Antitumor Activity and Metal Complexes of the First Transition Series. *Trans*-bis(salicylaldoximato)copper(II) and Related Copper(II) Complexes, a Novel Group of Potential Antitumor Agents

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The antitumor activity of forty nine different metal complexes of the first transition series against mouse leukemia L 1210 cells and of two of the complexes against Ehrlich ascites carcinoma have been tested in vitro *by the method described in this paper. Eight complexes showed a 50% inhibition of tumor cell division at concentration level 5–6 μg/ml of the complex for the former and two most effective complexes also for the latter. The* trans-*bis(salicylaldoximato)copper(II) and* trans-*bis(resorcylaldoximato)copper(II) complexes were found to possess the highest antitumor activity.*

Introduction

The discovery and development of new, better cancer medicines is one of the main goals of present day medicine and chemical investigations. In recent years the exposition of the antitumor effects of inorganic and particularly metal complexes and their possible use to cure cancer diseases have received increasing attention.

The potential of metal complexes as anticancer drugs became widely known about fourteen years ago when Rosenberg and Van Camp with their co-workers published observations on strong antitumor effects of certain Pt compounds against mouse sarcoma 180 and leukemia [1]. Since then intensive studies of Pt complexes as possible anticancer agents have continued [2, 3]. Complexes of other platinum metals have also been tested in this respect, but not as extensively [2, 4–6].

Tests of other metal complexes have so far been rather few and further studies are needed [2, 4]. The antitumor effects of copper complexes of different thiosemicarbazones seem to have been investigated most intensively [2, 4, 7], whereas studies of other copper and metal complexes of the first transition series are rather few [2, 4].

The discovery of the antitumor effect of Pt complexes meant new advances in cancer medicine research. The Pt complexes are, however, rather poisonous, especially for the kidneys (nephrotoxicity). The upper limit of the dose used is determined on this basis [2, 4, 8]. The platinum metals are unnatural from the biological viewpoint and the body has not as effective mechanisms for their rejection as for the rejection of those metals which occur naturally in the body, *e.g.* copper and iron [7]. The toxicity of the Pt compounds may, however, be mitigated through abundant hydratation and mannitol diuretics [4, 8].

It would, however, be useful to find complexes of the naturally occurring metals which have strong antitumoral activity, but the decomposition products of which the organism is capable of removing easily through its normal mechanisms. Such metal complexes may possibly exist among those of the first transition series.

During the past thirty years we have prepared and studied in different physicochemical ways a great number of metal complexes of the first transition series. From these we have picked some groups and investigated their antitumor activity through cell culture *in vitro*. Some of the results of these experiments are reported and discussed in this paper.

Experimental

Metal Complexes

Altogether 48 selected metal complexes of earlier preparations [9] were tested plus one derivative of an effective proved compound, whose synthesis is described here.

Trans-bis(resorcylaldoximato)copper(II), complex No. 49, was prepared for comparison. According to the literature the complex dissolves in absol. ethan-

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TABLE I. Pyrazole Complexes. Cell Strain: L 1210. Complex Solvent: DMSO. Complex/Cell Culture = 25 μl/5 ml. CC = Complex Concentration in Cell Culture. B = Initial Density. A = Cell Density. Pyrazole = $C_3H_4N_2$,

No.	CC (μg/ml)	B (X × 10^6 cell/ml)	A (X × 10^6 cell/ml)		Y (%)			Ref.
			1 day	2 day	1 day	2 day	Complex	
Control	–	0.222	0.619	1.564				
Control	–	0.222	0.632	1.567				
Solvent								
Control	–	0.222	0.564	1.408	100	100		
Solvent								
Control	–	0.222	0.623	1.420	100	100		
1	108	0.222	0.226	0.215	1	−1	$Co(C_3H_4N_2)_4Br_2$	9
1	27.0	0.222	0.441	0.734	59	43	$Co(C_3H_4N_2)_4Br_2$	
1	5.41	0.222	0.551	1.230	89	85	$Co(C_3H_4N_2)_4Br_2$	
2	158	0.217	0.214	0.209	−1	−1	$Co(C_3H_4N_2)_7(SCN)_2$	9
2	39.4	0.217	0.271	0.263	19	5	$Co(C_3H_4N_2)_7(SCN)_2$	
2	7.88	0.217	0.469	0.913	89	79	$Co(C_3H_4N_2)_7(SCN)_2$	
3	51.7	0.211	0.500	1.121	69	75	$Ni(C_3H_4N_2)_4Cl_2$	9
3	17.2	0.211	0.597	1.317	92	91	$Ni(C_3H_4N_2)_4Cl_2$	
3	5.74	0.211	0.602	1.496	93	106	$Ni(C_3H_4N_2)_4Cl_2$	
4	101	0.213	0.398	0.617	50	38	$Ni(C_3H_4N_2)_4Br_2$	9
4	25.3	0.213	0.582	1.425	101	114	$Ni(C_3H_4N_2)_4Br_2$	
4	5.05	0.213	0.610	1.427	109	114	$Ni(C_3H_4N_2)_4Br_2$	
5	102	0.213	0.392	0.609	49	37	$Ni(C_3H_4N_2)_4(NO_3)_2$	9
5	25.5	0.213	0.536	1.383	88	110	$Ni(C_3H_4N_2)_4(NO_3)_2$	
5	5.10	0.213	0.558	1.404	95	112	$Ni(C_3H_4N_2)_4(NO_3)_2$	
6	25.8	0.213	0.559	1.291	95	101	$Ni(C_3H_4N_2)_4SO_4$	9
6	5.15	0.213	0.588	1.396	103	111	$Ni(C_3H_4N_2)_4SO_4$	

ol [10]. The synthesis method was a modification of the literature method [10]. 1.5315 g (10 mmol) of resorcylaldoxime was dissolved in 175 ml of absol. ethanol plus 25 ml of 0.01 *M* HCl. The solution was heated to 60 °C. To this a solution which was prepared by dissolving 0.9989 g (5 mmol) of copper(II) diacetate monohydrate (Merck, *p.a.*) in 50 ml of 0.01 *M* HCl was added slowly. The solution turned brown-green and a dark precipitate separated. The solution was allowed to cool slowly. After standing one week the solution was filtered, the precipitate washed three times with water and allowed to dry on the sinter. The dried product was grey-brown. This was used in experiments *in vitro*. The TG curve showed no escape of crystal water at 90–95 °C in contrast to the results reported in the literature [10]. The product decomposed suddenly at 230–250 °C. According to the TG curve the product contained no crystal water.

