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Cytotoxicity of Copper Complexes of 2-Furaldehyde Oxime Derivatives in Murine and Human Tissue Cultured Cell Lines

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Abstract. *The copper complexes of furan oxime derivatives were found to be potent cytotoxic agents in both murine and human tissue cultured cell lines which were either suspended or solid tumors. The ED_{50} values were frequently improved over the clinically useful antineoplastic agents. These copper complexes of 2-furaldehyde oximes were effective inhibitors of L1210 lymphoid leukemia DNA synthesis followed by RNA synthesis. Purine synthesis regulatory enzyme activities were markedly reduced by the compounds with marginal inhibition of t-RNA polymerase, and nucleoside kinases activities. L1210 DNA topoisomerase II activity was markedly reduced with IC_{50} values better than the standard VP-16, etoposide. Yet, the copper complexes caused no further protein linked breaks than VP- 16 did, but did block phosphorylation activation of the topoisomerase II enzyme.*

A series of metallic complexes of furan oximes have recently been synthesized. Based on the cytotoxicity of other copper, cobalt and iron metal complexes of trimethylamine carboxyboranes[1,2] and copper and nickel complexes of heterocyclic thiosemicarbazones[3,4] a pilot study on the metal complexes of furan oximes [5] to determine their ability to retard tumor cell growth was undertaken. These complexes are unlike cis-platinum derivatives in that the agents were inhibitors of tumor cell metabolic enzymes, *e.g.* purine regulatory enzymes and DNA topoisomerase II activity.

Mode of action studies in murine L1210 lymphoid leukemia cells showed that the compounds suppressed DNA, RNA and protein synthesis after 60 minutes at 100 μM. Inhibition of purine and pyrimidine *de novo* syntheses, as well as inhibition of ribonucleoside reductase and nucleoside kinase activities with DNA strand scission occurred after 60 minutes incubation. All of these effects of the complexes probably added to their ability to cause cell death but most important was the inhibition of DNA topoisomerase II activity with IC_{50} values lower than those afforded by VP-16, the standard, which causes apoptosis. Currently, four additional copper complexes of furan oximes have become available; thus, we have conducted a cytotoxic and mode of action study with these new derivatives in L1210 leukemic cells.

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Key Words: Copper, complexes of furan oximes, IMP dehydrogenase, purine inhibitors, cytotoxicity, nucleic acid inhibitors.

Materials and Methods

Source of compounds. Synthesis of [$CuCl_2$(2-furaldehyde oxime)2] **1**, [$CuCl_2$(5-ethyl 2-furaldehyde oxime)2] **2**, [$CuCl_2$ (3-(5-methyl 2-furyl) prop 2-ene aldoxime)2] **3**, and [$CuCl_2$(2-furaldehyde semicarbazone)2] **4** (Figure 1), were conducted as previously reported in that the compounds were obtained by reacting the ligands and copper chloride [analytical grade] in stoichiometric amounts in refluxing anhydrous ethanol [6,7].

All radioisotopes were purchased from New England Nuclear (Boston, MA) unless otherwise indicated. Radioactivity was determined in Fisher Scintiverse scintillation fluid with correction for quenching. Substrates and cofactors were obtained from Sigma Chemical Co. (St. Louis, MO).

Pharmacological methods. Compounds **1-4** (Table I) were tested for cytotoxic activity by homogenizing drugs in a 1 mM solution in 0.05% Tween 80/H_2O. These solutions were sterilized by passing them through an acrodisc filter (0.45μ). The following cell lines were maintained by literature techniques: murine L1210 lymphoid leukemia, P-388 lymphocytic leukemia, human Tmolt3 acute lymphoblastic T cell leukemia, HL-60 promyelocytic leukemia, colorectal adenocarcinoma SW480, lung A549 carcinoma, rat osteosarcoma UMR-106, rat adrenal pheochromocytoma PC-12, mouse L929 fibrosarcoma, human KB epidermoid nasopharynx, epidermoid skin A-431, HeLa-S^3 suspended and solid cervical carcinoma, and glioma EH 118 MG. Geran *et al* 's protocol [8] was used to assess the cytotoxicity of the compounds and standards in each cell line. Values for cytotoxicity were expressed as ED_{50}= μg/ml, *i.e.* the concentration of the compound inhibiting 50% of cell growth. ED_{50} values were determined by the trypan blue exclusion technique. A value of less than 4 μg/ml was required for significant activity of growth inhibition. Solid tumor cytotoxicity was determined by Liebovitz *et al* 's method[9] utilizing crystal violet/MeOH and read at 580 nm (Molecular Devices).

