Synthesis, Characterization and Antitumour Properties of some Metal(II) Complexes of 2-Pyridinecarboxaldehyde 2′-Pyridylhydrazone and Related Compounds

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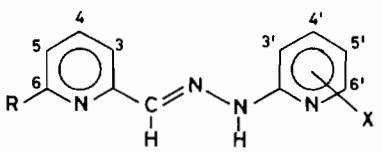
Abstract

Metal complexes of 2-pyridinecarboxaldehyde 2′-pyridylhydrazone (PCPH) and related compounds with manganese(II), iron(II), cobalt(II), nickel(II), copper(II), zinc(II) and platinum(II) were synthesized and characterized by magnetic susceptibility measurements down to liquid nitrogen temperature and also by electronic, infrared, electron spin resonance and Mössbauer spectra. All the metal(II) complexes appeared to be monomeric, high-spin, five-coordinate (square-pyramidal) ($X = Cl^-$ or OAc^-), except for $Ni(PCPH)Cl_2$ which is polymeric, high-spin, six-coordinate. Each ligand behaved as a tridentate NNN donor, via the pyridine nitrogen, azomethine nitrogen, and pyridine or quinoline nitrogen. One of the most active agents of this series, $Cu(PCPH)Cl_2$, showed antitumour activity against a variety of transplanted tumours, including Sarcoma 180, Ehrlich carcinoma and L1210 leukaemia sensitive to α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones. This agent caused inhibition of ^{3}H-thymidine and ^{3}H-uridine incorporation into DNA and RNA, respectively, of Sarcoma 180 ascites cells; protein biosynthesis was relatively insensitive to the action of this agent.

Introduction

There is a class of compounds cytotoxic to tumour cells that comprises agents that have been shown to or are thought to bind metals as part of their mechanism of action. Principal members of this class are the *cis*-dichlorodiammineplatinum(II) complexes, CuKTS (3-ethoxy-2-oxobutyraldehyde-bis(thiosemicarbazone)copper(II), α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones, the rhodium carboxylates and copper bleomycin [1–7]. These are ligands and metal complexes involving a diversity of metals, which display a rich variety of chemical properties. The novelty of metallo-drugs is underscored by the fact that the success of these compounds has not led to a broad, systematic investigation of metal complexes as potential drugs within the fields of medicinal chemistry and cancer chemotherapy. Thus, despite the paucity of intense, systematic effort to explore this area, there is ample precedent to encourage us to focus on metal complexes and metal binding ligands.

Aroylhydrazones have been shown to exhibit a significant inhibiting effect on DNA synthesis and cell growth in a number of human and rodent cell lines grown in culture [8]. A copper(II) complex of the most potent of the chelators, salicylaldehyde benzoylhydrazone (SBH), has greater inhibitory activity than does SBH itself [9]. Although the bioactive forms and mechanism of action of these compounds are uncertain, their cytotoxic activity is equal or more than that of many chelators and chelates previously known to have such activity, including agents clinically used. These interesting results stimulated us to study the antitumour activity of related ligands and their metal chelates. This paper describes the synthesis, characterization and antitumour activity of 2-pyridinecarboxaldehyde 2′-pyridylhydrazone and related ligands **I–VII** and their metal(II) chelates.



PCPH

I: R = H, X = H (PCPH)
II: R = CH_3, X = H (6-MePCPH)
III: R = H, X = 3′-CH_3 (3′-MePCPH)
IV: R = H, X = 4′-CH_3 (4′-MePCPH)
V: R = H, X = 5′-CH_3 (5′-MePCPH)
VI: R = H, X = 6′-CH_3 (6′-MePCPH)

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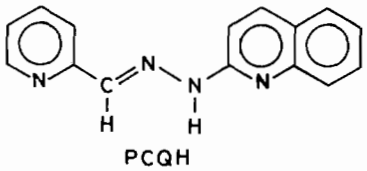


PCQH

VII: 2-Pyridinecarboxaldehyde 2'-quinolylhydrazone

Experimental

Conductance measurements were carried out in ethanol at 10^{-3} M on a Toshniwal conductivity bridge type Cl 01/01. All magnetic susceptibilities were measured on polycrystalline samples with a vibrating sample magnetometer. The X-band ESR spectra were recorded on a Varian spectrophotometer in the solid state as polycrystalline samples using DPPH as a reference material. The diffuse reflectance spectra of the compounds were measured on a Cary 14 spectrophotometer equipped with a diffuse reflectance accessory, using MgO as a reference. The spectra of the free ligands and their complexes in the 4000–200 cm^{-1} range were measured on a Perkin-Elmer 1430 spectrophotometer in KBr. Elemental analyses for carbon, hydrogen and nitrogen were performed on a Perkin-Elmer 240-C elemental analyzer. The data for the compounds are reported in Table I.