Cell Culture

Primary screening was done using leukemia L 1210 cells. For some experiments, Ehrlich ascites carcinoma cells were employed. The cells were cultivated in RPMI 1640 medium (Gibco) supplemented with 5% (v/v) of pooled human serum (Transfusion Service, Finnish Red Cross, Helsinki, Finland), 2 g of sodium bicarbonate (Merck, *pro analysi*) per litre, 50 mg of the sodium salt of penicillin G (Hoechst) per litre and 50 mg of streptomycin per litre. Cells were counted using a particle counter (Coulter Counter, model Industrial D). A confluent cell suspension was diluted, the cells were counted and 5 ml aliquots of the diluted suspension were pipetted into sterile disposable plastic tubes of 10 ml volume (Tube 144 AS, Sterilin Ltd.). The test substances were added either as solutions (most in dimethylsulfoxide (Merck, *p.a.*), some in absolute ethanol or in water) or as suspensions in dimethylsulfoxide (DMSO). The volume added was normally 25 μl. Only aqueous solutions were sterilized (filter sterilization). Controls (nothing added) and solvent controls were employed for each cultivation series. The tubes were incubated at 37 °C for 2 or 3 days, with the corks closed. The cells were counted after 1, 2 and 3 days. All cultivations were performed as blind experiments.

TABLE II. Pyrazole Complexes. Cell Strain: L 1210. Complex Solvent: DMSO. Complex/Cell Culture = 25 μl/5 ml. Pyrazole = $C_3H_4N_2$,

No.	CC (μg/ml)	B (X × 10^6 cell/ml)	A (X × 10^6 cell/ml)		Y (%)			Ref.
			1 day	2 day	1 day	2 day	Complex	
7	112	0.217	0.213	0.237	−1	2	$Cu(C_3H_4N_2)_4Cl_2$	9
7	28.1	0.217	0.216	0.224	0	1	$Cu(C_3H_4N_2)_4Cl_2$	
7	5.63	0.217	0.434	0.752	77	61	$Cu(C_3H_4N_2)_4Cl_2$	
8	107	0.217	0.218	0.219	0	0	$Cu(C_3H_4N_2)_4(NO_3)_2$	9
8	26.8	0.217	0.220	0.216	1	0	$Cu(C_3H_4N_2)_4(NO_3)_2$	
8	5.35	0.217	0.477	0.940	92	82	$Cu(C_3H_4N_2)_4(NO_3)_2$	
9	105	0.213	0.211	0.218	−1	0	$Cu(C_3H_4N_2)_4Br_2$	9
9	26.1	0.213	0.252	0.263	11	5	$Cu(C_3H_4N_2)_4Br_2$	
9	5.23	0.213	0.536	1.094	88	83	$Cu(C_3H_4N_2)_4Br_2$	
10	105	0.213	0.199	0.201	−4	−1	$Cu(C_3H_4N_2)_4SO_4 \cdot H_2O$	9
10	26.1	0.213	0.204	0.214	−2	0	$Cu(C_3H_4N_2)_4SO_4 \cdot H_2O$	
10	5.23	0.213	0.536	0.968	88	71	$Cu(C_3H_4N_2)_4SO_4 \cdot H_2O$	
11	21.9	0.213	0.230	0.232	5	2	$Cu(C_3H_4N_2)_4SO_4 \cdot H_2O$	9
11	5.48	0.213	0.482	0.942	74	68	$Cu(C_3H_4N_2)_4SO_4 \cdot H_2O$	
12	104	0.222	0.229	0.241	19	2	$Zn(C_3H_4N_2)_6SO_4 \cdot H_2O$	9
12	26.1	0.222	0.353	0.693	35	40	$Zn(C_3H_4N_2)_6SO_4 \cdot H_2O$	
12	5.21	0.222	0.588	1.422	99	101	$Zn(C_3H_4N_2)_6SO_4 \cdot H_2O$	

Results and Discussion

To avoid personal influence on the measurements the complexes were studied using code numbers. The results are represented in Tables I–XVI, but rearranged according to the organic and inorganic ligand type and the transition metal. Control measurements were performed in connection with every measurement series, but are given as an example only in Table I for the first complex.

The Tables include for each sample the order number, the concentration of the complex, the cell density at the beginning and after 1 and 2 days, the complex formula, its reference and the quantity Y in per cent. The extent Y is calculated as follows:

$$Y\,(\%) = ((A - B)/(C - B)) \times 100 \qquad (1)$$

where A = the cell density of the sample tube, B = the initial cell density, and C = the mean cell density of the solvent controls.

In our opinion the quantity Y represents well the ability of the complex to prevent the increase of the number of cells, because it indicates the increase of the cell density of the sample tube in percent compared with the increase which occurs when the tube does not contain the complex. In the test instructions of antitumor activity from the National Cancer Institute of the U.S.A. [24] a similar function with the symbol Y, which is based on protein content, is used.

In *in vitro* experiments there are difficulties in specifying the lower limits of concentration at which the compounds should be effective in order to extend experiments *in vivo*. The definition of exact concentration limits may be considered as arbitrary but rough limits can be outlined. It should be noted, however, that the usual cell culture methods do not give any indication of the general toxicity of the compound for animals or man, or of the optimum effective dose which determines how serviceable the compound is.