Table I. *The cytotoxicity of copper complexes of 2-furaldehyde oxime derivatives in murine and human tissue cultured cells.*

	ED_{50} = μg													
Cpd	L1210 Leukemia	Tmolt$_3$ Leukemia	P388 Leukemia	HeLa-S^3 Uterine	HL-60	HeLa solid	SW480 colon	Skin A431	Glioma PC12	Adrenal A549	Lung A549	L929 fibro sarc	UMR-106 Osteo	KB Naso phar.
1	3.42	2.89	2.71	2.77	3.64	4.35	2.54	0.13	7.45	20.	0.45	5.50	5.23	0.33
2	2.86	1.93	0.99	1.42	1.95	3.96	3.95	0.69	2.81	5.94	0.47	4.99	5.11	1.22
3	2.53	3.08	1.44	1.72	3.77	3.46	1.50	0.15	4.26	10.72	0.52	2.97	3.45	0.02
4	4.41	2.70	2.38	1.64	2.02	3.89	5.37	0.71	3.94	20.1	1.64	5.53	3.71	0.60
6MP	2.43	1.62	2.16	2.12		5.61	3.61	3.42	4.46		4.71	5.41	9.13	11.04
Ara-C	2.42	2.67	2.38	2.13		4.74	3.42	0.92	1.88		6.28	3.87	0.86	2.84
5FU	1.41	2.14	1.41	2.47		4.11	3.09	0.61	1.28		3.58	5.41	3.52	1.25
VP-16	1.83		3.03	7.87		3.05	3.34	0.71	2.44		4.74	5.13	3.57	3.32
Actin	1.98		1.41	5.88		2.46	3.18	0.30	1.15		0.90	3.11	0.33	0.93
AzaU	1.20	1.54	1.41	2.48		4.69	5.73	1.09	1.93		2.63	4.27	4.02	3.57
HU	2.67	4.47	1.30	1.96		8.12	7.33	3.21	2.27		8.89	6.68	2.87	5.27
AMA	1.79		1.08	5.83		4.29	6.44	2.28	3.90		6.69	3.90	2.05	5.40
CyH	2.99		1.84	3.57		0.86	3.62	0.61	2.04		1.34	1.70	0.60	0.57

Incorporation studies. Incorporation of labeled precursors into ^{3}H-DNA, ^{3}H-RNA and ^{3}H-protein for 106 L-1210 cells was determined [10]. The concentration response at 25, 50 and 100 μM for inhibition of DNA, RNA and protein syntheses was determined after 60 minute incubations. The incorporation of ^{14}C-glycine (53.0 mCi/mmol) into purines was obtained by the method of Cadman *et al* [11]. Incorporation of ^{14}C-formate (53.0 mCi/mmol) into pyrimidines was determined by the method of Christopherson *et al* [12].

Enzyme assays. Inhibition of various enzyme activities was performed by first preparing the appropriate L-1210 cell homogenates or subcellular fractions, 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 of compounds 1-4 after 60 minute incubations. DNA polymerase α activity was determined in cytoplasmic extracts isolated by Eichler *et al* 's method [13]. Nuclear DNA polymerase beta was determined by isolating nuclei [14]. The polymerase assay for both alpha and beta was described by Sawada *et al* [15] with ^{3}H-TTP. Messenger-, ribosomal and transfer-RNA polymerase enzymes were isolated with different concentrations of ammonium sulfate; individual RNA polymerase activities were determined using ^{3}H-UTP [16,17]. Ribonucleoside reductase activity was measured using ^{14}C-CDP with dithioerythritol [18]. The deoxyribonucleotides ^{14}C-dCDP were separated from the ribonucleotides by TLC on PEI plates. Thymidine, TMP and TDP kinase activities were determined using ^{3}H-thymidine (58.3 mCi/mmol) in the medium of Maley and Ochoa[19]. Carbamyl phosphate synthetase activity was determined with the method of Kalman *et al* [20]; citrulline was determined colorimetrically[21]. Asparate transcarbamylase activity was measured by the method of Kalman *et al* [20]; carbamyl asparate was determined colorimetrically [22]. Thymidylate synthetase activity was analyzed by Kampf *et al* 's method [23]. 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* [24]. PRPP amidotransferase activity was determined by Spassova *et al* 's method [25]; IMP dehydrogenase activity was analyzed with 8- ^{14}C-IMP (54 mCi/mmol) (Amersham, Arlington Heights, IL) after separating XMP on PEI plates (Fisher Scientific) by TLC [26]. Protein content was determined for the enzymatic assays by the Lowry technique [27].

After deoxyribonucleoside triphosphates were extracted [28], levels were determined by the method of Hunting and Henderson [29] with calf thymus DNA, *E. coli* DNA polymerase I, non-limiting amounts of the three deoxyribonucleoside triphosphates not being assayed, and either 0.4 mCi of (^{3}H-methyl)-dTTP or (5-^{3}H)-dCTP.

