PHYSICOCHEMICAL AND STRUCTURAL STUDIES

ON TWO TRIDENTATE ANTITUMOUR LIGAND SYSTEMS

A thesis presented in partial fulfilment of the requirements for the degree of

Doctor of Philosophy in Chemistry at Massey University.

JOHN DAVID RANFORD

1988

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DEDICATION

To all the people, especially Mona and Alan Wong, who constantly remind me how silly I must be to have to stay at school for **SO** long.

ABSTRACT

This work is an investigation into the physicochemical and structural properties of two tridentate, antitumour ligand systems and is divided into two sections. In the first (Chapters 1 to 4), the ligand 2-formylpyridine thiosemicarbazone (LH - containing an NNS donor set), several of its congeners and a range of complexes (predominantly Cu(II)) were prepared. The second section (Chapters 5 and 6) deals with a range of ligands based on salicylaldehyde benzoylhydrazone (sbH_2 - containing an ONO donor set), their complexes (predominantly Cu(II)) and the cytotoxicity data for all of this work.

In Chapter 1, complexes of the general formulation $[CuLX]_2$ for the deprotonated and $[Cu(LH)X]_2X_2$ for the neutral, protonated ligand were prepared (where X = e.g. halide, pseudohalide, NO_3^-, ClO_4^-, CH_3COO^-, CF_3COO^-). The complexes formed are very stable in strong, non-oxidising acid solutions and with mildly reducing anions, but are susceptible to oxidising acids and anions. The crystal structures of the neutral ligand, dimeric, one-atom anion bridged complex $[Cu(LH)(CF_3COO)]_2(CF_3COO)_2$ and the monomeric complex $[Cu(LH)(ClO_4)_2H_2O]\cdot 2H_2O$ with axially coordinated perchlorato groups were determined.

In Chapter 2, the possibility that *in vivo* S and N donor atom adducts of CuL^+ may form was investigated *in vitro*. Stable complexes containing a copper(II)-thiolato bond were isolated at ambient temperatures, under aerobic conditions. The e.s.r. parameters for these were very similar to a species formed from the interaction of CuL^+ with human blood components. Ternary, Lewis-base adducts of nitrogen donor atoms were also isolated, and the crystal structures for two of these, $[CuL(2,2'\text{-bipyridyl})]ClO_4$ and $[CuL(saccharinato)H_2O]\cdot \frac{1}{2}H_2O$, were solved.

The possibility of CuL^+ interacting with O donor groups (in particular phosphates) *in vivo* was investigated *in vitro* in Chapter 3. The ternary complexes isolated contain the anions mono-

and dihydrogenphosphate, pyrophosphate, phenolate and molybdate. The crystal structure of $[Cu(LH)(H_2PO_4)]_2(H_2PO_4)_2(H_3PO_4)_2{\cdot}2H_2O$ showed the complex is dimeric, having a unique one-atom dihydrogenphosphate bridge, three inequivalent phosphates and a very strong interphosphate hydrogen-bond. In contrast, the ternary, pyrophosphato complex $[(CuL)_4P_2O_7]{\cdot}12H_2O$ is a tetramer, with each Cu(II) centre having a one-atom S, a three-atom pyrophosphato and two five-atom pyrophosphato bridges.

The low temperature magnetic properties of $[CuL(CH_3COO)]_2$ fit the Bleany-Bowers expression well, whereas for $[(CuL)_4P_2O_7]{\cdot}12H_2O$ a very weak interaction through the five-atom pyrophosphato bridge may account for the non-dimeric behaviour observed. Both complexes are weakly antiferromagnetic ($-2J \sim 6$ cm^{-1}).

In Chapter 4, four variations on the ligand LH and a representative series of their Cu(II) complexes were synthesised. Reduction potentials for a Cu(II) complex of each ligand, as well as for two thiolato and a Lewis-base adduct of CuL^+, were measured. N.m.r. spectroscopy was used to characterise the ligands and pKa values for both the ligands and their Cu(II) complexes were determined. No correlation between any of these values and the cytotoxicities was found.

In Chapter 5, Section 2, a range of ligands based on sbH_2 (salicylaldehyde benzoylhydrazone) and their transition metal complexes (predominantly Cu(II)) were synthesised for cytotoxicity trials (on the cell line HCT-8). A number of the Cu(II) complexes had depressed room temperature magnetic moments and displayed e.s.r. spectral features which were attributed to magnetic interactions in the solid state. The crystal structure of $[Cu(sbH)ClO_4(EtOH)]_2$ revealed it to be a planar, side-by-side dimer with $Cu(sbH)^+$ moieties bridged via the phenolato-oxygens.

Depending upon the pH, sbH_2 can coordinate as either a neutral, monoanionic or dianionic moiety to transition metals. The interaction of $CuF_2.2H_2O$ in HF with sbH_2 resulted in the *in*

situ formation of H_2SiF_6. The crystal structure of the resulting complex, $[(Cu(sbH)H_2O)_2SiF_6]\cdot 2H_2O$, showed it to be a dimer, with the Cu(II) centres linked by the coordinated SiF_6^{2-} anion. The crystal structure of a cytotoxically inactive $Cu(sbH)^+$ analogue, $[Cu(saH)Cl(H_2O)]H_2O$ was also solved.

In the final chapter, the cytotoxicity data for all compounds tested are presented. The copper(II) complexes generally showed activities different to the metal free ligands. For LH congeners the complexes were no better than the ligands; in contrast to the sbH_2 analogues where the Cu(II) chelates were statistically more cytotoxic. Transition metals other than Cu(II) either did not improve the activity or resulted in a reduction or loss of cytotoxicity.

