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The cytotoxicity of copper(II) complexes of heterocyclic thiosemicarbazones and 2-substituted pyridine *N*-oxides

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Thiosemicarbazone complexes of copper(II) were shown to be potent cytotoxic/antineoplastic agents against the growth of murine and human tumor cells. Selectivity of some agents was demonstrated against specific solid tumor growth. In L1210 lymphoid leukemia cells the copper complexes preferentially inhibited DNA synthesis with their major effects on the purine *de novo* pathway at PRPP amido transferase, IMP dehydrogenase and dihydrofolate reductase. The reductions of purines correlated positively with inhibition of DNA synthesis and cytotoxicity of the agents tested. DNA itself was fragmented after incubation with the drug; however, no binding of the agent to nucleotide bases or intercalation between base pairs was evident.

Key words: Copper complexes, IMP dehydrogenase, L1210 leukemia, purine synthesis inhibitors, thiosemicarbazones.

Introduction

Copper(II) complexes of 2-acetylpyrazine, ^{2}N-methyl, ^{4}N-dimethyl and 3-hexamethyleneiminyl thiosemicarbazones[1] as well as copper(II) complexes of 2-butyl, 2-isobutyl, 2-sec-butyl and 2-tert-butylaminopyridine N-oxides (syntheses[1–14]) have demonstrated potent antifungal activity against *Aspergillus niger* and *Paecilomyes variotii*.[2] Since the agents were effective in inhibiting growth of cells, they were also tested for cytotoxicity in human and murine tissue culture cell lines where they demonstrated potent activity.[2] Among the groups of thiosemicarbazone derivatives tested were two copper(II) complexes showing good cytotoxic activity. Hence, an expanded group of copper(II) complexes of heterocyclic thiosemicarbazones was tested and their mode of action was examined. Selected for this study were (i) copper complexes with both neutral and anionic ligands, (ii) complexes with thiosemicarbazones derived from 2-acetylpyridine, 2-formylpyridine and acetylpyrazine, (iii) complexes that feature changes in the nature and bulkiness of the ^{4}N-substituent, and (iv) two copper(II) complexes of 2-substitued pyridine N-oxides.

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Materials and methods

Source of compounds

The following compounds, previously synthesized and reported, were used (Figure 1):

[Cu(L4DM)Cl] **1**[3]
[Cu(L4DP)Cl] **3**[4]
[Cu(Lpo)Cl] **5**[6]
[Cu(Lhexim)Cl] **7**[8]
[Cu(H4DM)Cl_2] **9**[10]
[Cu(H4DP)Cl_2] **11**[10]
[Cu(Pz4DM)Cl] **13**[1]
[Cu(Pzbcn)Cl] **15**[11]
[Cu(4DP)Cl] **17**[10]
[Cu(Lpip)Br] **19**[7]
[Cu(Lppz4M)Cl] **21**[12]
[Cu(OS-H)$_2$] **23**[14]
[Cu(L4DE)Cl] **2**[4]
[Cu(L4DCH)Cl] **4**
[Cu(Lpip)Cl] **6**[7]
[Cu(Lbcn)Cl] **8**[9]
[Cu(H4DE)Cl_2] **10**[10]
[Cu(HPz4M)Cl_2] **12**[1]
[Cu(Pzhexim)Cl] **14**[1]
[Cu(4DM)Cl] **16**[10]
[Cu(L4DP)Br] **18**[4]
[Cu(HLppz4M)Cl_2] **20**[12]
[Cu(SB-H)$_2$] **22**[13]

Cytotoxic activity

Compounds **1**–**23** were tested for cytotoxic activity by preparing a 1 mM solution of each of the drugs in 0.05% Tween 80/H_2O by homogenization. The drug solutions were sterilized by passing them through an Acrodisc 45 μM. The following cell lines were maintained by the literature techniques:[15] murine L1210 lymphoid leukemia, rat UMR 106 osteosarcoma, human Tmolt$_3$ acute lymphoblastic T cell leukemia, colorectal adenocarcinoma SW480, lung bronchogenic MB-9812, osteosarcoma TE418, KB epidermoid nasopharynx, A431 epidermoid carcinoma, HeLa-S^3 suspended and solid cervical carcinoma, and glioma EH 118 MG. The protocol

