementary sequence $d(CGCATATGCG)_2$, maintaining the pD at 6.0-7.0, showed no evidence for the formation of a metallocene-oligonucleotide complex and only peaks arising from hydrolysis of Cp_2MoCl_2 **4** were detected. A similar result was obtained in titration experiments with the single stranded sequence d(ATGGTA) at pD 6.5-7.0. However, at pD 3.0, new signals assigned to a molybdocene-oligonucleotide complex(es), which was stable for hours at pD 3.0, were detected; while at pD ~7 the complex is destabilised and only peaks arising from hydrolysis of Cp_2MoCl_2 **4** were detected. Titration experiments at low pD with Cp_2MoCl_2 **4** and the dinucleotide dCG were consistent with formation of a complex arising due to coordination of molybdenum to guanine N7 and/or cytosine N3. The results obtained showed that stable oligonucleotide adducts were not formed in 50 mM salt at pD ~7 and hence it is highly unlikely that formation of molybdocene-DNA adducts *in vivo* is the primary action that is responsible for the antitumour properties of Cp_2MoCl_2 **4**.

The rate of hydrolysis of the aromatic rings of Cp_2TiX_2 (X = Cl **1**, $OCOCH_2NH_3Cl$ **27**) and the dimethylsubstituted derivatives $(MeCp)_2TiX_2$ (X = Cl **34**, $OCOCH_2NH_3Cl$ **41**), in aqueous solutions at pD 2-8 was studied by ^1H NMR spectroscopy. Rapid hydrolysis of both the halide/glycine and Cp ligands in Cp_2TiX_2 (X = Cl **1**, $OCOCH_2NH_3Cl$ **27**) occurred and predominantly gave a precipitate at pD ~7. In contrast, under the same experimental conditions, the predominant species present in aqueous solutions of $(MeCp)_2TiX_2$ (X = Cl **34**, $OCOCH_2NH_3Cl$ **41**) at pH 2-8 contained both MeCp rings metal bound. At pD < 5, Cp_2TiX_2 (X = Cl **1**, $OCOCH_2NH_3Cl$ **27**) and $(MeCp)_2TiX_2$ (X = Cl **34**, $OCOCH_2NH_3Cl$ **41**) formed similar complex(es) with purine nucleotides. However, at pD >5, stable adducts between nucleotides and Cp_2TiX_2 (X = Cl **1**, $OCOCH_2NH_3Cl$ **27**) were not formed. In contrast, $(MeCp)_2TiX_2$ (X = Cl **34**, $OCOCH_2NH_3Cl$ **41**) formed complex(es) with 5'-dAMP or 5'-dGMP, which were stable for 24 h. These results suggest that formation of stable chelates between $(MeCp)_2TiX_2$ (X = Cl **34**, $OCOCH_2NH_3Cl$ **41**) and nucleic acid constituents *in vivo* is possible. However, the methyl substituted derivatives **34** and **41** did not show any antitumour activity against EAT in mice when administered in either 10%DMSO/90%saline or in water at pH 6.2-6.4, which suggests that the labile Cp-Ti bond present in Cp_2TiCl_2 **1** is required for antitumour activity.

The synthesis of a range of Cp substituted titanocene derivatives was investigated in an attempt to prepare derivatives with modified Cp stability in comparison to the methyl substituted derivatives. The synthesis of derivatives $(CpCH_2Y)_2TiCl_2$ where Y = -CHO **43**, $-CONMe_2$ **44**, $-NO_2$ **45**, $(RCp)_2TiCl_2$ where R = -COMe **46**, -COOMe **47** or $-CONMe_2$ **48**, $(CpNMe_2)_2TiCl_2$ **62** and $(Cp(CH_2)_2NMe_2)_2TiCl_2$ **63** was unsuccessful, due to the presence of coordinating

substituents on the Cp rings and poor stability in polar, protic solvents. Hence, these derivatives were excluded from further studies.