The effects of compounds 1-4 on DNA strand scission was determined by the methods of Suzuki *et al* [30], Pera *et al* [31], and Woynarowski *et al* [32]. L1210 leukemia cells were incubated with 10μCi thymidine methyl-^{3}H, 84.0 Ci/mmol for 24 hours at 37°C. L-1210 cells (10^7) were harvested and then centrifuged at 600 g × 10 minutes in PBS. They were later 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); this was followed by 0.2 ml of the cell preparation. After the gradient was incubated for 2.5 hours at room temperature, it was centrifuged at 12,000 RPM at 20°C for 60 min (Beckman rotor SW60). Fractions (0.2 ml) were collected from the bottom of the gradient, neutralized with 0.2 ml of 0.3 N HCl, and measured for radioactivity. Thermal calf thymus DNA denaturation

studies, U.V. absorption studies and DNA viscosity studies were conducted after incubation of compounds 1-4 at 100 μM at 37°C for 24 hours [33].

Isolation of L-1210 topoisomerase II enzyme. The procedure for isolating L-1210 DNA topoisomerase II enzyme has been published previously [34]. The isolated enzyme was used for the P4 phage DNA unknotting assay and drugs were incubated at 25 to 100 μM concentration. Inhibition of the activity was noted as a smear of the DNA in the gel as opposed to DNA knotting at the top of the gel.

DNA topoisomerase II, activity, inhibition. L-1210 cells were pelleted and the DNA topoisomerase II enzyme was isolated from the nuclei as outlined bv the published method of Liu *et al* [35,36]. The inhibition by compounds 1-4 of DNA strand-passing activity were determined by a standard relaxation and unknotting assay for DNA topoisomerases [36]. Briefly, the P4 knotted-DNA was prepared from the phage heads of infected *E coli* (C117) cells and was used as the substrate. The enzyme reaction contained a unit of DNA topoisomerase II [that amount which catalyzed the unknotting of 0.2 μg of P4 DNA] in 20 μl of 50 mM Tris-HCl, pH 7.5 buffer, 100 mM KCl, 10 mM $MgCl_2$, 0.5 mM DTT, 0.5 mM Na_3EDTA, BSA (30 μg/ml) and 1 mM ATP. The reaction was incubated for 60 minutes at 37°C then was stopped by adding 5 μl of 5% SDS (w/v), 50 mM Na_3EDTA, 20% sucrose (w/v), and 0.05 mg/ml bromophenol blue. The reaction product was analyzed on 0.7% (w/v) agarose gel in 89 mM Tris-borate buffer, pH 8.2 + 2 mM Na_3EDTA, electrophoresed at 23 v for 18 hours, and then stained with ethidium bromide. The IC-50 values for inhibition were determined using a densitometer and a photograph with UV at 300 nm illumination was made as permanent records. Photographs were obtained with Polaroid Type 667 film.

Cleavage-interference in L-1210, cells. Whole L-1210 cells or prenared nuclei from cells in log growth were pre-labeled with ^{3}H-thymidine (60-90 Ci/mMol., NEN) for 24 hr. The reaction medium [250 μl] contained whole cells or isolated nuclei in buffer supplemented with 3 mM ATP, compounds 1-4 at 100 μM for 30 minutes. Additional tubes were incubated with VP-16 at 40 μM for an additional 60 minutes. After incubation at 37°C, cells or nuclei were collected by centrifugation [500 G × 2 min] and analyzed for protein-linked DNA breaks by the method detailed by Rowe *et al* [37] and counted.

Phosphorylation of the isolated L-1210 DNA topoisomerase II enzyme was measured by the method of DeVore *et al* [38] using [γ ^{32}P]-ATP (3000 Ci/mmol) (Dupont) for 30 or 60 minutes. The reaction was inactivated by 10% TCA and filtered on GF/A glass fiber filters and counted. Standard protein kinase C inhibitors, A3 and bisindolylmaleimide [Calbiochem-Novabiochem.] were also used.

Statistics. The mean and standard deviation are designated by "X+SD." The probable level of significance (p) between test and control samples was determined by the Student's "t" or Dunnett's test with the raw data.

Results

The copper complexes of 2-furaldehyde oximes demonstrated potent cytotoxic action against the growth of suspended as well as solid cultured tumor cells [Table I]. Compounds **1, 2** and **3** were active against murine L1210 lymphoid leukemia and all four compounds were active in murine P388 lymphocytic leukemia, human $Tmolt_3$ T cell leukemia and human HL-60 leukemia. HeLa-S^3 suspended uterine growth was reduced significantly by all four compounds but in the HeLa solid uterine carcinoma less activity was shown with only compounds **2, 3** and **4** demonstrating marginal activity. SW480 colon adenocarcinoma growth was significantly