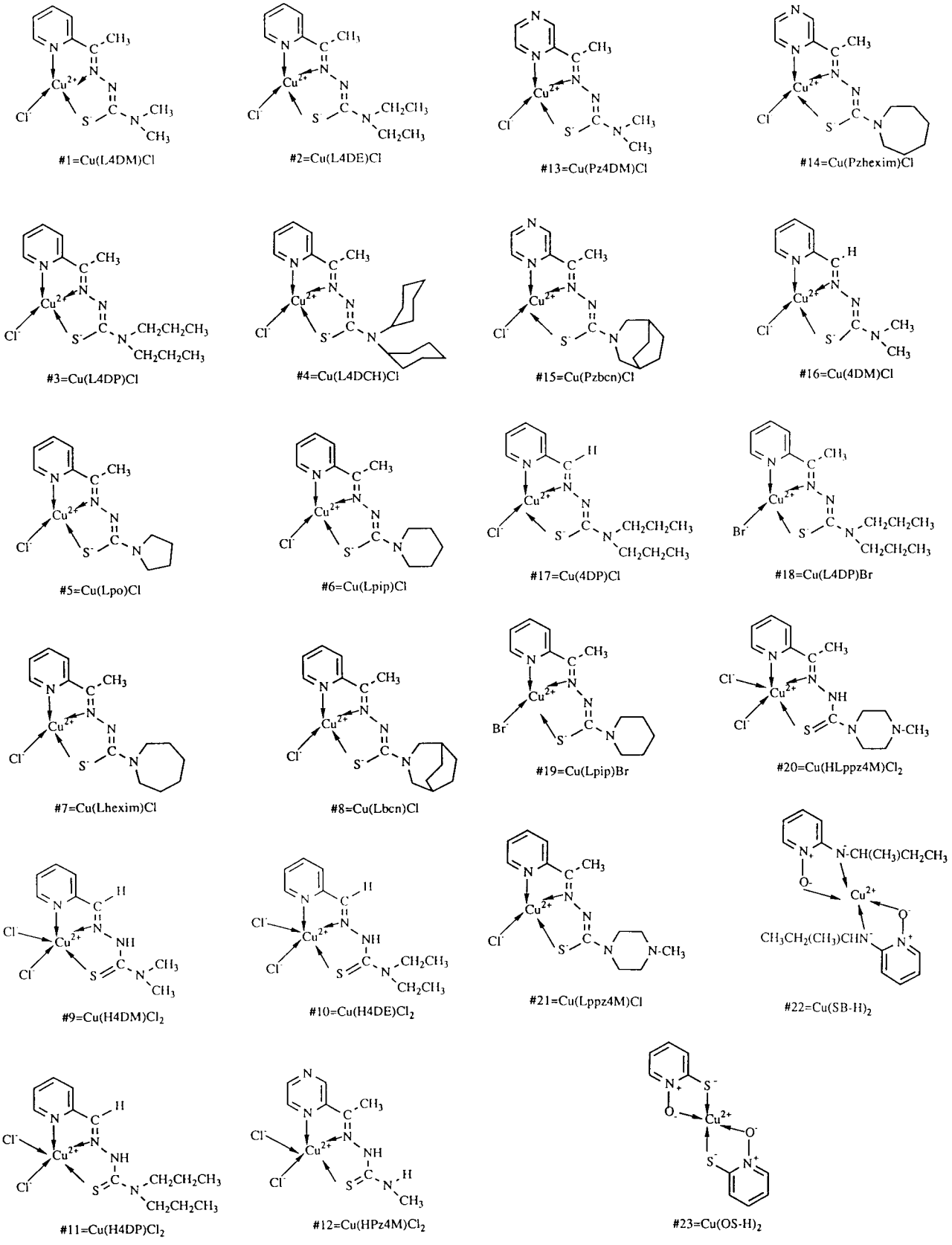

Figure 1. Structures of copper(II) complexes of heterocyclic thiosemicarbazones and 2-substituted pyridine *N*-oxides.

for assessment of cytotoxicity was that of Geran *et al.*[15] Standards were determined in each cell line. The compounds' cytotoxicities were expressed as ED_{50} values, i.e. the concentration which inhibits 50% of cell growth determined by the Trypan blue exclusion technique, in μg/ml. Solid tumor cytotoxicity was determined by the method of Leibovitz *et al.*[16] using crystal violet/MeOH staining and evaluation at 580 nm.