TABLE I. Transition Metal Complexes of 2-Pyridinecarboxaldehyde 2'-Pyridylhydrazone and Related Compounds

Compound	Formula	Colour	Yield (%)	Found (%)				Calculated (%)			
				C	H	N	M	C	H	N	M
1	$Mn(PCPH)Cl_2$	orange needles	78	40.54	3.21	17.32	17.02	40.74	3.09	17.28	16.98
2	$Fe(PCPH)Cl_2$	dark red plates	70	40.82	3.20	17.11	17.11	40.61	3.08	17.23	17.23
3	$Co(PCPH)Cl_2$	dark green prisms	80	40.62	3.17	17.20	17.82	40.24	3.05	17.07	17.99
4	$Ni(PCPH)Cl_2$	green needles	85	40.60	3.15	17.21	17.82	40.24	3.05	17.07	17.99
5	$Cu(PCPH)Cl_2$	dark green plates	90	39.28	3.15	16.75	18.82	39.76	3.01	16.87	18.98
6	$Zn(PCPH)(OAc)_2$	yellow needles	75	46.83	4.05	14.65	17.18	47.24	4.20	14.70	17.06
7	$Pt(PCPH)Cl_2$	dark red	80	28.22	2.02	12.12	42.14	28.45	2.16	12.07	42.03
8	$Mn(6\text{-}MePCPH)Cl_2$	orange needles	76	42.15	3.45	16.68	16.35	42.60	3.55	16.57	16.27
9	$Fe(6\text{-}MePCPH)Cl_2$	dark red plates	85	42.01	3.43	16.63	16.68	42.48	3.54	16.52	16.52
10	$Co(6\text{-}MePCPH)Cl_2$	green prisms	85	42.62	3.40	16.25	17.38	42.11	3.51	16.37	17.25
11	$Ni(6\text{-}MePCPH)Cl_2$	green needles	79	42.62	3.40	16.25	17.39	42.11	3.51	16.37	17.25
12	$Cu(6\text{-}MePCPH)Cl_2$	green plates	75	41.12	3.51	16.03	18.32	41.62	3.47	16.18	18.21
13	$Zn(6\text{-}MePCPH)(OAc)_2$	yellow needles	80	48.22	4.12	14.34	16.67	48.85	4.07	14.25	16.54
14	$Pt(6\text{-}MePCPH)Cl_2$	red plates	75	30.62	2.62	11.81	40.82	30.13	2.50	11.72	40.80
15	$Mn(3'\text{-}MePCPH)Cl_2$	orange needles	79	42.12	3.42	16.68	16.39	42.60	3.55	16.57	16.27
16	$Fe(3'\text{-}MePCPH)Cl_2$	dark red plates	80	42.02	3.41	16.63	16.66	42.48	3.54	16.52	16.52
17	$Co(3'\text{-}MePCPH)Cl_2$	green prisms	87	42.60	3.61	16.21	17.36	42.11	3.51	16.37	17.25
18	$Ni(3'\text{-}MePCPH)Cl_2$	green prisms	85	42.62	3.62	16.22	17.38	42.11	3.51	16.37	17.25
19	$Cu(3'\text{-}MePCPH)Cl_2$	dark green prisms	93	41.12	3.58	16.26	18.06	41.62	3.47	16.18	18.21
20	$Zn(3'\text{-}MePCPH)(OAc)_2$	pale yellow needles	80	48.26	4.12	14.35	16.40	48.85	4.07	14.25	16.54

(continued)

