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DNA interaction with metal complexes and salts of substituted boranes and hydroborates in murine and human tumor cell lines

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A series of metal complexes and sodium salts of substituted boranes and hydroborates was shown to have cytotoxicity in murine and human tumor screens. Most of these agents were active against the growth of L-1210, $Tmolt_3$ and Hela-S^3. Selected agents demonstrated activity against the growth of monolayer human cell lines derived from solid tumors. Interestingly, many of the compounds demonstrated even lower ED_{50} values in the solid tumor than the L-1210 leukemic screen. Four compounds, $Cu_2(m\text{-}CH_3)_3NBH_2CO_2)_4 \cdot 2(CH_3)NBH_2COOH$ (I), $[Fe_3O((CH_3)_3NBH_2CO_2)_6(CH_3OH)_3]NO_3 \cdot CH_3CN$ (II), $cis\text{-}[Co(en)_2((CH_3)_3N \cdot BH_2CO_2)_2]Cl \cdot 2.5\ H_2O \cdot 0.5\ CH_3OH$ (V), and $Na(CH_3)_3NBH_2CO_2 \cdot 0.25\ CH_3OH$ (IX) were shown preferentially to inhibit DNA synthesis of L-1210 cells with only moderate inhibition of RNA and protein synthesis. In preliminary studies these agents effectively inhibited the activities of regulatory enzymes involved in the purine pathway and nucleoside kinases resulting in the suppression of d(NTP) pool levels. The boron derivatives also caused L-1210 DNA strand scission. These drugs may act together to inhibit DNA synthesis and induce cytotoxicity.

Key words: Boron metallic complexes, cytotoxicity, DNA strand scission, DNA synthesis inhibition, hydroborates.

Introduction

Metal complexes which have previously been reported to show effective antineoplastic activity include rhodium and ruthenium,[1] gold,[2,3] gallium,[4] copper,[5] iridium,[6] calcium, iron, magnesium, manganese and zinc,[7] titanium and vanadium,[8] nickel and silver,[9] palladium and platinum.[10] Two metal complexes of trimethylamine-substituted borane adducts have also previously demonstrated cytotoxicity and antineoplastic activity *in vivo* against murine tumors. A zinc chloride complex of trimethylamine–cyanoborane at a concentration of 20 mg/kg/day inhibits Ehrlich ascites tumor growth by 96%[11] and a copper complex, tetrakis-μ-(trimethylamine-boranecarboxylato)-bis(trimethylamine-carboxyborane) dicopper (II) inhibited Ehrlich ascites growth by 99% when administered ip at a dose of 10 mg/kg/day.[12] Subsequently, a series of complexes of trimethylamine-carboxyborane ligands with iron (III), chromium (III), cobalt (III), cobalt (II), calcium (II)[13,14] and a series of hydroborates[13,15,16] have been synthesized. Their cytotoxicity and effects on nucleic acid and protein metabolism in L-1210 lymphoid leukemia are described here.

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Materials and methods

Source of compounds

The synthesis of the following compounds was conducted by procedures previously outlined in the literature: $Cu_2(m\text{-}(CH_3)_3NBH_2CO_2)_4 \cdot 2(CH_3)_3NBH_2COOH$ (I),[12] $[Fe_3O((CH_3)_3NBH_2CO_2)_6(CH_3OH)_3]NO_3 \cdot CH_3CN$ (II), $[Fe_3O(CH_3)_3NBH_2CO_2)_6(CH_3OH)_3]Cl$ (III), $[Cr_3O((CH_3)_3NBH_2CO_2)_6(H_2O)_3]NO_3 \cdot CH_3OH \cdot CH_3CN$ (IV),[13] $cis\text{-}[Co(en)_2((CH_3)_3\ NBH_2CO_2)_2]Cl \cdot 2.5H_2O \cdot 0.5CH_3OH$ (V),[14] $ZnCl_2 \cdot 2Me_3NBH_2CN$ (VI),[11] $Ca((CH_3)_3N \cdot BH_2CO_2)NO_3 \cdot CH_3\ COCH_3 \cdot 0.5H_2O$ (VII),[14] $Na(NH_3 \cdot BH_2CN)_6$ (VIII),[15] $Na(CH_3)_3NBH_2CO_2 \cdot 0.25CH_3$