According to the test instructions of the National Cancer Institute [24] a synthesized compound is studied further if the concentration of the compound causing a 50% inhibition of the growth in cell culture is in the first test $\leqq 6$ μg/ml and as a mean of two tests $\leqq 4$ μg/ml. This has also been the criterion in the present study, although the test methods are not identical.

Considering the results in Tables I–XVI it is possible to make certain conclusions as to the strength of the inhibition tendency of the compounds and its dependence on the nature of the compounds, their ligands, metals, structure and bond type. The tested metal complexes may then be divided into three main groups which are now examined.

TABLE III. 2-Aminopyrimidine Complexes. Cell Strain: L 1210. Complex Solvent: DMSO or Absol. Ethanol*. Complex/Cell Culture = 25 μl/5 ml. *m*-Chloraniline = C_6H_6ClN.

No.	CC (μg/ml)	B (X × 10^6 cell/ml)	A (X × 10^6 cell/ml)		Y (%)			Ref.
			1 day	2 day	1 day	2 day	Complex	
13	100	0.217	0.190	0.172	−10	−5	$Co(C_4H_5N_3)SO_4 \cdot 4H_2O$	9
13	25.0	0.217	0.316	0.481	35	30	$Co(C_4H_5N_3)SO_4 \cdot 4H_2O$	
13	5.00	0.217	0.474	0.845	91	71	$Co(C_4H_5N_3)SO_4 \cdot 4H_2O$	
14	20.9	0.273	0.648	1.486	110	96	$Ni(C_4H_5N_3)_2Cl_2 \cdot 4H_2O$	11
14	5.22	0.273	0.659	1.518	114	99	$Ni(C_4H_5N_3)_2Cl_2 \cdot 4H_2O$	
15	66.2	0.273	0.504	0.932	68	52	$Ni(C_4H_5N_3)_2Br_2 \cdot 4H_2O$	11
15	22.1	0.273	0.630	1.513	105	98	$Ni(C_4H_5N_3)_2Br_2 \cdot 4H_2O$	
15	5.52	0.273	0.630	1.642	105	108	$Ni(C_4H_5N_3)_2Br_2 \cdot 4H_2O$	
16*	81.4	0.345	0.526	0.687	41	33	$Ni(C_4H_5N_3)_2(SCN)_2 \cdot 4H_2O$	9
16*	16.3	0.345	0.785	1.417	99	95	$Ni(C_4H_5N_3)_2(SCN)_2 \cdot 4H_2O$	
16*	3.25	0.345	0.753	1.500	92	102	$Ni(C_4H_5N_3)_2(SCN)_2 \cdot 4H_2O$	
17	48.4	0.222	0.520	0.987	80	64	$Ni(C_4H_5N_3)_2(NO_3)_2 \cdot H_2O$	9
17	16.1	0.222	0.607	1.261	104	87	$Ni(C_4H_5N_3)_2(NO_3)_2 \cdot H_2O$	
17	5.38	0.222	0.584	1.169	97	79	$Ni(C_4H_5N_3)_2(NO_3)_2 \cdot H_2O$	
18	162	0.217	0.293	0.317	27	11	$Ni(C_4H_5N_3)_2SO_4 \cdot 4H_2O$	9
18	40.4	0.217	0.461	0.925	87	80	$Ni(C_4H_5N_3)_2SO_4 \cdot 4H_2O$	
18	6.73	0.217	0.525	1.287	109	121	$Ni(C_4H_5N_3)_2SO_4 \cdot 4H_2O$	

TABLE IV. 2-Aminopyrimidine Complexes. Cell Strain: L 1210. Complex Solvent: DMSO. Complex/Cell Culture = 25 μl/5 ml. 2-Aminopyrimidine = $C_4H_5N_3$.

No.	CC (μg/ml)	B (X × 10^6 cell/ml)	A (X × 10^6 cell/ml)		Y (%)			Ref.
			1 day	2 day	1 day	2 day	Complex	
19	108	0.267	0.497	0.578	42	26	$Cu_2(C_4H_5N_3)_5(ClO_4)_2 \cdot 2H_2O$	9
19	26.9	0.267	0.376	0.393	20	13	$Cu_2(C_4H_5N_3)_5(ClO_4)_2 \cdot 2H_2O$	
19	5.38	0.267	0.814	1.333	101	89	$Cu_2(C_4H_5N_3)_5(ClO_4)_2 \cdot 2H_2O$	
20	103	0.267	0.440	0.488	32	19	$Cu(C_4H_5N_3)_2SO_4 \cdot 3H_2O$	11
20	25.8	0.267	0.366	0.415	18	12	$Cu(C_4H_5N_3)_2SO_4 \cdot 3H_2O$	
20	5.15	0.267	0.907	1.331	118	89	$Cu(C_4H_5N_3)_2SO_4 \cdot 3H_2O$	
21	100	0.211	0.265	0.247	13	3	$Cu(C_4H_5N_3)_2(NO_3)_2$	11
21	25.0	0.211	0.261	0.269	12	5	$Cu(C_4H_5N_3)_2(NO_3)_2$	
21	5.0	0.211	0.545	0.804	80	49	$Cu(C_4H_5N_3)_2(NO_3)_2$	

A. Mixed Coordination Complexes in which the Organic Ligands are Coordinated to the Central Metal Atom through Nitrogen Donor Atoms and the Metal Charge is Compensated through Inorganic Anions (Tables I–IX)

In this group complexes 1, 2, 7–11, 13, 28, 30 and 33 (Tables I–III and VII–IX) are able to prevent the cell division of L 1210 completely at a concentration level of about 100 μg/ml. At 25 μg/ml level this is achieved with complexes 7, 8, 10 and 11 (Table II).

If the criterion is 50% inhibition at a concentration of 5–6 μg/ml then only complex No. 28 (Table VII) fulfils this requirement.

In considering the pyrazole complexes in Tables I and II it may be concluded that the cell division preventing effect increases for the same inorganic anion containing complexes in the order Ni < Co < Zn < Cu when the number of unpaired 3d electrons changes as follows: 2, 3, 0, 1.