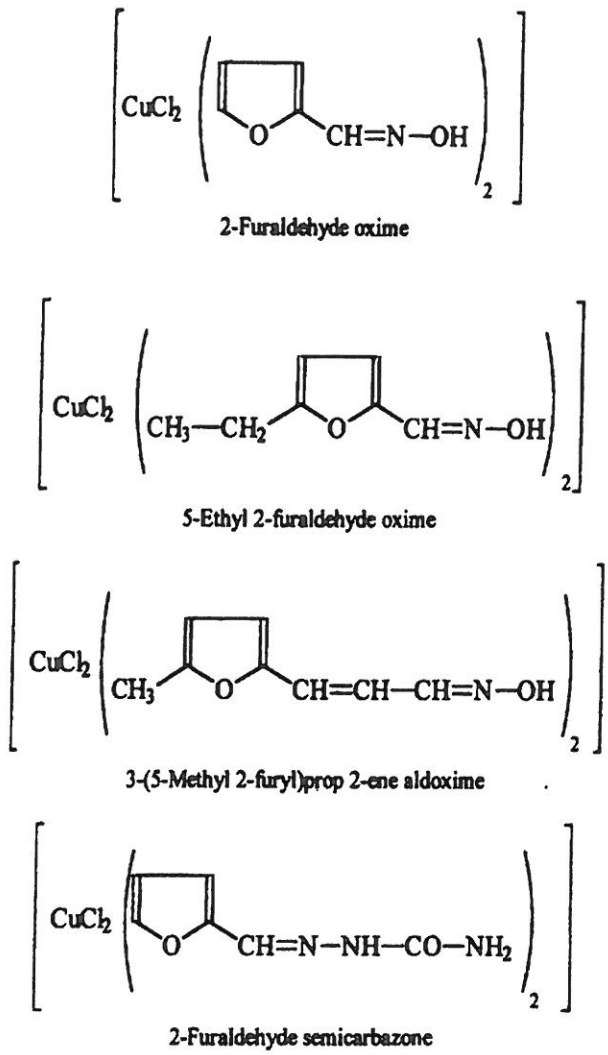

Figure 1. *Structures of the copper complexes.*

reduced by compounds **1** and **3** with marginal inhibition of **2**. Skin epidermoid carcinoma, KB nasopharynx and lung A549 growth was reduced markedly by all four compounds. Only compound **2** was active against glioma growth with marginal activity for compound **4**. None of the compounds were active against adrenal pheochromocytoma growth. L929 fibrosarcoma growth was only reduced by compound **3**. Rat UMR-106 osteosarcoma growth was reduced marginally by compounds **3** and **4**.

In the L-1210 leukemic mode of action study all four compounds significantly reduced DNA synthesis after 60 minutes with compounds **1-3** causing at least 80% inhibition at 100 μM [Tables II-V]. RNA synthesis was reduced by all of the compounds but compounds **1** and **2** afforded at least 70% reduction. Protein synthesis was only marginally reduced 20-32% after 60 minutes DNA polymerase α activity was only marginally reduced by 7-33% and m-RNA polymerase activity was slightly increased by compounds **2-4**. r-RNS polymerase

Table II. *Effects [$CuCl_2$(2-furaldehyde oxime)$_2$] on L-1210 leukemia cell metabolism over 60 min.*

(N=6)Assay	Percent of control (X + SD)			
	Control	25 μM	50 μM	100 μM
DNA synthesis	100±5[a]	36±3*	26±4*	17±2*
RNA synthesis	100±6[b]	94±4	45±4*	27±4*
Protein synthesis	100±5[c]	88±5	80±4*	68±5*
DNA polymerase alpha	100±6[d]	80±6	75±5*	67±4*
mRNA polymerase	100±7[e]	142±6*	129±5*	91±5
rRNA polymerase	100±4[f]	110±5	106±5	102±6
tRNA polymerase	100±7[g]	84±5	43±3*	31±2*
Ribonucleoside reductase	100±5[h]	73±3*	51±3*	25±2*
Dihydrofolate reductase	100±5[i]	91±5	83±4	67±3*
Purine *de novo* synthesis	100±5[j]	18±3*	11±2*	2±1*
PRPP amido transferase	100±6[k]	126±5*	94±3	79±5*
IMP dehydrogenase	100±5[l]	49±3*	47±4*	42±4*
Pyrimidine *de novo* synthesis	100±5[m]	179±6*	149±5*	79±5*
Carbamyl phosphate synthetase	100 ±7[n]	110±6	104±4	94±4
Aspartate transcarbamylase	100±6[o]	96±6	88±5	87±5
Thymidylate synthase	100±5[p]	104±6	109±5	120±6
Thymidine kinase	100±6[q]	36±3*	26±4*	17±3*
Thymidine monophosphate kinase	100±7[r]	115±4	67±4*	31±2*
Thymidine diphosphate kinase	100±6[s]	148±7*	146±8*	83±5
d(ATP)	100±5[t]			99±5
d(GTP)	100±6[u]			118±4
d(CTP)	100±5[v]			88±5
d(TTP)	100±4[w]			99±6