TABLE I. *(continued)*

Compound	Formula	Colour	Yield (%)	Found (%)				Calculated (%)			
				C	H	N	M	C	H	N	M
21	Pt(3′-MePCPH)Cl_2	dark red needles	85	30.28	2.42	11.81	40.61	30.13	2.50	11.72	40.80
22	Mn(4′-MePCPH)Cl_2	pale yellow needles	87	42.41	3.66	16.74	16.38	42.60	3.55	16.57	16.27
23	Fe(4′-MePCPH)Cl_2	dark red plates	90	42.52	3.64	16.64	16.68	42.48	3.54	16.52	16.52
24	Co(4′-MePCPH)Cl_2	green plates	90	42.00	3.62	16.19	17.12	42.11	3.51	16.37	17.25
25	Ni(4′-MePCPH)Cl_2	yellowish green plates	89	42.02	3.61	16.42	17.34	42.11	3.51	16.37	17.25
26	Cu(4′-MePCPH)Cl_2	green hexagonal plates	93	41.51	3.35	16.02	18.10	41.62	3.47	16.18	18.21
27	Zn(4′-MePCPH)$(OAc)_2$	yellow needles	80	48.62	4.18	14.37	16.42	48.85	4.07	14.25	16.54
28	Pt(4′-MePCPH)Cl_2	red square plates	90	30.26	2.58	11.87	40.19	30.13	2.50	11.72	40.08
29	Mn(5′-MePCPH)Cl_2	pale yellow plates	85	42.48	3.42	16.42	16.13	42.60	3.55	16.57	16.27
30	Fe(5′-MePCPH)Cl_2	dark red needles	80	42.34	3.58	16.43	16.41	42.48	3.54	16.52	16.52
31	Co(5′-MePCPH)Cl_2	dark green plates	92	42.00	3.42	16.19	17.37	42.11	3.51	16.37	17.25
32	Ni(5′-MePCPH)Cl_2	green needles	89	42.25	3.40	16.24	17.11	42.11	3.51	16.37	17.25
33	Cu(5′-MePCPH)Cl_2	green plates	92	41.50	3.51	16.29	18.32	41.62	3.47	16.18	18.21
34	Zn(5′-MePCPH)$(OAc)_2$	pale yellow needles	85	48.62	4.16	14.31	16.66	48.85	4.07	14.25	16.54
35	Pt(5′-MePCPH)Cl_2	dark red	89	30.00	2.53	11.84	40.19	30.13	2.50	11.72	40.08
36	Mn(6′-MePCPH)Cl_2	pale yellow needles	89	42.80	3.67	16.69	16.39	42.60	3.55	16.57	16.27
37	Fe(6′-MePCPH)Cl_2	dark red plates	90	42.62	3.67	16.67	16.61	42.48	3.54	16.52	16.52
38	Co(6′-MePCPH)Cl_2	green square plates	93	41.92	3.62	16.12	17.12	42.11	3.51	16.37	17.25
39	Ni(6′-MePCPH)Cl_2	green hexagonal plates	90	42.00	3.62	16.49	17.13	42.11	3.51	16.37	17.25
40	Cu(6′-MePCPH)Cl_2	green needles	90	41.51	3.33	16.02	18.06	41.62	3.47	16.18	18.21
41	Zn(6′-MePCPH)$(OAc)_2$	orange needles	84	48.67	4.18	14.37	16.62	48.85	4.07	14.25	16.54
42	Pt(6′-MePCPH)Cl_2	dark red prisms	79	30.23	2.62	11.81	40.19	30.13	2.50	11.72	40.08
43	Mn(PCQH)Cl_2	orange needles	85	48.26	3.37	15.02	14.83	48.13	3.21	14.97	14.71
44	Fe(PCQH)Cl_2	dark red square plates	79	48.21	3.35	15.02	15.12	48.00	3.20	14.93	14.93
45	Co(PCQH)Cl_2	green needles	90	47.51	3.28	14.93	15.72	47.62	3.17	14.81	15.61
46	Ni(PCQH)Cl_2	dark green plates	93	47.53	3.30	14.67	15.73	47.62	3.17	14.81	15.61
47	Cu(PCQH)Cl_2	green plates	89	47.00	3.31	14.76	16.62	47.12	3.14	14.66	16.50
48	Zn(PCQH)$(OAc)_2$	yellow needles	94	53.25	3.84	13.17	15.27	53.15	3.73	13.05	15.15
49	Pt(PCQH)Cl_2	dark hexagonal plates	89	35.17	2.47	11.02	38.13	35.02	2.33	10.89	37.94