For the same metal-including complexes the inhibiting effect increases depending on the inor-

TABLE V. Aniline and *o*-Chloraniline Complexes. Cell Strain: L 1210. Complex Solvent: DMSO. Complex/Cell Culture = 25 μl/5 ml. Aniline = C_6H_7N. *o*-Chloroaniline = C_6H_6ClN.

No.	CC (μg/ml)	B (X × 10^6 cell/ml)	A (X × 10^6 cell/ml)		Y (%)			Ref.
			1 day	2 day	1 day	2 day	Complex	
23	33.7	0.269	0.686	1.085	76	65	$Ni(C_6H_7N)_2Br_2 \cdot 2H_2O$	12
23	13.5	0.269	0.757	1.376	89	88	$Ni(C_6H_7N)_2Br_2 \cdot 2H_2O$	
23	5.4	0.269	0.723	1.457	83	95	$Ni(C_6H_7N)_2Br_2 \cdot 2H_2O$	
24	13.4	0.269	0.706	1.328	80	85	$Ni(C_6H_7N)_2SO_4$	12
24	5.4	0.269	0.762	1.497	90	98	$Ni(C_6H_7N)_2SO_4$	
25	34.0	0.273	0.593	1.140	94	69	$Ni(C_6H_6ClN)_2Br_2$	12
25	11.3	0.273	0.603	1.355	97	86	$Ni(C_6H_6ClN)_2Br_2$	
25	5.67	0.273	0.655	1.365	113	87	$Ni(C_6H_6ClN)_2Br_2$	

TABLE VI. *m*-Chloraniline Complexes. Cell Strain: L 1210. Complex Solvent: Ethanol (Absol.). Complex/Cell Culture = 25 μl/5 ml. *m*-Chloraniline = C_6H_6ClN.

No.	CC (μg/ml)	B (X × 10^6 cell/ml)	A (X × 10^6 cell/ml)		Y (%)			Ref.
			1 day	2 day	1 day	2 day	Complex	
26	35.3	0.200	0.345	0.482	64	79	$Ni(C_6H_6ClN)_4Br_2$	13
26	17.6	0.200	0.357	0.485	70	80	$Ni(C_6H_6ClN)_4Br_2$	
26	8.81	0.200	0.368	0.493	75	82	$Ni(C_6H_6ClN)_4Br_2$	
26	4.41	0.200	0.358	0.502	70	84	$Ni(C_6H_6ClN)_4Br_2$	
26	2.20	0.200	0.368	0.507	75	86	$Ni(C_6H_6ClN)_4Br_2$	
26	1.10	0.200	0.375	0.510	78	87	$Ni(C_6H_6ClN)_4Br_2$	
26	0.55	0.200	0.386	0.542	83	96	$Ni(C_6H_6ClN)_4Br_2$	
27	50	0.215	0.516	0.939	79	73	$Ni(C_6H_6ClN)_4I_2$	14
27	20	0.215	0.645	1.437	113	123	$Ni(C_6H_6ClN)_4I_2$	
27	8.0	0.215	0.596	1.155	100	95	$Ni(C_6H_6ClN)_4I_2$	
27	3.2	0.215	0.597	1.384	101	118	$Ni(C_6H_6ClN)_4I_2$	
27	1.3	0.215	0.629	1.461	109	125	$Ni(C_6H_6ClN)_4I_2$	
27	0.51	0.215	0.596	1.377	100	117	$Ni(C_6H_6ClN)_4I_2$	
27	0.10	0.215	0.612	1.297	104	109	$Ni(C_6H_6ClN)_4I_2$	
27	0.02	0.215	0.597	1.274	101	106	$Ni(C_6H_6ClN)_4I_2$	

TABLE VII. Quinoxaline Complexes. Cell Strain: L 1210. Complex Solvent: Ethanol (Absol.) or Water*. Complex/Cell Culture = 25 μl/5 ml. Quinoxaline = $C_8H_6N_2$.

No.	CC (μg/ml)	B (X × 10^6 cell/ml)	A (X × 10^6 cell/ml)		Y (%)			Ref.
			1 day	2 day	1 day	2 day	Complex	
28	232	0.200	0.201	0.148	0	−15	$Co(C_8H_6N_2)_2(OH)(ClO_4)_2 \cdot 6H_2O$	9
28	128	0.200	0.164	0.149	−16	−14	$Co(C_8H_6N_2)_2(OH)(ClO_4)_2 \cdot 6H_2O$	
28	50	0.200	0.265	0.250	29	14	$Co(C_8H_6N_2)_2(OH)(ClO_4)_2 \cdot 6H_2O$	
28	25	0.200	0.278	0.312	35	31	$Co(C_8H_6N_2)_2(OH)(ClO_4)_2 \cdot 6H_2O$	
28	12.5	0.200	0.304	0.394	46	54	$Co(C_8H_6N_2)_2(OH)(ClO_4)_2 \cdot 6H_2O$	
28	6.25	0.200	0.291	0.403	40	57	$Co(C_8H_6N_2)_2(OH)(ClO_4)_2 \cdot 6H_2O$	

(continued overleaf)

TABLE VII. *(continued)*

No.	CC (μg/ml)	B (X × 10^6 cell/ml)	A (X × 10^6 cell/ml)		Y (%)			Ref.
			1 day	2 day	1 day	2 day	Complex	
28	3.13	0.200	0.314	0.424	51	63	$Co(C_8H_6N_2)_2(OH)(ClO_4)_2 \cdot 6H_2O$	9
28	1.56	0.200	0.365	0.465	73	74	$Co(C_8H_6N_2)_2(OH)(ClO_4)_2 \cdot 6H_2O$	
28	0.78	0.200	0.356	0.524	69	91	$Co(C_8H_6N_2)_2(OH)(ClO_4)_2 \cdot 6H_2O$	
29*	310	0.345	0.383	0.387	7	3	$Ni(C_8H_6N_2)_2(ClO_4)_2 \cdot 6H_2O$	9
29*	31	0.345	0.846	1.563	93	99	$Ni(C_8H_6N_2)_2(ClO_4)_2 \cdot 6H_2O$	
29*	3.1	0.345	0.908	1.485	105	93	$Ni(C_8H_6N_2)_2(ClO_4)_2 \cdot 6H_2O$	

TABLE VIII. Comparative Measurements. Cell Strain: L 1210. Complex Solvent: Ethanol (Absol.). Complex/Cell Culture = 25 μl/5 ml. Quinoxaline = $C_8H_6N_2$. COP = $Co(ClO_4)_2 \cdot 6H_2O$ (G. F. Smith Co.). SOP = $NaClO_4$ (G. F. Smith Co.).