Control values for 10^6 cells/hr
a7719 dpm g400 dpm m19758 dpm s1891 dpm
b1014 dpm h48780 dpm n 0.807 mol N-carbamyl aspartate t17.07 p moles
c17492 dpm 10.144 DO.D. units o0.273 mmol citrulline u13.58 pmoles
d9019 dpm j28624 dpm p77616 dpm v33.60 pmoles
e1343 dpm k0.0878 O.D. units q1371 dpm w31.04 pmoles
f325 dpm 119575 dpm. units r1179 dpm
*P = 0.001 Student's "t" test

Table III. *The effects of [$CuCl_2$ (5-ethyl 2-furaldehyde oxime)$_2$] on L-1210 cell metabolism over 60 min.*

Assay N=6	Percent of control			
	Control	25μM	50μM	100μM
DNA synthesis	100±5	32±3*	16±3*	6±2*
RNA synthesis	100±6	87±5	52±3*	30±2*
Protein synthesis	100±5	88±4	81±5	80±6
DNA polymerase alpha	100±6	106±6	97±5	93±6
mRNA polymerase	100±7	122±5	110±4	102±4
rRNA polymerase	100±4	111±5	102±5	81±5
tRNA polymerase	100±7	61±4*	48±3*	41±4*
Ribonucleoside reductase	100±5	95±5	91±5	57±4*
Dihydrofolate reductase	100±5	88±6	55±4*	32±2*
Purine *de novo* synthesis	100±5	29±3*	24±3*	21±2*
PRPP amido transferase	100±6	92±5	79±4*	75±4*
IMP dehydrogenase	100±5	62±4*	52±3*	50±3
Pyrimidine *de novo* synthesis	100±5	106±5	91±4	67±4*
Carbamyl phosphate synthetase	100±7	116±5	108±5	86±5
Aspartate transcarbamylase	100±6	94±5	94±4	88±5
Thymidylate synthase	100±5	104±5	106±5	120±7
Thymine kinase	100±6	32±3*	16±2*	6±2*
Thymidine monphosphate kinase	100±7	108±5	94±5	7±2*
Thymidine diphosphate kinase	100±6	123±7	104±6	55±4*
d[ATP]	100±5			118±6
d[GTP]	100±6			105±4
d[CTP]	100±5			95±7
d[TTP]	100±4			

activity was marginally reduced by compounds **2** and **3** 19% and 40% respectively. t-RNA polymerase activity was reduced significantly by all four compounds 59-69% after 60 minutes at 100 μM. Ribonucleoside reductase was reduced 75% by compound **1** and 43% by compound **2** but compounds **3** and **4** were not effective at 100 μM. *De novo* purine synthesis was markedly inhibited by the compounds. Compounds **1** and **4** afforded greater than 90% inhibition with compound **2** causing 79% and compound **3** causing 53% after 60 minutes at 100 μM. PRPP-amido transferase activity was marginally reduced 15-58% by the compounds after 60 minutes. IMP dehydrogenase activity was reduced 47-61% and dihydrofolate reductase activity was reduced 33-79% after 60 minutes at 100 μM. *De novo* pyrimidine synthesis was reduced 33% by compound **1** and **2** and by 27% by compound **4**, but the three regulatory enzymes of the pathway, *i.e.* carbamyl phosphate synthetase, aspartate transcarbamylase and thymidylate synthase were only affected marginally by the compounds. Thymidine kinase activity was reduced significantly where compound **1** caused 83% reduction, compound **2** 94%, compound **3** 80% and compound **4** only

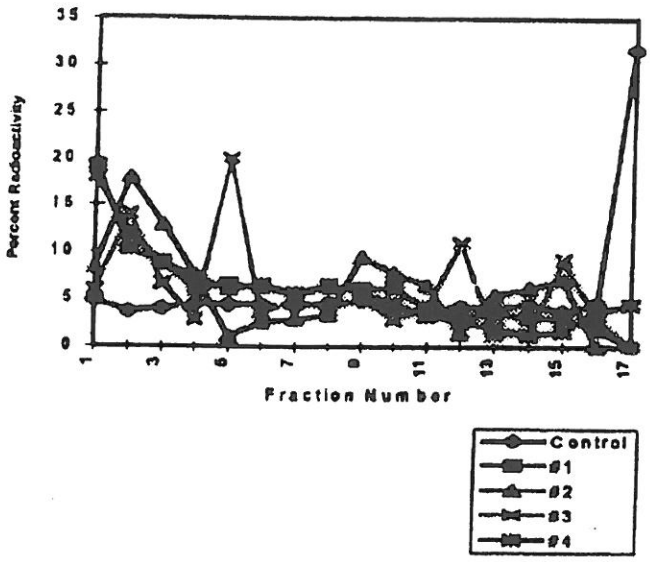

Figure 2. *L1210 DNA Strand Scission After 24 Hours Incubation of Compounds at 100 μM. In the alkaline sucrose gradient the high molecular weight DNA is located at the bottom of the tube, i.e.the higher numbered fractions and the low molecular weight DNA, i.e. DNA fragments is located in the lower numbered fractions.*