In vivo anti-neoplastic screens

Ehrlich ascites carcinoma cells (2×10^6) were implanted into CF_1 male mice on day 0. Drugs were administered intraperitoneally at 8 mg/kg/day from day 1–9. On day 10 the mice were sacrificed and the volume of tumor and packed cell volume was determined.[17]

Incorporation studies

Incorporation of labeled precursors into [^{3}H]DNA, [^{3}H]RNA and [^{3}H]protein for 10^6 L1210 cells was determined by the method of Liao *et al.*[18] The concentration response for inhibition of DNA, RNA and protein syntheses was determined at 60 min at 25, 50 and 100 μM with compounds **22** and **23**. [1-^{14}C]Glycine (53.0 mCi/mol) incorporation into purines was determined by the method of Cadman *et al.*[19] [^{14}C]Formate (53.0 mCi/mol) incorporation into pyrimidines was determined by the method of Christopherson *et al.*[20]

Enzyme assays

Inhibition of various enzyme activities was carried out by first preparing the appropriate L1210 cell homogenate or subcellular fraction, then adding the drug to be tested during the enzyme assay. For the concentration response studies, inhibition of enzyme activity was determined at 25, 50 and 100 μM after incubation for 60 min. DNA polymerase α activity was determined in a cytoplasmic extract isolated by the method of Eichler *et al.*[21] Nuclear DNA polymerase was determined by isolating nuclei.[22] The polymerase assay for both α and β was that of Sawada[23] with [^{3}H]TTP. Messenger, ribosomal and transfer RNA polymerase enzymes were isolated with different concentrations of ammonium sulfate[24,25] and the individual RNA polymerase activities were determined using [^{3}H]UTP. Ribonucleoside reductase activity was measured with [^{14}C]CDP with and without dithioerythritol.[26] The deoxyribonucleotides labeled with [^{14}C]dCDP were separated from [^{14}C]r-CDP from the ribonucleotides by thin layer chromatography (TLC) on PEI plates. Thymidine, TMP and TDP kinase activities were measured with [^{3}H]thymidine (58.3 mCi/mol) in the medium of Maley and Ochoa.[27] PRPP amidotransferase activity was determined by the method of Spassova *et al.*[28] and IMP dehydrogenase activity was determined with [^{14}C]IMP (Amersham, Arlington Heights, IL) where XMP was separated on PEI plates (Fisher Scientific) by TLC.[29] Carbamyl phosphate synthetase activity was determined by the method of Kalman *et al.*[30] and citrulline was determined colorimetrically.[31] Aspartate transcarbamylase activity was determined by the method of Kalman *et al.*[30] and carbamyl aspartate was determined colorimetrically.[32] OMP decarboxylase activity was determined by the method of Appel.[33] Thymidylate synthetase activity was analyzed by the method of Kampf *et al.*[34] The ^3H_2O measured was proportional to the amount of TMP formed from [^{3}H]dUMP. Dihydrofolate reductase activity was determined by the spectrophotometric method of Ho *et al.*[35] Protein was determined for all of the enzymatic assays by the Lowry technique.[36]

Deoxyribonucleoside triphosphates were extracted by the method of Bagnara and Finch.[37] Deoxyribonucleoside triphosphates were determined by the method of Hunting and Henderson[38] with calf thymus DNA, *Escherichia coli* DNA polymerase I, non-limiting amounts of the three deoxyribonucleoside triphosphates not being assayed, and either 0.4 μCi of [^{3}H-methyl]dTTP or [5-^{3}H]dCTP.

DNA assays

The effects of compounds **22** and **23** on DNA strand scission was determined by the methods of Suzuki *et al.*,[39] Pera *et al.*[40] and Woynarowski *et al.*[41] L1210 lymphoid leukemia cells were incubated with 10 μCi [methyl-^{3}H]thymidine, 84.0 Ci mmol and drug at 100 μM for 24 h at 37°C. After harvesting the L1210 cells (10^7), the cells were centrifuged at 600 *g* for 10 min in phosphate buffered saline (PBS), washed and suspended in 1 ml of PBS. Lysis buffer (0.5 ml: 0.5 M NaOH, 0.02 M EDTA, 0.01% Triton X-100 and 2.5% sucrose) was layered onto a 5–20% alkaline–sucrose gradient (5 ml: 0.3 M NaOH, 0.7 KCl and 0.01 M EDTA) followed by 0.2 ml cell

preparation. After incubating 2.5 h at room temperature, the gradient was centrifuged at 12 000 r.p.m. at 20°C for 60 min (Beckman rotor SW60). Fractions (0.2 ml) were collected from the top of the gradient, neutralized with 0.2 ml of 0.3N HCl and radioactivity measured. Thermal calf thymus DNA denaturation studies, UV absorption studies and DNA viscosity studies were conducted after incubating **22** and **23** at 100 μM in PBS buffer pH 7.2 at 37°C for 24 h.[42]