Preparation of Hydrazones

2-Pyridinecarboxaldehyde 2′-pyridylhydrazone (PCPH) was prepared according to the reported method [10] and methyl substituted PCPH derivatives by analogous condensation reactions. The following compounds were recrystallized to constant melting point from ethanol: 6-methylpyridine-2-carboxaldehyde 2′-pyridylhydrazone (melting point (m.p.) 210–211 °C), 2-pyridinecarboxaldehyde 4′-methyl-2′-pyridylhydrazone (m.p. 183–184 °C), 2-pyridinecarboxaldehyde 5′-methyl-2′-pyridylhydrazone (m.p. 158–159 °C) and 2-pyridinecarboxaldehyde 6′-methyl-2′-pyridylhydrazone (m.p. 149–150 °C). 2-Pyridinecarboxaldehyde 3′-methyl-2′-pyridylhydrazone was obtained as a yellow oil after purification by chromatography on alumina. 2-Pyridinecarboxaldehyde 2′-quinolylhydrazone was purchased from Aldrich Chemical Co., Wisconsin, and was used as such. In each case, elemental analyses confirmed the identity of the product.

Preparation of Metal(II) Complexes

All the metal(II) chelates were prepared by the following general method. A hot solution of ligand (1 mmol) in 95% ethanol (25 ml) was added to a hot solution of metal(II) salt (1 mmol) in ethanol (15 ml) or an aqueous solution of K_2PtCl_4 (0.415 g, 1 mmol) and the resulting solution mixture was heated under reflux for 15–30 min. The boiling solution, on cooling to room temperature, afforded fine crystalline solids which were filtered off, washed with ethanol and diethylether and dried over P_2O_5 under vacuum.

Evaluation of Antitumour Activity

The antitumour activity of the ligands **I–VII** and their metal(II) chelates was determined at the National Cancer Institute, Bethesda, MD, by the standard screening procedure (*cf.* instruction 14) in the P388 lymphocytic leukaemia test system. The P388 lymphocytic leukaemia screen was carried out on $CD_2F_1(CDF_1)$ mice (male or female). On day 0, 1×10^6 ascites cells were injected intraperitoneally (ip). Test compounds were suspended in Saline with Tween-80 and administered ip on days 1 through 5. Six mice were used per test compound, and a *T/C* of greater than 125% was considered significant activity against P388 tumour growth. The ascites cell forms of Sarcoma 180, and L 1210 leukaemia were propagated in $CD_2F_1(CDF_1)$ mice (female), while Ehrlich carcinoma were grown in male BDF 1 mice. Transplantation was carried out by withdrawing peritoneal fluid from donor mice bearing 7-day tumour growths. The experiments were carried out according to the reported procedure [11]. The suspension was centrifuged for 2 min, the supernatant peritoneal fluid was decanted, and a 10-fold dilution with isotonic saline was made. The cell number was counted and the cell population was adjusted to a level of 10^6 cells/ml. A portion (0.1 ml) of the resulting cell suspension was injected intraperitoneally (ip) into each recipient animal. Dosage levels of all compounds were administered over a range of 20–60 mg/kg by ip injection, beginning 24 h after tumour implantation, once daily for 6 consecutive days. The drugs were injected ip as a fine suspension following homogenization in 2–3 drops of Tween 80 and then making up to volume with isotonic saline. The mice were weighed throughout the course of the experiments and the percentage change in body weight from onset to termination of therapy was used as an indication of drug toxicity. Determination of the sensitivity of ascitic neoplasms to these agents was based on the prolongation of survival time afforded by the drug treatments.

Incorporation of Radioactive Precursors into DNA, RNA and Protein

The effect of **5** on macromolecular synthesis of DNA, RNA and protein was determined by injecting either 200 μg of ^{3}H-thymidine (2.2×10^4 cpm/μg), 200 μg of ^{3}H-uridine (1.3×10^4 cpm/μg) or 125 μg of DL-^{14}C-leucine (1.7×10^4 cpm/μg) intraperitoneally into mice bearing 6 days accumulations of Sarcoma 180 ascites cells that were either untreated or treated for various periods of time with a single intraperitoneal injection of drug at a level equimolar to 25 mg of IQ-1/kg. The radioactive tracers were allowed 1 h to be incorporated and the specific radioactivity was then measured according to described methodology [12].