The rate of hydrolysis of the Cp rings of Cp_2TiX_2 (X = Cl **1**, $OCOCCl_3$ **22** and $OCOCH_2NH_3Cl$ **27**) in aqueous solutions, 10%DMSO/90%D_2O and 100% DMSO was monitored by ^1H NMR spectroscopy. Rapid hydrolysis of both the carboxylate and Cp ligands of Cp_2TiX_2 ($OCOCCl_3$ **22** and $OCOCH_2NH_3Cl$ **27**) occurred in DMSO to give biologically inactive species. The rate of these reactions were concentration dependent as dilution of these samples with saline or water to give the therapeutic conditions of 10%DMSO/90%D_2O slowed the hydrolysis chemistry. In contrast, samples of Cp_2TiX_2 (X = Cl **1** and $OCOCH_2NH_3Cl$ **27**) dissolved in water, gave solutions containing the presumed antitumour active species in which the halide or glycine ligands have been hydrolysed but the Cp rings remain metal bound. Thus, charged X ligands may be incorporated into Cp_2TiX_2 and will give comparable activity to Cp_2TiCl_2 **1** provided the samples are administered in water.

The antitumour metallocenes Cp_2MCl_2 (M = Ti **1**, V **2**, Nb **3**, Mo **4**) and the inactive derivative $(MeCp)_2TiCl_2$ **34** were found to inhibit the relaxation of supercoiled plasmid DNA pBR322 by human topoisomerase II *in vitro*. These results implicated the inhibition of topoisomerase II in the mechanism of antitumour activity although there was no direct correlation between the *in vitro* results with biological activity against EAT *in vivo*.

UV spectroscopy confirmed that the metallocenes Cp_2MCl_2 (M = Ti **1**, Mo **4**) became associated with and were stabilised to hydrolysis by calf thymus DNA but not with human serum albumin. ICP-AES was used to measure the amount of metal associated with either DNA or human serum albumin after incubation with Cp_2MCl_2

(M = Ti **1**, Nb **3**, Mo **4**) and dialysis of these solution. The results confirmed that DNA stabilises or becomes associated with the metallocenes. However, errors associated with the ICP-AES measurements did not allow these results to be quantified. ^{1}H NMR spectroscopy was used to show that the antitumour metallocene Cp_2MoCl_2 **4** formed an adduct with glutathione **72** in the pH range 3-7 through the sulfur donor group. In comparison, the antitumour metallocenes Cp_2MCl_2 (M = Ti **1**, Nb **3**) showed limited adduct formation with glutathione **72** at pH ~3 and no adducts were detected at pH > 5.5.

Abbreviations

AAS	atomic absorption spectroscopy
*m*AMSA	4'-(9-acridinylamino)-*N*-(methanesulfonyl)-*m*-anisidine or amsacrine
Cp	cyclopentadienyl (η^5-C_5H_5)
CCC	covalently closed circular
5'-dAMP	deoxyadenosine 5'-monophosphate
5'-dCMP	deoxycytidine 5'-monophosphate
5'-dGMP	deoxyguanosine 5'-monophosphate
DNA	deoxyribonucleic acid
DMSO	dimethyl sulfoxide
5'-dUMP	deoxyuridine 5'-monophosphate
EAT	Ehrlich ascites tumour
equiv.	mole equivalent
EPR	electron paramagnetic resonance
HSA	human serum albumin
hTF	human serum transferrin
ICP-AES	inductively coupled plasma – atomic emission spectroscopy
LD_{50}	lethal dose required to kill 50% of the population
MTD	maximum tolerable dose
MWCO	molecular weight cut off
NMR	nuclear magnetic resonance
RNA	ribonucleic acid
OCR	optimum cure rate
ODR	optimum dose range
pD	$\log_{10}[D^+]$ where D refers to deuterium
THF	tetrahydrofuran
UV	ultra-violet