Statistics

The means and standard deviations are designated by '$\bar{X} \pm$ SD'. The probability of differences between the control and treated groups was determined by the Student's *t*-test.

Results

Cytotoxicity

Anti-neoplastic activity was demonstrated *in vivo* at 8 mg/kg/day (i.p.) in the Ehrlich ascites carcinoma screens by compounds **2–4**, **6**, **7**, **8**, **12**, **18**, **19** and **23** (Tables 1 and 2). Many of the compounds demonstrated toxicity at 4 and 8 mg/kg/day *in vivo*, including **1**, **5**, **9–11**, **13–17**, **20** and **21**. These latter compounds have LD_{50} values between 5 and 10 mg/kg (i.p.) in male mice. In the cytotoxicity screens, most of the compounds with the exception of **21** demonstrated significant activity against L1210 lymphoid leukemia growth, i.e. less than 4 μg/ml. Compounds **1**, **3**, **4**, **7**, **8**, **10–12**, **14** and **15** resulted in ED_{50} values of less than 2 μg/ml. Rat UMR 106 osteosarcoma growth was inhibited by all compounds tested except **2** and **22**. In the human $Tmolt_3$ T leukemia cell series, **7**, **10–14**, **19–21** and **23** afforded ED_{50} values of 2 μg/ml or less. Compound **1** was not active against $Tmolt_3$ leukemia growth. Colon adenocarcinoma growth was significantly inhibited by **3–8** and **10–23**, with ED_{50} values less than 1 μg/ml. HCT-8 ileum mucosa growth was inhibited significantly by all of the compounds. In this cell line, only compounds **1** and **18** had ED_{50} values above 1 μg/ml. KB nasopharyngeal growth was reduced by all of the compounds; only compounds **2** and **22** had ED_{50} values greater than 1 μg/ml. HeLa-S^3 uterine carcinoma growth was inhibited by **4**, **8**, **16–21**, and **23** with ED_{50} values of less than 2 μg/ml. HeLa solid uterine carcinoma growth was inhibited by all of the compounds except **2**, with ED_{50} values below those observed in the assay for the suspended HeLa cells. Lung bronchogenic growth was inhibited by all of the compounds with ED_{50} values between 0.94 and 3.52 μg/ml. Skin A431 growth was reduced by **1–3**, **5–15**, **22** and **23**. ED_{50} values were less than 1 μg/ml for these agents. Bone osteosarcoma growth was significantly inhibited by **1–3**, **6**, **9**, **11–13** and **15–22** with ED_{50} values of less than 1 μg/ml. Brain glioma growth was significantly inhibited by **3**, **5** and **10** with ED_{50} values below 1 μg/ml; compounds **1–15**, **22** and **23** afforded ED_{50} values less than 2.0 μg/ml.

Table 1. Antineoplastic activity of copper(II) complexes at 2, 4, and 8 mg/kg/day (i.p.) in CF_1 mice preinoculated with Ehrlich ascites