Results and Discussion

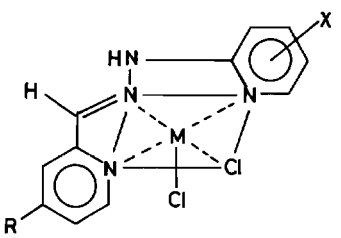
The ligands **I–VII** were prepared according to the reported procedures [10]. When equimolar ethanolic solutions of the ligands were combined with a metal(II) salt in ethanol or water, the complexes of the general formula M(ligand)X_2 separated from the solution. All the compounds are quite stable at room temperature and do not show decomposition after standing for a long period. The compounds are characterized by their high melting points (>300 °C) and low solubilities in polar but unreactive solvents. The molar conductance values are in the 10.7–15.7 ohm^{-1} cm^2 mol^{-1} range at 27 °C for the complexes in ethanol, indicating their non-electrolytic behaviour [13].

The products that were obtained from Mn(II), Fe(II), Co(II), Ni(II), Cu(II), Zn(II) and Pt(II) are monoligand complexes that have been assigned the distorted five-coordinate (square-pyramidal) structures represented by **VIII**, except for Ni(PCPH)Cl_2 which is the chloro-bridged, high-spin, *trans*-

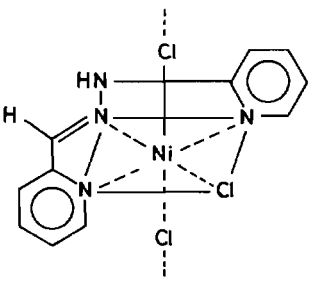
TABLE II. ESR Spectral Data for Cu(II) Complexes of 2-Pyridinecarboxaldehyde 2'-Pyridylhydrazone and Related Compounds

Compound	$g_\perp$	$g_\parallel$
$Cu(PCPH)Cl_2$	2.052(g_1) 2.100(g_2)	2.216(g_3)
$Cu(6\text{-}MePCPH)Cl_2$	2.031	2.246
$Cu(3'\text{-}MePCPH)Cl_2$	2.042	2.238
$Cu(4'\text{-}MePCPH)Cl_2$	2.039	2.242
$Cu(5'\text{-}MePCPH)Cl_2$	2.040	2.238
$Cu(6'\text{-}MePCPH)Cl_2$	2.038	2.243
$Cu(PCQH)Cl_2$	2.041	2.237

distorted six-coordinate complex **IX**. This conclusion is based on micro-analytical data, magnetic measurements and spectral studies, which agree well with the reported value for $M(PCPH)Cl_2$ [14]. An approximate square-pyramidal, five-fold coordination for $M((PCPH)X_2$ complexes was confirmed by a single crystal X-ray study [15, 16]. The far-infrared spectra of $M(ligand)Cl_2$ complexes exhibited bands at *ca.* 290s [ν_{sym}(M–Cl)], *ca.* 260m [ν_{asym}(M–Cl)], *ca.* 250s [ν(M–ligand)] and *ca.* 240 m [ν(M–Cl)] cm^{-1}, whereas the $Ni(PCPH)Cl_2$ complex exhibited bands at 298s [$\nu(Ni–Cl_{terminal})$], 250s [ν(Ni–ligand)] and 235 [$\nu(Ni–Cl_{bridging})$] cm^{-1} [17, 18]. The monodentate coordination of acetato groups in Zn(II) complexes was confirmed by the appearance of ν_{as}(COO) and ν_s(COO) bands at 1560sb and 1415s cm^{-1}, respectively [19].