Table IV. *The effects of [$CuCl_2$(3-(5-methyl 2-furyl)prop 2-ene aldoxime)$_2$] on L-1210 cell metabolism over 60 min.*

(N=6)	Percent of control			
Assay	Control	25 μM	50 μM	100 μM
DNA synthesis	100±5	79±5*	55±4*	20±3*
RNA synthesis	100±6	78 4*	75±5*	51±5*
Protein synthesis	100±5	118±6	92±6	71±5*
DNA polymerase alpha	100±6	89 5	76±4*	74±5*
mRNA polymerase	100±7	187±7*	182±6*	147±5*
rRNA polymerase	100±4	110±5	65±5*	60±5*
tRNA polymerase	100±7	63±4*	48±4*	33±2*
Ribonucleoside reductase	100±5	131±5*	110±6	100±5
Dihydrofolate reductase	100±5	72±4*	36±4*	21±2*
Purine *de novo* synthesis	100±5	74±51.	69±4*	47±3*
PRPP amido transferase	100±6	130±6	105±4	85±5
IMP dehydrogenase	100±5	49±4*	45±3*	39±2*
Pyrimidine de novo synthesis	100±5	116±5	111±5	110±6
Carbamyl phosphate synthetase	100±8	108±6	102±5	91±5
Aspartate transcarbamylase	100±6	93±5	84±4	80±6
Thymidylate synthase	100±5	93±5	102±6	115±5
Thymidine kinase	100±6	79 4*	55±4*	20±3*
Thymidine monophosphate kinase	100±7	62±4*	25±3*	4±2*
Thymidine diphosphate kinase	100±6	11±6	77±4*	34±3*
d[ATP]	100			109±5
d[GTP]	100			107±6
d[CTP]	100			152±8*
d[TTP]	100			112±6

Table V. *The effects of [$CuCl_2$ (2-furaldehyde semicarbazone)$_2$] on L-1210 nucleic acid metabolism after 60 min.*

(N=6)	Percent of control			
Assay	Control	25 μM	50 μM	100 μM
DNA synthesis	100±5	75±4*	71±3*	50±3*
RNA synthesis	100±6	84±5	74±5*	66±4*
Protein synthesis	100±5	80±5	80±6	79±4
DNA polymerase	100±6	93±6	93±4	73±5*
mRNA polymerase	100±7	149±7*	138±5*	120±5*
rRNA polymerase	100±4	131±4*	98±6	94±5
tRNA polymerase	100±7	44±4*	42±3*	34±2*
Ribonucleoside reductase	100±5	86±5	84±4	83±4
Dihydrofolate reductase	100±5	136±5*	103±6	12±3*
Purine *de novo* synthesis	100±5	61±4*	19±3*	7±2*
PRPP amido transferase	100±6	96±5	74±4*	42±3*
IMP dehydrogenase	100±5	60±4*	59±4*	53±3*
Pyrimidine *de novo* synthesis	100±5	84±5	78±6*	73±4*
Carbamyl phosphate synthesis	100±7	118±5	135±7*	138±6*
Aspartate transcarbamylase	100±6	97±5	88±5	80±4*
Thymidylate synthase	100±5	108±5	104±6	104±6
Tymidine kinase	100±6	75±4*	71±3*	50±3*
Thymidine monophosphate kinase	100±7	14±2*	13±3*	6±3*
Thymidine diphosphate kinase	100±6	42±3*	42±3*	42±2*
d[ATP]	100±5			119±8
d[GTP]	100±6			107±4
d[CTP]	100 ±5			131±6*
d[TTP]	100±4			115±5

50%. TMP kinase activity was reduced 69% by compound **1**, but compounds **2-4** caused greater than 92% reduction at 100 μM after 60 minutes. TDP kinase activity was not inhibited as much from 17% by compound **1** to 66% by compound **3**. d[NTP] pools were not reduced after 60 minutes incubation with the compounds in fact some elevation occurred. L-1210 DNA strand scission did occur after 24 hours at 100 μM (Figure 2). There was no evidence that ct-DNA absorption from 220 -340 nm was changed with the compounds; however ct-DNA thermal denaturation values changed after 24 hour incubation at 100 μM of agent. The Tm for the control was 70°C, whereas values of 79.5, 85, 87.5 and 85 °C were obtained for compounds **1-4**, respectively. ct-DNA viscosity was slightly reduced with the presence of compounds from 294 seconds for the control and 268, 298, 273 and 258 seconds for the treated samples with compounds **1-4**. L-1210 DNA topoisomerase activity was inhibited with IC_{50}, values of 5, 17.5, 24.9 and 1.75 μM for compounds **1**, **2**, **3** and **4**, respectively compared to 23.5 μM for the standard VP-16 (Figure 3). L1210 DNA protein linked break was not induced by the compounds from 1-24 hours nor were there increased breaks when incubated with VP- 16 at 40 μM (Figure 4). Some of the compounds actually reduced the DNA protein