Compound		Percent inhibition of growth at dose (mg/kg/day, i.p.)		
		8	4	2
1	[Cu(L4DM)Cl]	toxic	toxic	toxic
2	[Cu(L4DE)Cl]	99.5	—	94.0
3	[Cu(L4DP)Cl]	99.9	—	96.0
4	[Cu(L4DCH)Cl]	86.0	—	—
5	[Cu(Lpo)Cl]	toxic	toxic	89.0
6	[Cu(Lpip)Cl]	99.3	—	97.0
7	[Cu(Lhexim)Cl]	99.6	—	99.0
8	[Cu(Lbcn)Cl]	99.9	—	89.0
9	[Cu(H4DM)Cl_2]	toxic	—	—
10	[Cu(H4DE)Cl_2]	toxic	toxic	—
11	[Cu(H4DP)Cl_2]	toxic	toxic	—
12	[Cu(HPz4M)Cl_2]	99.9	—	—
13	[Cu(Pz4DM)Cl]	toxic	toxic	—
14	[Cu(Pzhexim)Cl]	toxic	toxic	—
15	[Cu(Pzbcn)Cl]	toxic	toxic	—
16	[Cu(4DM)Cl]	toxic	—	33.0
17	[Cu(4DP)Cl]	toxic	—	96.0
18	[Cu(L4DP)Br]	99.9	—	69.0
19	[Cu(Lpip)Br]	99.4	—	—
20	[Cu(HLppz4M)Cl_2]	toxic	—	toxic
21	[Cu(Lppz4M)Cl]	toxic	—	toxic
22	[Cu(SB-H)$_2$]	17.0	—	—
23	[Cu(OS-H)$_2$]	96.3	—	—
	6-MP	—	—	99.9

Mode of action

Compounds **22** and **23**, the two pyridine *N*-oxide complexes, were selected to elevate their effects on metabolic and synthetic events in L1210 lymphoid leukemia cells. Compounds **22** and **23** effectively inhibited L1210 DNA synthesis by 53 and 88%, respectively, at 100 μM after 60 min incubations (Table 3). RNA synthesis was inhibited 26–28% at

Table 2. Cytotoxicity of copper compounds in rodent and human tissue culture lines (ED_{50} = μg/ml)

Compound	Rodent		Human									
	murine lymphoid L1210	rat UMR-106	T leukemia $Tmolt_3$	colon colorectal	HCT-8 ileum mucosa	uterine HeLa-S^3	HeLa-S^3 solid	KB nasopharynx	skin A431	bronchogenic lung	bone osteosarcoma	glioma brain
1	1.68	0.27	5.91	2.24	1.27	2.17	0.08	0.94	0.06	3.03	0.98	1.50
2	2.08	3.16	2.75	1.54	0.42	2.41	7.35	1.98	0.88	2.15	0.69	1.93
3	1.76	0.32	2.74	0.30	0.31	2.80	0.05	0.81	0.01	2.38	0.72	1.40
4	1.84	2.37	2.56	0.41	0.88	1.99	0.06	0.46	3.07	2.82	1.01	0.55
5	2.80	0.46	2.64	0.27	0.44	4.85	0.05	0.27	0.01	1.97	1.24	0.27
6	2.32	0.44	2.32	0.34	0.32	3.11	0.08	0.15	0.17	3.16	0.85	1.11
7	1.84	0.39	1.84	0.20	0.46	3.11	0.08	0.67	0.01	3.52	1.08	1.24
8	1.76	0.37	2.56	0.18	0.30	1.94	0.14	0.31	0.01	3.16	1.01	1.76
9	2.08	0.38	2.00	1.41	0.39	2.72	0.03	0.32	0.01	2.93	0.59	1.26
10	1.68	0.37	1.36	0.18	0.35	2.25	0.08	0.25	0.01	1.76	1.04	0.53
11	1.84	0.43	0.80	0.39	0.34	3.03	0.07	0.27	0.01	1.71	0.78	1.11
12	1.60	0.24	1.44	0.73	0.39	3.26	0.10	0.26	0.01	2.85	0.69	1.60
13	2.00	0.23	1.84	0.47	0.29	3.11	0.12	0.48	0.02	3.29	0.62	1.98
14	1.76	0.32	0.72	0.45	0.39	2.49	0.10	0.27	0.01	2.74	1.44	1.28
15	1.76	0.21	2.16	0.21	0.34	2.64	0.13	0.39	0.01	2.36	0.72	1.47
16	3.21	0.40	2.12	0.19	0.21	2.06	1.20	0.32	3.08	1.86	—	0.46
17	2.35	0.01	2.24	0.24	0.35	1.64	1.26	0.32	2.94	1.88	—	0.43
18	2.66	0.18	2.63	0.14	1.05	1.86	0.98	0.33	2.94	1.69	—	0.81
19	2.03	0.20	1.93	0.16	0.38	1.54	1.09	0.47	2.95	1.86	—	0.86
20	3.54	0.13	1.97	0.16	0.36	1.82	0.47	0.27	3.44	1.86	—	0.99
21	4.21	0.18	2.00	0.16	0.41	1.82	0.45	0.28	3.47	1.77	—	0.89
22	3.03	6.81	2.61	0.99	0.47	2.12	7.29	1.32	0.47	2.15	0.21	1.67
23	2.71	0.30	1.89	0.07	0.44	1.57	0.03	0.17	0.44	0.94	1.93	1.07
5-FU	1.41	—	2.14	3.09	—	2.47	—	1.25	—	5.69	—	1.28
ARA C	2.76	—	2.67	3.42	—	2.13	—	2.84	—	4.60	—	1.88
Hydroxyurea	2.67	—	3.18	4.74	—	1.96	—	5.29	—	7.37	7.57	2.27