VIII



IX

TABLE III. Mössbauer Spectral Parameters for Fe(II) Complexes of 2-Pyridinecarboxaldehyde 2'-Pyridylhydrazone and Related Compounds

Compound	T (K)	δ[a] (mm/s)	ΔEQ (mm/s)	Γ_1[b] (mm/s)	Γ_2[b] (mm/s)
$Fe(PCPH)Cl_2$	78	1.14	3.98	0.28	0.27
	RT	1.10	3.87	0.25	0.24
$Fe(6\text{-}MePCPH)Cl_2$	78	1.16	4.00	0.27	0.27
	RT	1.13	3.89	0.25	0.25
$Fe(3'\text{-}MePCPH)Cl_2$	78	1.16	4.00	0.26	0.24
	RT	1.12	3.90	0.24	0.24
$Fe(4'\text{-}MePCPH)Cl_2$	78	1.14	3.98	0.29	0.26
	RT	1.10	3.86	0.27	0.26
$Fe(5'\text{-}MePCPH)Cl_2$	78	1.16	4.02	0.29	0.28
	RT	1.09	3.90	0.26	0.26
$Fe(6'\text{-}MePCPH)Cl_2$	78	1.14	3.98	0.29	0.28
	RT	1.10	3.87	0.27	0.27
$Fe(PCPQH)Cl_2$	78	1.16	4.02	0.30	0.27
	RT	1.10	3.89	0.24	0.24

[a]Relative to natural iron foil. [b]Full width at half-maximum for low-velocity line (Γ_1) and high-velocity line (Γ_2).

The ^{57}Fe Mössbauer spectral parameters, the chemical isomer shift values, δ, and the quadrupole splitting values, ΔE_Q, measured at room temperature and 78 K (Table III) were consistent with those found for distorted five-coordinate iron(II) complexes having a $^5B_2$ ground state [20]. Electron spin resonance spectra of polycrystalline samples of some copper(II) complexes are presented in Fig. 1. The ESR spectra of all copper(II) complexes were anisotropic and were consistent with a square-pyramidal structure having a $d_{x^2-y^2}$ ground state [21].

Biological Activity

The ligands **I–VII** and their metal(II) chelates were tested for antitumour activity against P388 lymphocytic leukaemia cells in mice by the National Cancer Institute, Bethesda, MD. The results (Table IV) indicated that 2-pyridinecarboxaldehyde 2'-pyridylhydrazone, **I**, (PCPH) was an inhibitor of P388, having a T/C value of 138% at a dose level of 60 mg/kg. Substitution in the pyridine rings and the replacement of the pyridine ring by quinoline resulted in complete loss of antitumour activity.

Most of the complexes investigated showed no significant antitumour activity ($T/C \leqslant 125\%$); the highest level of activity was, however, exhibited by complex **5**. Since **5** was one of the most active agents of the series in the P388 lymphocytic leukaemia test system, it was also tested against a variety of other transplanted tumour systems, *i.e.*, Sarcoma 180, Ehrlich carcinoma and leukaemia L 1210 (Table V). Compound **5** showed activity in each of these test