No.	CC (μg/ml)	B (X × 10^6 cell/ml)	A (X × 10^6 cell/ml)		Y (%)			Ref.
			1 day	2 day	1 day	2 day	Complex	
30	50.0	0.325	0.357	0.343	11	2	$Co(C_8H_6N_2)_2(OH)(ClO_4)_2 \cdot 6H_2O$	9
30	6.25	0.325	0.510	0.845	63	57	$Co(C_8H_6N_2)_2(OH)(ClO_4)_2 \cdot 6H_2O$	
30	1.55	0.325	0.643	1.128	109	88	$Co(C_8H_6N_2)_2(OH)(ClO_4)_2 \cdot 6H_2O$	
COP	132	0.345	0.323	0.314	−5	−3	$Co(ClO_4)_2 \cdot 6H_2O$	–
COP	28.6	0.325	0.334	0.295	3	−3	$Co(ClO_4)_2 \cdot 6H_2O$	
COP	3.55	0.325	0.545	0.923	75	67	$Co(ClO_4)_2 \cdot 6H_2O$	
COP	1.77	0.345	0.751	1.579	91	109	$Co(ClO_4)_2 \cdot 6H_2O$	
COP	0.890	0.325	0.599	1.065	94	81	$Co(ClO_4)_2 \cdot 6H_2O$	
COP	0.887	0.345	0.701	1.368	80	91	$Co(ClO_4)_2 \cdot 6H_2O$	
COP	0.443	0.345	0.765	1.513	95	104	$Co(ClO_4)_2 \cdot 6H_2O$	
SOP	19.2	0.325	0.564	1.169	82	92	$NaClO_4$	–
SOP	2.40	0.325	0.568	1.037	83	78	$NaClO_4$	
SOP	0.600	0.325	0.658	1.163	114	92	$NaClO_4$	

TABLE IX. Nicotinic Acid Amide and Hydrazide Complexes. Cell Strain: L 1210. Complex Solvent: DMSO. Complex/Cell Culture = 25 μl/5 ml. Nicotinic Acid Amide = $C_6H_6N_2O$. Nicotinic Acid Hydrazide = $C_6H_7N_3O$. s = Suspension.

No.	CC (μg/ml)	B (X × 10^6 cell/ml)	A (X × 10^6 cell/ml)		Y (%)			Ref.
			1 day	2 day	1 day	2 day	Complex	
31	25.8s	0.273	0.588	1.069	93	63	$Ni(C_6H_6N_2O)_2Cl_2$	9
31	5.16s	0.273	0.558	1.291	84	81	$Ni(C_6H_6N_2O)_2Cl_2$	
32	67.2	0.273	0.530	0.869	76	47	$Ni(C_6H_6N_2O)_2SO_4 \cdot 6H_2O$	9
32	22.4	0.273	0.560	1.183	85	72	$Ni(C_6H_6N_2O)_2SO_4 \cdot 6H_2O$	
32	5.60	0.273	0.628	1.301	105	81	$Ni(C_6H_6N_2O)_2SO_4 \cdot 6H_2O$	
33	83.0	0.273	0.261	0.253	−4	−2	$Co(C_6H_7N_3O)_2Cl_2 \cdot 2H_2O$	9
33	27.7	0.273	0.444	0.623	50	28	$Co(C_6H_7N_3O)_2Cl_2 \cdot 2H_2O$	
33	5.53	0.273	0.523	1.105	74	66	$Co(C_6H_7N_3O)_2Cl_2 \cdot 2H_2O$	
34	1.9s	0.273	0.610	1.308	99	82	$Ni(C_6H_7N_3O)_2I_2$	9
35	37.9	0.273	0.573	1.142	88	69	$Ni(C_6H_7N_3O)_2(NO_3)_2 \cdot 3H_2O$	9
35	12.6	0.273	0.583	1.161	91	70	$Ni(C_6H_7N_3O)_2(NO_3)_2 \cdot 3H_2O$	
35	6.31	0.273	0.569	1.249	87	77	$Ni(C_6H_7N_3O)_2(NO_3)_2 \cdot 3H_2O$	

TABLE X. 2-Pyridine- and 2-Thiophenecarboxylic Acid Complexes. Cell Strain: L 1210. Complex Solvent: DMSO. Complex/Cell Culture = 25 μl/5 ml. 2-Pyridine carboxylic Acid = $C_6H_5NO_2$. 2-Thiophenecarboxylic Acid = $C_5H_4O_2S$. Ethanol = C_2H_6O. s = Suspension.