Table 3. The effects of thiosemicarbazone copper complexes on L1210 cell metabolism

Assay ($N = 6$)	Percent of control ($\bar{X} \pm$ SD)								
		Compound **22**				Compound **23**			
	control	10 μM	25 μM	50 μM	100 μM	10 μM	25 μM	50 μM	100 μM
DNA synthesis	100 ± 6[a]	88 ± 7	70 ± 5*	51 ± 4*	47 ± 4*	93 ± 6	62 ± 5*	13 ± 3*	12 ± 4*
RNA synthesis	100 ± 5[b]	86 ± 6	76 ± 5*	73 ± 6*	72 ± 5*	94 ± 7	88 ± 6	77 ± 5*	74 ± 6*
Protein synthesis	100 ± 7[c]	68 ± 6*	47 ± 5*	46 ± 6*	31 ± 5*	75 ± 6*	51 ± 5*	39 ± 4*	17 ± 2*
DNA polymerase α	100 ± 5[d]	93 ± 6	84 ± 5	77 ± 6*	75 ± 4*	130 ± 7	153 ± 6	127 ± 5	78 ± 6*
mRNA polymerase	100 ± 7[e]	103 ± 6	112 ± 7	68 ± 5*	54 ± 5*	99 ± 6	96 ± 7	90 ± 7	78 ± 6*
rRNA polymerase	100 ± 6[f]	99 ± 7	97 ± 6	91 ± 6	90 ± 5	110 ± 7	132 ± 6	119 ± 5	104 ± 6
tRNA polymerase	100 ± 5[g]	97 ± 6	95 ± 5	155 ± 8	184 ± 0	135 ± 6	187 ± 7	221 ± 8*	134 ± 7
Ribonucleoside reductase	100 ± 7[h]	72 ± 5*	65 ± 5*	61 ± 5*	20 ± 3*	66 ± 5*	38 ± 4*	31 ± 4*	28 ± 2
Purine *do novo* synthesis	100 ± 6[i]	95 ± 5	70 ± 5*	45 ± 6*	27 ± 3*	105 ± 6	73 ± 5*	54 ± 6*	24 ± 3*
PRPP amido transferase	100 ± 4[j]	68 ± 7*	50 ± 5*	38 ± 4*	34 ± 4*	72 ± 6*	55 ± 4*	40 ± 5	38 ± 4*
IMP dehydrogenase	100 ± 6[k]	71 ± 5*	52 ± 6*	48 ± 4*	47 ± 5*	61 ± 6*	53 ± 5*	53 ± 4*	45 ± 4*
Pyrimidines *de novo* synthesis	100 ± 7[l]	130 ± 7	150 ± 8	158 ± 7	149 ± 6	84 ± 7	106 ± 6	122 ± 7	144 ± 7*
Carbamyl phosphate synthetase	100 ± 7[m]	99 ± 8	92 ± 6	106 ± 8	117 ± 5	93 ± 6	87 ± 5	85 ± 6	84 ± 5
Aspartate transcarboxylase	100 ± 6[n]	101 ± 7	94 ± 6	79 ± 5*	67 ± 5*	85 ± 6	72 ± 5	71 ± 4	68 ± 4
OMP decarboxylase	100 ± 7[o]	83 ± 6	94 ± 7	108 ± 8	123 ± 6*	96 ± 6	97 ± 7	108 ± 7	110 ± 6
Thymidine kinase	100 ± 5[p]	128 ± 7	126 ± 6	103 ± 5	88 ± 5	106 ± 8	99 ± 8	83 ± 5	67 ± 3*
TMP kinase	100 ± 5[q]	69 ± 6*	56 ± 5*	20 ± 4*	20 ± 2*	81 ± 7	62 ± 6*	53 ± 6*	21 ± 4*
TDP kinase	100 ± 6[r]	149 ± 8	98 ± 7	62 ± 5*	47 ± 4*	59 ± 6*	44 ± 3*	33 ± 4*	27 ± 3*
Dihydrofolate reductase	100 ± 6[s]	80 ± 7	66 ± 5*	42 ± 6*	40 ± 4*	75 ± 6	54 ± 5*	47 ± 6*	30 ± 3*
Thymidylate synthetase	100 ± 8[t]	109 ± 7	130 ± 6	113 ± 7	102 ± 8	114 ± 6	133 ± 7	107 ± 6	103 ± 8
d(ATP)	100 ± 5[u]				49 ± 6*				185 ± 6*
d(GTP)	100 ± 7[v]				37 ± 8*				192 ± 7*
d(CTP)	100 ± 7[w]				25 ± 7*				146 ± 5*
d(TTP)	100 ± 6[x]				52 ± 6				56 ± 4*