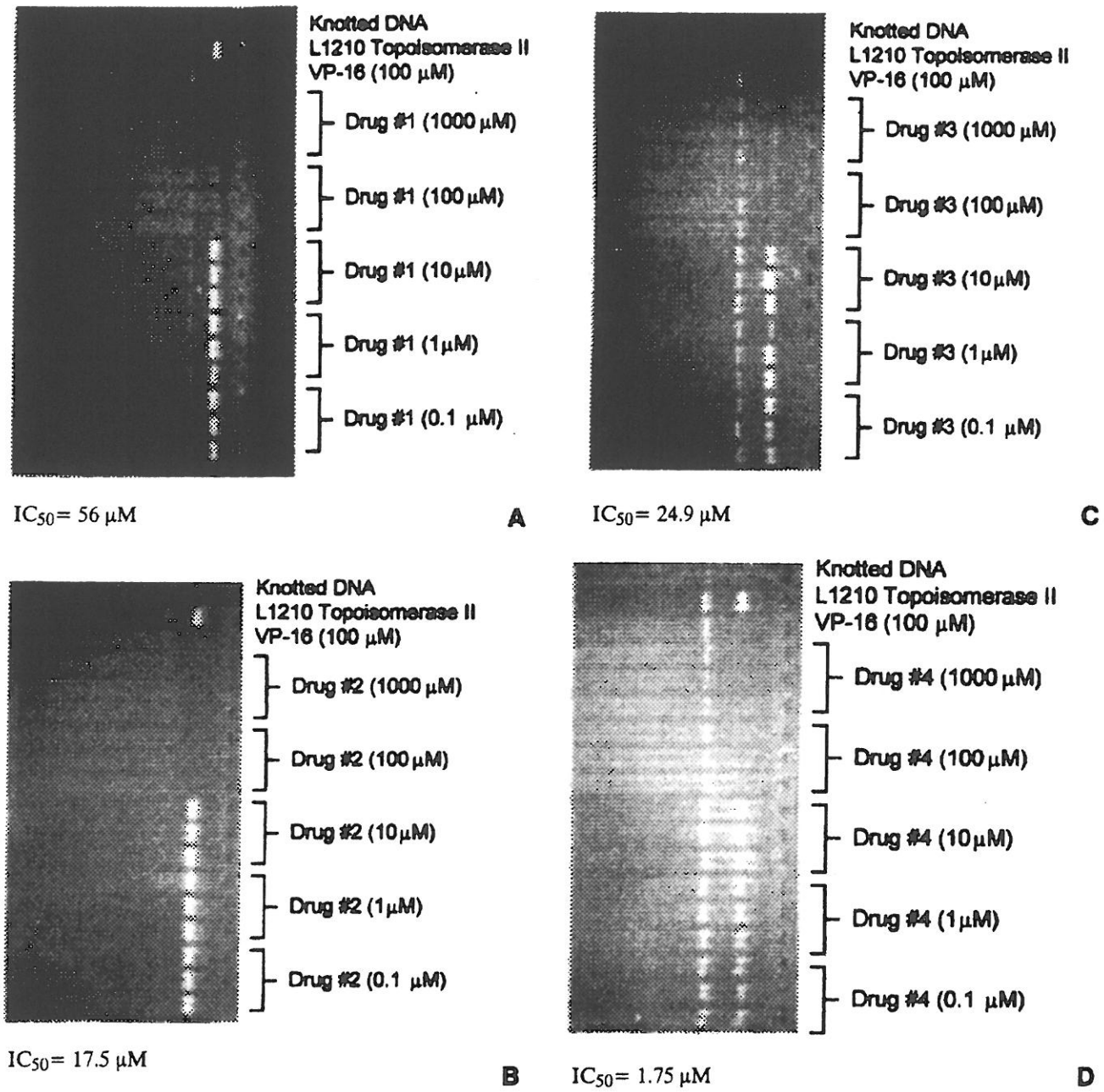

Figure 3. *L1210 DNA Topoisomerase II Activity Over 60 Minutes Drugs were incubated from 1000 to 0.1 μM.*

breaks at 14 hours (Figure 5). Phosphorylation of L1210 topoisomerase enzyme was reduced 94%, 91%,96% and 97% by compounds 1-4 at 100 μM, respectively at 30 minutes and 77%, 94%, 88% and 89% by compounds 1-4 at 100 μM at 1 hour (Figure 6). The standards A3 at 100 μM afforded 23% inhibition and bisindolylmaleimide at 20 nM resulted in 84% inhibition of DNA topoisomerase II phosphorylation at 30 minutes.