No.	CC (μg/ml)	B (X × 10^6 cell/ml)	A (X × 10^6 cell/ml)		Y (%)			Ref.
			1 day	2 day	1 day	2 day	Complex	
36	7.4	0.222	0.451	0.553	62	28	$Cu(C_6H_4NO_2)_2 \cdot H_2O$	9, 15
36	2.5	0.222	0.546	0.937	87	60	$Cu(C_6H_4NO_2)_2 \cdot H_2O$	
37	29.8s	0.222	0.254	0.238	9	13	$Co(C_5H_3O_2S)Cl \cdot (C_2H_6O)(H_2O)$	16, 17
37a	14.9s	0.222	0.437	0.679	58	38	$Co(C_5H_3O_2S)Cl \cdot (C_2H_6O)(H_2O)$	
38	16.8s	0.211	0.377	0.556	40	29	$Co(C_5H_3O_2S)Cl \cdot (C_2H_6O)(H_2O)$	
38	5.59s	0.211	0.488	1.000	66	65	$Co(C_5H_3O_2S)Cl \cdot (C_2H_6O)(H_2O)$	
38	1.86s	0.211	0.550	1.402	81	99	$Co(C_5H_3O_2S)Cl \cdot (C_2H_6O)(H_2O)$	
39	19s	0.211	0.634	1.360	101	95	$Ni(C_5H_3O_2S)Cl \cdot (C_2H_6O)(H_2O)$	16, 17
39	6.3s	0.211	0.645	1.454	104	103	$Ni(C_5H_3O_2S)Cl \cdot (C_2H_6O)(H_2O)$	
39	6.9	0.211	0.647	1.483	104	105	$Ni(C_5H_3O_2S)Cl \cdot (C_2H_6O)(H_2O)$	
40	14.8	0.265	0.285	0.290	5	2	$Cu(C_5H_3O_2S)_2 \cdot H_2O$	16, 17
40	4.9	0.265	0.624	1.108	95	64	$Cu(C_5H_3O_2S)_2 \cdot H_2O$	
40	1.6	0.265	0.665	1.526	106	96	$Cu(C_5H_3O_2S)_2 \cdot H_2O$	

TABLE XI. 2-Indole-, 2-Quinoline- and 8-Quinolinecarboxylic Acid Complexes. Cell Strain: L 1210. Complex Solvent: DMSO. Complex/Cell Culture = 25 μl/5 ml. 2-Indolecarboxylic Acid = $C_9H_7NO_2$. 2- and 8-Quinolinecarboxylic Acid = $C_{10}H_7NO_2$. s = Suspension.

No.	CC (μg/ml)	B (X × 10^6 cell/ml)	A (X × 10^6 cell/ml)		Y (%)			Ref.
			1 day	2 day	1 day	2 day	Complex	
41	30.3	0.265	0.343	0.375	21	8	$Co(C_9H_6NO_2)_2 \cdot 2H_2O$	17, 18
41	10.1	0.265	0.524	0.933	69	51	$Co(C_9H_6NO_2)_2 \cdot 2H_2O$	
41	3.36	0.265	0.595	1.134	87	66	$Co(C_9H_6NO_2)_2 \cdot 2H_2O$	
42	9.55	0.265	0.679	1.677	110	107	$Ni(C_9H_6NO_2)_2 \cdot 2H_2O$	17, 18
42	3.82	0.265	0.658	1.544	104	97	$Ni(C_9H_6NO_2)_2 \cdot 2H_2O$	
43	21.3	0.265	0.273	0.274	2	1	$Cu(C_9H_6NO_2)_2 \cdot 2H_2O$	17, 18
43	19.8	0.213	0.266	0.267	15	5	$Cu(C_9H_6NO_2)_2 \cdot 2H_2O$	
43	10.7	0.265	0.484	0.628	58	28	$Cu(C_9H_6NO_2)_2 \cdot 2H_2O$	
43	6.58	0.213	0.483	0.815	74	56	$Cu(C_9H_6NO_2)_2 \cdot 2H_2O$	
43	5.3	0.265	0.572	0.892	81	48	$Cu(C_9H_6NO_2)_2 \cdot 2H_2O$	
44	2.75	0.211	0.559	0.904	83	57	$Cu(C_{10}H_6NO_2)_2$ 2-Q.c.a.	19, 20
45	7.5	0.273	0.474	0.646	59	30	$Cu(C_{10}H_6NO_2)_2$ 8-Q.c.a.	19, 21

ganic anion in the order: $NO_3^- < SCN^- < Br^- < SO_4^{2-} < Cl^-$, but the differences are small.

Among the 2-aminopyrimidine complexes (Tables III and IV) inhibition of cell division increases in correlation with the metal ions in the order: Ni < Cu < Co, but with respect to the anions in the order: $SO_4^{2-} < ClO_4^- < Cl^- < Br^- < SCN^- < NO_3^-$. The differences, however, are very small here also.

The aniline and *o*- and *m*-chloraniline Ni(II) complexes (Tables V and VI) seem to be about equally ineffective against tumor cell proliferation.

The tests in Tables VII and VIII indicate that the obstructing property of the quinoxaline complex of No. 28 is dependent on its special character and not on the metal ion (Co^{3+}) or ligands only.

The nicotinic acid amide and hydrazide complexes (Table IX) do not show any recognizable inhibiting effect on tumor cell division.

On the basis of these results it may be concluded that the complexes which can dissociate into the inorganic anions and the central ion coordinated with the organic ligands do not easily

TABLE XII. Salicylaldoxime Complexes and the Ligand. Cell Strain: L 1210. Complex Solvent: DMSO. Complex/Cell Culture = Z or 25 μl/5 ml. Salicylaldoxime = $C_7H_7NO_2$. S-W = $C_7H_6NO_2Na$ in Water. S-D = $C_7H_6NO_2Na$ in DMSO.