Control values for 10^6 cells/h: [a]26125 d.p.m., [b]4851 d.p.m., [c]7164 d.p.m., [d]47804 d.p.m., [e]1502 d.p.m., [f]4239 d.p.m., [g]6400 d.p.m., [h]2744 d.p.m., [i]92551 d.p.m., [j]0.121 OD 340/h/mg protein, [k]76058 d.p.m., [l]13680 d.p.m., [m]0.392 mol citrulline, [n]1.064 mol, [o]44743 d.p.m., [p]0.867 OD 340/h/mg protein, [q]0.625 OD 340/h/mg protein, [r]0.121 OD 340/h/mg protein, [t]0.868 OD units/h/mg protein, [s]18463 d.p.m.,[u]6.17 pmol, [v]5.27 pmol, [w]6.87 pmol, [x]6.94 pmol.

100 μM. Protein synthesis was inhibited 69 and 83%, respectively, after 60 min at 100 μM. Further studies showed that DNA polymerase α activity, but not DNA polymerase β activity, was inhibited 22–25% at 100 μM. mRNA polymerase activity was inhibited 22–46% by the compounds. rRNA and tRNA polymerase activities were not significantly reduced by compounds **22** and **23**. Ribonucleoside reductase activity was inhibited in a concentration-dependent manner with 72–80% reduction at 100 μM of the agents. Dihydrofolate reductase activity was inhibited 60 and 70% by compounds **22** and **23**, respectively, after 60 min.

De novo synthesis of purines was inhibited 73 and 76% by **22** and **23**, respectively, after 60 min (Table 3). Both PRPP amido transferase and IMP dehydrogenase activities were inhibited greater than 50% by **22** and **23**. *De novo* pyrimidine synthesis and related enzyme activities, i.e. carbamyl transcarboxylase, OMP decarboxylase and thymidylate synthetase activities, were not significantly affected by compounds **22** and **23**. Only aspartate transcarboxylase activity was inhibited 33 and 32% by these compounds after 60 min at 100 μM. TMP and TDP kinase activities were inhibited in a concentration-dependent manner with greater than 50% inhibition at 100 μM of the agents. d(TTP) pool levels were reduced 48 and 44% by compounds **22** and **23**. All other d(NTP) pools were also reduced by compound **22**, whereas only the d(TTP) level was significantly reduced by compound **23**. cDNA UV absorption was not affected after drug incubation at 100 μM for 24 h. T_m values for thermal denaturation of cDNA were not affected by incubation with compounds **22** and **23**. cDNA viscosity was affected compared to the control value (271 s); compound **22** was 243 s and compound **23** was 239 s. In L1210 cells incubated with **22** or **23** at 100 μM for 24 h, DNA strand scission occurred with **22** markedly (Figure 2); however, **23** only demonstrated marginal DNA fragmentation (Figure 3).