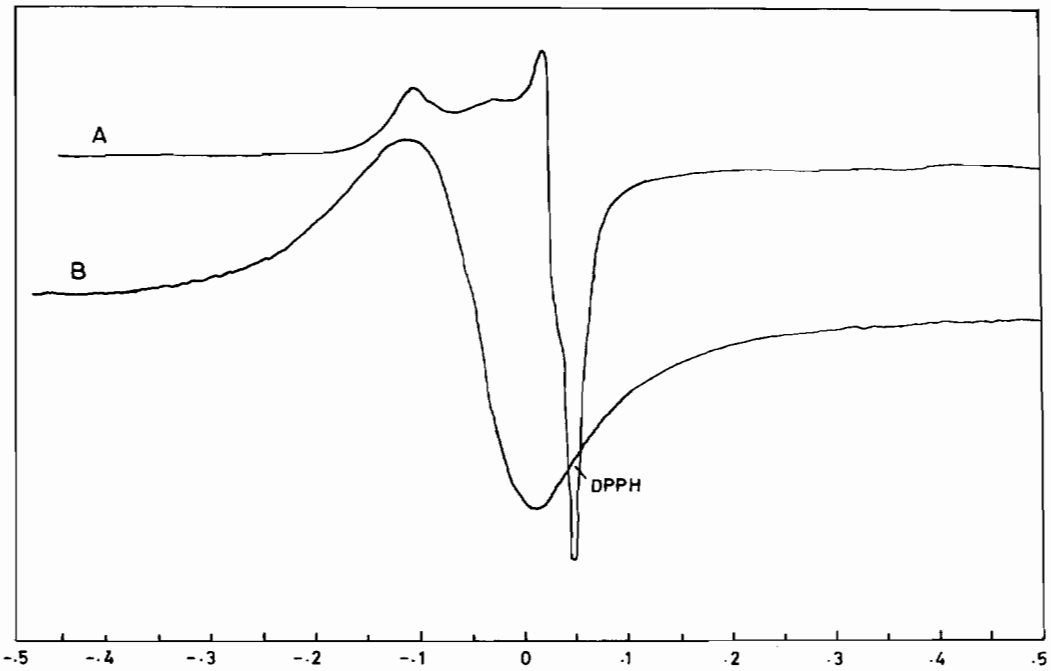


Fig. 1. ESR spectrum of $Cu(PCPH)Cl_2$ (A) and $Cu(3'\text{-}MePCPH)Cl_2$ (B) at RT.

TABLE IV. Antitumour Activity of 2-Pyridinecarboxaldehyde 2'-Pyridylhydrazone and Related Compounds and their Transition Metal Complexes Against the P388 Lymphocytic Leukaemia Test System in Mice

No.	Max. effective daily dose (mg/kg)[a]	Weight difference $(W - C)$[b] (%)	T/C[c] (%)
I	60	−7.5	138
II	60	−3.1	118
III	60	−1.1	120
IV	40	+0.7	107
V	40	−2.0	115
VI	60	+0.9	125
VII	60	−1.1	118
1	20	+1.6	107
2	20	+2.1	105
3	20	+2.6	107
4	40	+0.0	99
5	60	−3.1	180
6	40	−1.2	130
7	20	+0.8	125
8	20	+0.3	100
9	40	+1.2	99
10	40	−1.1	100
11	20	−2.1	117
12	40	−4.1	125
13	40	+0.7	127
14	20	−2.7	90
15	40	−3.3	100
16	40	+0.0	99
17	20	−5.9	112
18	60	−1.1	117
19	40	−4.5	129
20	40	+0.1	100
21	40	−3.2	98
22	20	+0.8	99
23	40	+0.2	112
24	60	−1.1	102
25	60	−1.0	99
26	40	+2.2	124
27	40	+2.0	116
28	60	−4.5	120
29	20	−3.0	100
30	40	−2.8	104
31	60	+0.2	118
32	40	+0.0	120
33	40	−3.1	127
34	60	−0.7	124
35	40	+0.4	125
36	20	−7.4	110
37	40	−5.2	107
38	40	−4.5	112
39	60	−5.7	124
40	40	−2.0	120
41	40	+1.1	117
42	20	+0.7	99
43	40	−2.2	99
44	60	−1.9	110
45	20	+2.2	120
46	40	+0.2	118
47	60	−3.1	126

TABLE IV. *(continued)*

(continued)

TABLE IV. *(continued)*

No.	Max. effective daily dose (mg/kg)[a]	Weight difference ($W - C$)[b] (%)	T/C[c] (%)
48	40	−2.1	119
49	20	+0.8	110

[a]Administered once daily for six consecutive days beginning 24 h after tumour transplantation. [b]Average change in body weight (%) from onset to termination of drug therapy. [c]T/C (%) = treated/control × 100.

systems, indicating that the agent possessed a wide spectrum of antitumour activity.

Essentially complete inhibition of the incorporation of ^{3}H-thymidine into DNA of Sarcoma-180 ascites cells occurred when the radioactive precursor was injected intraperitoneally into mice bearing 6-day accumulations of neoplastic cells 15 min after the administration of 1-formylisoquinoline thiosemicarbazone, IQ-1, at a dose level of 25 mg/kg and was allowed 1 h to be utilized [12]. This degree of blockade persisted for up to 12 h after IQ-1 but 24 h after the chelating agent inhibition was completely relieved. The data (Table VI) indicate that at the same level of dosage **5** caused essentially complete inhibition of ^{3}H-thymidine into DNA at 6 h. Twelve hours after, however, the inhibition by **5** was decreased, but after 24 h inhibition was completely terminated. As with α-(*N*)-heterocyclic thiosemicarbazones, the inhibition of the biosynthesis of RNA and protein was also produced by **5**; however, these metabolic processes were considerably less sensitive than was the replication of DNA [22]. Maximum inhibition of RNA synthesis was produced by IQ-1 3 h after administration of the drug, and the level of the inhibition decreased slowly thereafter [12]. Under essentially the same conditions, **5** inhibited the RNA synthesis to the same degree 12 h after administration of the drug, but after 18 h inhibition by **5** was decreased. Thus, the degree of inhibition of DNA and RNA synthesis of Sarcoma 180 cells *in vivo* was very comparable to that observed for α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones. These agents have long been