For LH congeners, changes in cytotoxicity could be related to altered electronic and steric properties, whereas for the sbH_2 series of compounds, statistical analysis showed the lipophilicity conferred by a substituent to be the dominant factor. Comparisons with proven anticancer drugs are made and possible future studies to maximise the biological activity are suggested. All of the compounds tested for their antiviral activity were either cytotoxic or inactive at the concentrations used.

ACKNOWLEDGEMENTS

Much of this work would not have been possible without the gratefully acknowledged contributions from the following people:

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I would also like to sincerely thank everyone else who contributed with helpful discussions and I am also indebted to Massey University for the position of Graduate Assistant during my studies.

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ABBREVIATIONS

a.a.	atomic absorption
a.m.u.	atomic mass units
bipy[a]	2, 2′-bipyridyl
cisplatin[b]	*cis*-diamminedichloroplatinum(II)
c.t.	charge transfer
dips	diisopropylsalicylic acid
dmap	4-N,N-dimethylaminopyridine
dmf	dimethylformamide
dmso	dimethylsulphoxide
DNA	deoxyribonucleic acid
edta	ethylenediaminetetraacetic acid
en	ethylenediamine
e.s.d.	estimated standard deviation
e.s.r.	electron spin resonance
H	in a ligand or complex refers to an ionisable proton
Hb	haemoglobin
IC_{50}	inhibitory concentration to 50%; the concentration required to inhibit cell growth to 50% compared with that of a control
ir	infrared
LD_{50}	lethal dose to 50%; the single injected dose that kills 50% of the animals
LH[a,c]	2-formylpyridine thiosemicarbazone
2′L[c]	2-formylpyridine 2′-methylthiosemicarbazone
4′LH[c]	2-formylpyridine 4′-methylthiosemicarbazone
6LH[c]	6-methyl-2-formylpyridine thiosemicarbazone
mbtH[a]	2-mercaptobenzothiazole
miH[a]	2-mercaptoimidazole
mmiH[a]	2-mercapto-1-methylimidazole

mpH_2[a]	2-mercapto-3-pyridinol
m.t.	mull transmittance
mttH[a]	4-methyl4H-1,2,4-triazole-3-thiol
n.m.r.	nuclear magnetic resonance
ntpH	4-nitrothiophenol
pbH[a]	2-formylpyridine benzoylhydrazone
pctpH	pentachlorothiophenol
pftpH	pentafluorothiophenol
phen[a]	1,10-phenanthroline
ptpH[a]	paratritylphenol
py	pyridine
rdr[e]	ribonucleoside diphosphate reductase (ribonucleotide reductase)
RNA	ribonucleic acid
saH_2[a,d]	salicylaldehyde acetylhydrazone
sbH_2[a,d]	salicylaldehyde benzoylhydrazone
spy	square-pyramidal
tipH	2,4,6-triiodophenol
tby	trigonal-bipyramidal
TMS	tetramethylsilane
uv/vis	ultraviolet/visible

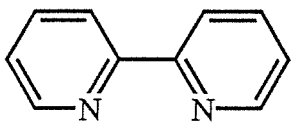
a structure abbreviated on following page

b see Figure 1.1

c see Figure 4.1 in Chapter 4 introduction

d see Figure 2.5.11 for this and all other structurally related ligands for Section 2

e see Figure 1.4



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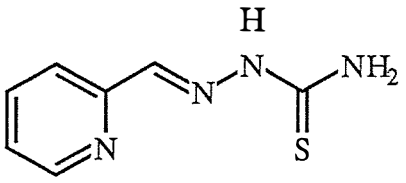

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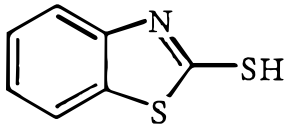

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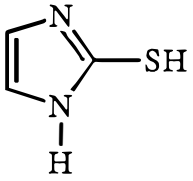

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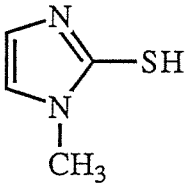

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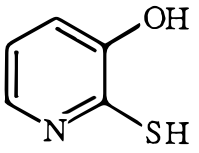

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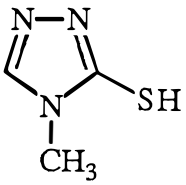

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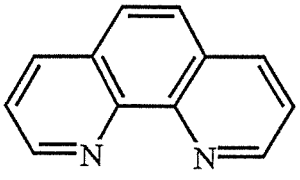

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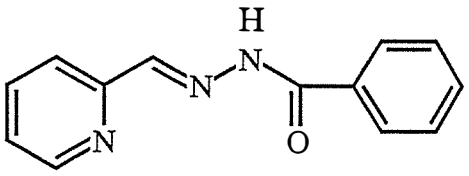

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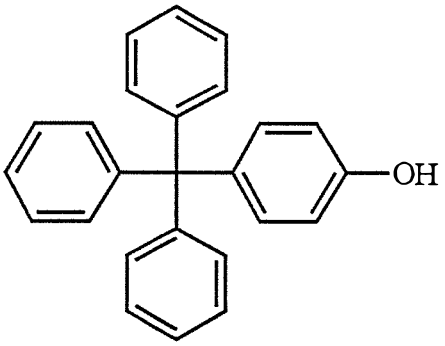

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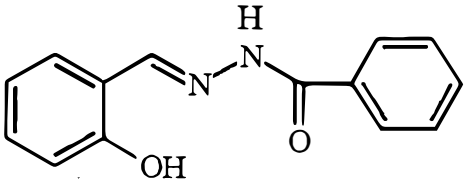

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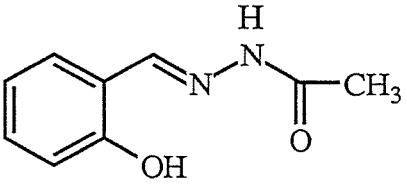

saH_2

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