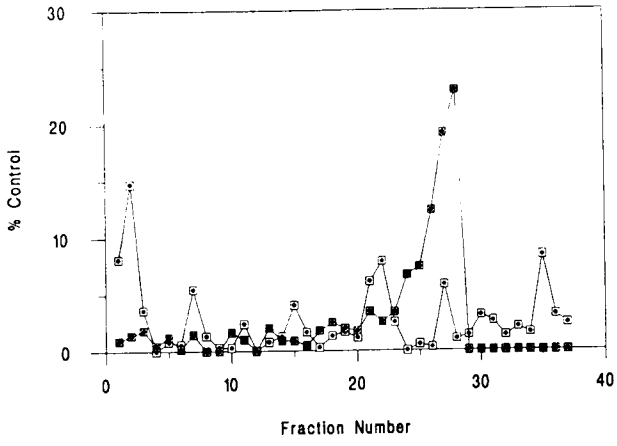

Figure 2. DNA strand scission: ▣, compound **22**; ▩, control.

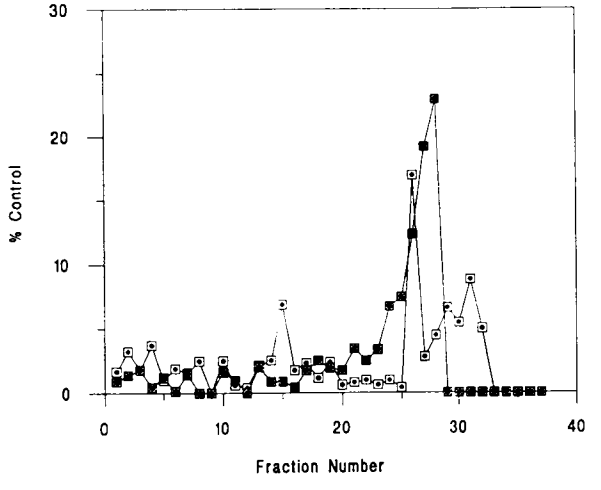

Figure 3. DNA strand scission: ▣, compound **23**; ▩, control.

Discussion

Copper(II) complexes of thiosemicarbazones and substituted pyridine N-oxides proved to be potent cytotoxic agents. Particularly good activity was observed for inhibition of growth of some solid tumors, e.g. human osteosarcoma. The compounds were also active against the growth of lung bronchogenic and adenocarcinoma colon cancer. These solid tumor models have few clinical agents which specifically block their growth. *In vivo* anti-neoplastic activity was demonstrated against Ehrlich ascites carcinoma growth in the dosage range of 4–8 mg kg day. The copper(II) complexes of the thiosemicarbazones appeared to be more toxic *in vivo* compared to the nickel and nickel cobalt complexes previously reported.[43]

The primary site of inhibition by these agents in L1210 lymphoid leukemia cells was DNA synthesis. *De novo* synthesis of purines appeared to be the major site where the copper complexes significantly inhibited both enzymatic regulatory sites, PRPP amidotransferase and IMP dehydrogenase. The inhibition of this pathway by the agents was of sufficient magnitude to account for the observed DNA synthesis inhibition. The inhibition of dihydrofolate reductase activity, which would reduce one carbon transfer for purine synthesis, would also play a role in reducing the overall purine levels in the cells. However, dATP and dGTP pools were elevated in L1210 cells after incubations with compound **23**. These elevations were probably due to the agents' inhibiting DNA polymerase α activity. If d(NTP) was not incorporated into newly-synthesized DNA, then it could accumulate in the cell leading to elevated pool levels. Over time, d(NTP) pools would be reduced dependent on the normal endogenous levels in the given mammalian cells and the efficiency of the retrieval pathway between the various deoxyribonucleotides. Since TMP and TDP kinases were reduced significantly by the agents, decrements in d(TPP) levels would be expected to be afforded rapidly. Reductions in d(TTP) levels were indeed observed after treatment with **22** and **23**. After DNA fragmentation by these compounds, DNA does not serve as a good template; this would lead to less DNA synthesis. It should be noted that the copper complexes did not appear to interact with DNA causing intercalation. The possibility exists that the copper(II) complexes interacted with DNA in an unstable manner, thus causing fragmentation. RNA synthesis was reduced in L1210 cells by the agents; this action may have been due to reduction of purine synthesis and reduced activity of mRNA polymerase. The DNA, if fragmented by the presence of these drugs, may not have been functioning as a good template for either mRNA polymerase or DNA polymerase α. Thus, the copper(II) complexes of thiosemicarbazones appeared to effect metabolism of L1210 cells by multiple mechanisms which are additive in bringing about cell death and cytotoxic activity.

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