TABLE V. Effects of Compound **5** on the Survival Time of Mice Bearing either Sarcoma 180, Ehrlich Carcinoma, or Leukaemia L 1210 cells

Daily dosage (mg/kg)[a]	Sarcoma 180		Ehrlich ascites carcinoma		Leukaemia L 1210	
	Weight difference ($W - C$) (%)[b]	Average Survival time, days ±SE[c]	Weight difference ($W - C$) (%)	Average Survival time, days ±SE	Weight difference ($W - C$) (%)	Average Survival time, days ±SE
Control	+18.4	13.5 ± 0.7	+24.5	12.6 ± 1.2	+13.2	10.0 ± 1.4
20	+16.0	24.5 ± 2.5	+2.0	36.4 ± 6.0(10)	+8.9	11.4 ± 0.8
40	−3.3	31.4 ± 2.0(10)	−3.1	45.8 ± 2.0(50)	+3.0	12.5 ± 0.4
60	−7.2	32.5 ± 2.4(20)	−2.0	42.7 ± 3.2(40)	−4.5	14.2 ± 1.2

[a]Administered once daily for six consecutive days, beginning 24 h after tumour transplantation. [b]Average change in body weight (%) from onset to termination of drug therapy. [c]Percentage of mice that survived more than 50 days; these animals were calculated as 50-day survivors in determination of the average survival time.

TABLE VI. Incorporation of ^{3}H-Thymidine, ^{3}H-Uridine and DL-^{14}C-Leucine into DNA, RNA and Protein, respectively, of Sarcoma 180 Ascites Cells Treated with Compound **5**[a]

Dose (mg/kg)	Pretreatment (h) before radioactive precursor	% Inhibition		
		^{3}H-Thymidine into DNA	^{3}H-Uridine into RNA	DL-^{14}C-Leucine into protein
36.2	6	96		
	12	70	44	28
	18	18	15	
	24	0		

[a]Mice bearing 6-day implants of Sarcoma 180 ascites cells received a single intraperitoneal injection of 5 per kilogram of body weight. At the indicated times, thereafter, animals received the appropriate radioactive tracer by ip injection, which was allowed 1 h to be incorporated. Control specific activities were 22.2 cpm/nmol, 11.7 cpm/nmol and 6520 cpm/mg for ^{3}H-thymidine, ^{3}H-uridine and ^{14}C-leucine incorporation, respectively. These values represent the mean (±standard error) or results obtained with 8–16 mice analysed separately.

recognized as potent cytotoxic agents which exhibit tumour-inhibitory actions that have been attributed to their abilities to function as tridentate chelators of transition metal ions [22–25]. Early structure–activity studies established that the conjugate N*–N*–S* tridentate ligand system was an essential feature of compounds exhibiting antitumour activity [22]. Thus, salicylaldehyde thiosemicarbazones, analogues of 2-pyridinecarboxaldehyde thiosemicarbazone, were found to have no antitumour activity. The above results, however, indicate that the ligands that are similarly tridentate but which bind metal through an N*–N*–N* array of donor atoms are also potent inhibitors of cell growth and that, as in the α-(*N*)-heterocyclic carboxaldehyde thiosemicarbazones, complexation to copper increases tumour inhibitory activity [23, 26].

The inhibitory activity of complex **5** may be related to the glycyl-L-histidyl-L-lysine–copper(II) (GHL–Cu) complex which has been shown to be a potent inducer of *in vivo* angiogenesis in rabbits [27]. Thus, the antitumour activity of complex **5** may either be due to its general cytotoxicity or interference with the mechanisms which concentrate the copper ions required for tumour angiogenesis.

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