No.	CC (μg/ml)	B (X × 10^6) cell/ml)	A (X × 10^6 cell/ml)		Y (%)			Ref.
			1 day	2 day	1 day	2 day	Complex	
46	103.5	0.291	0.278	0.273	−3	−2	$Co(C_7H_6NO_2)_2$	17, 22, 23
46	25.9	0.291	0.290	0.264	0	2	$Co(C_7H_6NO_2)_2$	
46	5.18	0.291	0.527	0.779	49	43	$Co(C_7H_6NO_2)_2$	
47	106	0.267	0.243	0.259	−4	−1	$Ni(C_7H_6NO_2)_2$	17, 22, 23
47, Z = 10	42.5	0.267	0.246	0.255	−5	−1	$Ni(C_7H_6NO_2)_2$	
47	26.6	0.267	0.264	0.296	−1	2	$Ni(C_7H_6NO_2)_2$	
47	5.31	0.267	0.767	1.602	92	112	$Ni(C_7H_6NO_2)_2$	
48, Z = 10	44.8	0.267	0.263	0.285	−1	2	$Cu(C_7H_6NO_2)_2$	17, 22, 23
48	28.0	0.267	0.270	0.275	1	1	$Cu(C_7H_6NO_2)_2$	
48	25.9	0.291	0.298	0.293	1	0	$Cu(C_7H_6NO_2)_2$	17
48	12.9	0.291	0.292	0.297	0	1	$Cu(C_7H_6NO_2)_2$	
48	6.46	0.291	0.282	0.299	−2	1	$Cu(C_7H_6NO_2)_2$	
48	5.61	0.267	0.256	0.244	−2	−2	$Cu(C_7H_6NO_2)_2$	
48	3.23	0.291	0.719	1.210	89	78	$Cu(C_7H_6NO_2)_2$	
S-W	21.1	0.291	0.743	1.147	94	73	$C_7H_6NO_2Na$	–
S-W	10.6	0.291	0.828	1.477	111	101	$C_7H_6NO_2Na$	
S-W	5.28	0.291	0.817	1.549	109	107	$C_7H_6NO_2Na$	
S-W	2.64	0.291	0.823	1.568	110	109	$C_7H_6NO_2Na$	
S-D	21.5	0.291	0.703	1.412	86	96	$C_7H_6NO_2Na$	
S-D	10.8	0.291	0.765	1.512	99	104	$C_7H_6NO_2Na$	
S-D	5.38	0.291	0.772	1.508	100	104	$C_7H_6NO_2Na$	
S-D	2.69	0.291	0.791	1.517	104	105	$C_7H_6NO_2Na$	

penetrate the tumor cell membrane to get into the protoplasm.

B. Metal Carboxylates of the Heterocylic Carboxylic acids (Tables X and XI)

Within this group all studied Cu(II) and Co(II) complexes (Tables X and XI), but not the Ni(II) complexes, gave 100% obstruction of the cell division of L 1210 at a concentration level of about 100 μg/ml. At a level of 25 μg/ml this effect is achieved with complexes 40 and 43.

50% prevention of the cell proliferation of L 1210 at the 5–6 μg/ml concentration level was achieved by the Cu(II) complexes 36 and 43–45 (Tables X and XI) after 2 days. This result is interesting, because the organic ligands are N-heterocyclic carboxylic acids and the complexes generally possess a planar *trans*-chelate structure and are Cu(II) complexes [17–21].

Obviously the metal carboxylates have greater permeability of the cell membrane of a tumor cell compared with the metal complexes of the preceding group (A).

C. Metal Aldoximates of Salicylaldoxime and Related Aldoximes (Tables XII–XVI)

This group, which comprises the metal aldoximates of salicylaldoxime and related aldoximes, is the most interesting and promising among those tested.

Within this group complexes 46 and 47 (Table XII) show 100% prevention of L 1210 cell division at the 25 μg/ml concentration level. At a concentration level of about 5 μg/ml only complexes 48 and 49 (Tables XII, XIII and XV) gave the same results. Only there complexes in this group, namely 46, 48 and 49 (Tables XII, XIII and XV) are able to show 50% obstruction of cell proliferation at the 5–6 μg/ml concentration level. They are all copper(II) complexes, except No. 46.

With respect to Ehrlich ascites carcinoma also, complexes 48 and 49 (Tables XIV and XVI) are able to obstruct cell division by 50% at a 5 μg/ml concentration of the metal complex *in vitro*.

The experiments on salicylaldoxime (Table XII) and copper(II) dichloride (Table XIII) with respect to L 1210 show that the prevention of tumor cell divi-

TABLE XIII. *Trans*-bis(salicylaldoximato)copper(II) and Copper(II) Dichloride Dihydrate. Cell Strain: L 1210. Complex Solvent: DMSO. Complex/Cell Culture = 25 μl/5 ml. CUC-W = $CuCl_2 \cdot 2H_2O$ in Water. CUC-D = $CuCl_2 \cdot 2H_2O$ in DMSO.

No.	CC (μg/ml)	B (X × 10^6 cell/ml)	A (X × 10^6 cell/ml)		Y (%)			Ref.
			1 day	2 day	1 day	2 day	Complex	
48	25.9	0.311	0.329	0.332	5	3	$Cu(C_7H_6NO_2)_2$	17, 22, 23
48	12.9	0.311	0.332	0.334	5	3	$Cu(C_7H_6NO_2)_2$	
48	6.46	0.311	0.320	0.315	2	1	$Cu(C_7H_6NO_2)_2$	
48	3.23	0.311	0.703	0.923	98	90	$Cu(C_7H_6NO_2)_2$	
48	1.62	0.311	0.678	0.796	92	72	$Cu(C_7H_6NO_2)_2$	
48	0.808	0.311	0.715	0.930	102	91	$Cu(C_7H_6NO_2)_2$	
48	0.404	0.311	0.736	1.005	107	103	$Cu(C_7H_6NO_2)_2$	
CUC-W	13.1	0.311	0.463	0.551	38	33	$CuCl_2 \cdot 2H_2O$	–
CUC-W	3.26	0.311	0.686	0.842	93	74	$CuCl_2 \cdot 2H_2O$	
CUC-D	13.1	0.311	0.468	0.536	39	33	$CuCl_2 \cdot 2H_2O$	
CUC-D	6.57	0.311	0.564	0.661	64	52	$CuCl_2 \cdot 2H_2O$	
CUC-D	3.28	0.311	0.660	0.726	88	61	$CuCl_2 \cdot 2H_2O$	
CUC-D	1.64	0.311	0.694	0.820	96	75	$CuCl_2 \cdot 2H_2O$	
CUC-D	0.821	0.311	0.702	0.843	98	79	$CuCl_2 \cdot 2H_2O$	
CUC-D	0.410	0.311	0.708	0.856	100	81	$CuCl_2 \cdot 2H_2O$	
OUC-D	0.205	0.311	0.700	0.944	98	94	$CuCl_2 \cdot 2H_2O$	

TABLE XIV. *Trans*-bis(salicylaldoximato)copper(II). Cell Strain: Ehrlich Ascites Carcinoma. Complex Solvent: DMSO. Complex/Cell Culture = 25 μl/5 ml.