Discussion

The copper complexes of 2-furaldehyde oximes proved to be effective cytotoxicity agents against the growth of murine, rat and human cultured tumor cell lines with more effective ED_{50} values than the currently useful clinical cancer chemotherapy agents. The copper complexes of 2-furaldehyde oximes were effective DNA synthesis inhibitors with moderate reduction

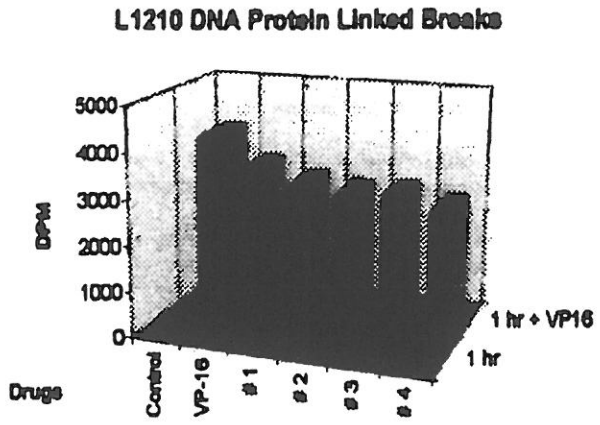

Figure 4. *L1210 DNA Protein Linked Breaks for 1 Hour. Front row compounds 1-4 incubated at 100 μM for 1 hour. Back row compounds 1-4 incubated 1 hour and then an additional hour with VP-16 at 40 μM.*

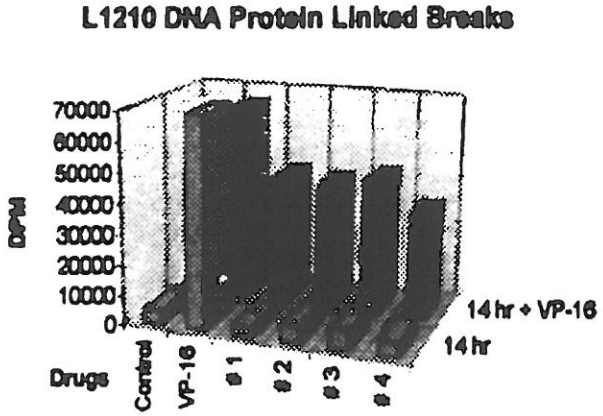

Figure 5. *L1210 DNA Protein Linked Breaks After 14 hours. Front row compounds 1-4 incubated at 100 μM for 14 hours. Back row compounds 1-4 incubated and then an additional hour with VP-16 at 40 μM.*

of RNA synthesis after 60 minutes incubation. The activities of regulatory enzymes, *i.e.* PRPP amido transferase, IMP dehydrogenase and dihydrofolate reductase, of the purine pathways were reduced by the compounds by a magnitude to account for the observed inhibition of nucleic acid synthesis caused by the compounds. One would expect to observe this inhibition in DNA synthesis before RNA since the pools in mammalian cancer have a ratio of 90:10 RNA:DNA pool levels. DNA synthesis would also be lowered by the inhibition of thymidine kinase and TMP kinase and ribonucleoside reductase activities. The DNA molecule itself did not appear to be a target of the agents since there did not appear to be alkylation of nucleoside bases, intercalation between base pairs, or cross linking of DNA strands. L1210 DNA topoisomerase II activity was inhibited by the complexes with observed DNA fragmentation after 24 hours. However, the agents did not produce cleavage products similar to VP-16, i.e. no DNA protein linked breaks were observed after 1-14 hours. This type of observation has been seen with fostriecin, novobiocin, merbarone, aclarubicin and bis-(2,6-dioxopiperazone) derivatives [40-43] indicating that these agents bring about DNA topoisomerase inhibition by some other mechanism than that observed for VP-16. Phosphorylation of DNA topoisomerase II was reduced by these copper complexes which leaves the enzyme in reduced energy state to conduct its catalytically function, thereby reducing enzyme activity. Interestingly other metal complexes like copper trimethylamine complexes reduce phosphorylation of L1210 DNA topoisomerase II[39]. Reduction in the phosphorylation of the DNA topoisomerase II enzyme has been related to increased DNA fragmentation and apoptosis of cancer cells.

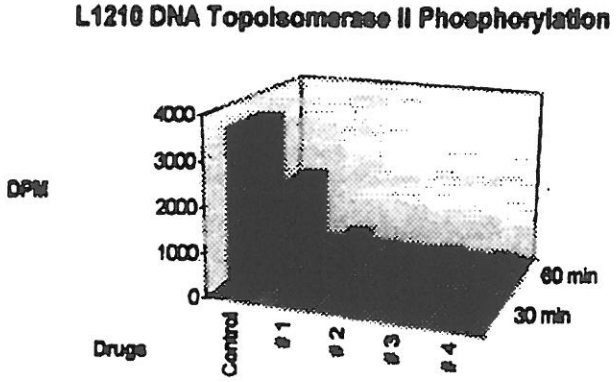

Figure 6. *L1210 DNA Topoisomerase II Enzyme Phosphorylation at 30 and 60 minutes by PKC. Drugs were incubated at 100 μM concentration.*

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