No.	CC (μg/ml)	B (X × 10^6 cell/ml)	A (X × 10^6 cell/ml)		Y (%)			Ref.
			1 day	2 day	1 day	2 day	Complex	
48	25.9	0.252	0.286	0.298	7	6	$Cu(C_7H_6NO_2)_2$	17, 22, 33
48	12.9	0.252	0.293	0.284	8	4	$Cu(C_7H_6NO_2)_2$	
48	6.46	0.252	0.297	0.320	9	9	$Cu(C_7H_6NO_2)_2$	
48	3.23	0.252	0.410	0.559	33	39	$Cu(C_7H_6NO_2)_2$	
48	1.62	0.252	0.462	0.708	44	58	$Cu(C_7H_6NO_2)_2$	

TABLE XV. *Trans*-bis(resolcylaldoximato)copper(II). Cell Strain: L 1210. Complex Solvent: DMSO. Complex/Cell Culture = 25 μl/5 ml, 2,4-Dihydroxybenzaldoxime = $C_7H_7NO_3$.

No.	CC (μg/ml)	B (X × 10^6 cell/ml)	A (X × 10^6 cell/ml)		Y (%)		
			1 day	2 day	1 day	2 day	Complex
49	29.6	0.269	0.279	0.304	2	3	$Cu(C_7H_6NO_3)_2$
49	14.8	0.269	0.264	0.294	−1	2	$Cu(C_7H_6NO_3)_2$
49	7.40	0.269	0.263	0.255	−1	−1	$Cu(C_7H_6NO_3)_2$
49	3.70	0.269	0.638	1.124	67	68	$Cu(C_7H_6NO_3)_2$
49	1.85	0.269	0.671	1.199	73	74	$Cu(C_7H_6NO_3)_2$

TABLE XVI. *Trans*-bis(resorcylaldoximato)copper(II). Cell Strain: Ehrlich Ascites Carcinoma. Complex Solvent: DMSO. Complex/Cell Culture = 25 μl/5 ml.

No.	CC (μg/ml)	B ($X \times 10^6$ cell/ml)	A ($X \times 10^6$ cell/ml)		Y (%)		
			1 day	2 day	1 day	2 day	Complex
49	29.6	0.244	0.304	0.292	16	8	$Cu(C_7H_6NO_3)_2$
49	14.8	0.244	0.296	0.289	14	7	$Cu(C_7H_6NO_3)_2$
49	7.40	0.244	0.266	0.291	6	8	$Cu(C_7H_6NO_3)_2$
49	3.70	0.244	0.511	0.724	72	77	$Cu(C_7H_6NO_3)_2$
49	1.85	0.244	0.563	0.853	86	98	$Cu(C_7H_6NO_3)_2$

sion is dependent on the metal complex and not only on the ligand or central metal ion.

The results in Table XII also indicate that the effect of the metal salicylaldoximates in inhibiting tumor cell division increases in the order Ni < Co < Cu, whereas the stability order in aqueous solutions is Co < Ni < Cu [25].

The experiments in Tables XV and XVI with respect to the copper(II) complex of 2,4-dihydroxybenzaldoxime (β-resolcylaldoxime) indicate that all copper(II) complexes of the ring substituted salicylaldoxime derivatives have antitumor activity. Insertion of hydrophilic substituents into the salicylaldoxime skeleton may make its metal complexes more soluble in water and thus also more suitable for antitumor purposes.

The strong inhibiting effect of the metal salicylaldoximates on tumor cell division is directly related to their high ability to penetrate the tumor cell membrane, the high stability of the chelate structure [17, 25], the uneven number of the unpaired 3d metal electrons [26], the *trans*-planar structure of the complexes [26] and the ionization behaviour of the organic ligand [22, 23].

After penetration of the tumor cell membrane the tumor growth inhibition agent may be *a*) the metal chelate itself, *b*) the metal(II) monosalicylaldoximato cation, c) the salicylaldoxime anion or radical, *d*) the metal ion. The three last mentioned cases require the dissociation of the metal(II) bissalicylaldoximato chelate in the tumor cell. In the three first mentioned cases (*a*–*c*) the mechanism of the inhibiting effect is directly related to the structure of the *trans*-bis(salicylaldoximato)metal(II) chelate resembling that of pyridoxal (a B_6 vitamin) and, especially, that of pyridoxaloxime, a well known inhibitor of homogeneous pyridoxal kinase catalysis [27, 28].

It is known that vitamin B_6 antagonists, *e.g.* 4-deoxypyridoxine [27] have growth inhibiting and antitumor effects. It therefore appears possible that the effect of *trans*-bis(salicylaldoximato)metal(II) chelates and, especially, that of the copper(II) chelate is, at least in part, due to vitamin B_6 antagonism.

This opinion is also supported by the fact that *in vivo* administration of *trans*-bis(salicylaldoximato)copper(II) to mice often causes swelling of the nose and severe weight loss, typical symptoms of vitamin B_6 deficiency.

This inhibition leads in the second stage to inhibition of transaminases, decarboxylases and generally of aminoacid and polyamine metabolism and therefore of the duplication of tumor DNA.

If the effecting species is, however, the metal(II) ion (case *d*) and, especially, the copper(II) ion, which is unlikely, this may prevent glutathione from acting as a biological reducing agent in thiol-dependent enzyme reactions of the tumor cells.

The mechanisms of the inhibiting effect will be discussed in a further paper as also will the L 1210 experiments *in vivo*. Further experiments have shown *trans*-bis(salicylaldoximato)copper(II) to have an antitumor effect against Ehrlich ascites carcinoma *in vivo*, also. These results will be published elsewhere.

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