OH (IX), $Na(CH_3)_2NBH_2CO_2 \cdot 0.45H_2O$ (X),[13] $Na(CH_3)_2NBH_3$ (XI), $NaBH_4$ (XII), $NaBH_2CN$ (XIII) (commercially available), $Na_2BH_3CO_2$ (XIV), [16] $Na_2B_{10}H_{10} \cdot H_2O$ (XV), $(Et_3NH)_2B_{10}H_{10}$ (XVI),[17] $(Et_3NH)B_{12}H_{12}$ (XVII).[18] All physical and chemical characteristics were identical to those already reported in the literature for these individual compounds.

Cytotoxic activity

All newly synthesized metal complexes and salts of boron derivatives were tested for cytotoxic activity by preparing a 1 mM solution of the drug in 0.05% Tween 80/H_2O by homogenization. The drug solutions were sterilized by passing them through an acrodisc 45 μM. The drugs were incubated with the appropriate cell line at 0.5, 1, 2, 4 and 10 μg/ml final concentration. The following cell lines were maintained by techniques described in the literature: murine L-1210 lymphoid leukemia, P-388 lymphocytic leukemia,[19] human $Tmolt_3$-acute lymphoblastic T cell leukemia,[20] colorectal adenocarcinoma SW-480,[21] lung bronchogenic MB-9812,[22] osteosarcoma TE-418,[23] KB epidermoid nasopharynx,[19,24] Hela-S^3 suspended cervical carcinoma,[25] and glioma EH 118 MG.[26] The protocol used to assess cytotoxicity was that of Geran *et al.*[19] Standards were determined in each cell line. Values are expressed for the drug's cytotoxicity as ED_{50} in μg/ml, i.e. the concentration which inhibits 50% of the cell growth determined by the Trypan Blue exclusion technique utilizing a hemocytometer on day 3 during log growth. Cytotoxicity was determined for the monolayer cells derived from solid tumors on day 4 or 5 by the method of Huang *et al.*[27] using Crystal Violet (0.8% in 50% EtOH). After 10 min the dye was washed off the cells and the density determined with a light box. Ehrlich ascites carcinoma *in vivo* tumor screens were conducted in CF_1 male mice (weighing ~28 g) with test drugs administered ip at a dose of 8 mg/kg/day.[28] The number of animals per group was six and the dose was based on previous studies of boron compounds.[29,30] 6-Mercaptopurine was used as a positive standard at a dose of 0.5 mg/kg/day ip.

Mode of action studies for compounds I, II, V and IX

All radioisotopes were purchased from New England Nuclear. All other chemicals were obtained from Sigma Chemical Company. GF/F and GF/B filters and PEI plates were purchased from Fisher Scientific. Compounds I, II, V and IX were selected for the L-1210 metabolic studies based on availability of drugs, representative examples of the chemical class of agents and the fact that the ED_{50} for each compound was <4 μg/ml in the L-1210 screen.

In vitro incorporation of labeled precursors into DNA, RNA and protein of L-1210 cells (10^6) was determined for 60 min by the method of Liao *et al.*[31] Drugs were present at 1, 2 and 3 times the concentration of their respective ED_{50} values in the tissue culture cells. The reaction mixtures were inactivated with acid. The DNA acid-insoluble precipitate was collected by vacuum suction on GF/F glass fiber discs which were washed with cold 10% perchloric acid containing 1% sodium pyrophosphate.[32] Acid-insoluble precipitates from the RNA and protein experiments were collected on GF/B and Whatman #1 filters, respectively, and were washed with 10% trichloroacetic acid. The filter discs were dried, placed in Scintiverse^R, and counted using a Packard Scintillation Counter. The following enzymatic activities were determined in cells at multiples of the ED_{50} values for each drug. DNA polymerase α activity was determined on a cytoplasmic fraction[33] using the incubation medium of Sawada *et al.*[34] with [^{3}H-methyl]dTTP (82.4 Ci/mmol), dCTP and dATP for 60 min at 37°C. The acid-insoluble precipitate was collected on glass fiber discs and counted. mRNA, rRNA and tRNA polymerase enzymes were separated by ammonium fractionation and their activities were determined using [^{3}H]UTP (23.2 Ci/mmol).[35] The reaction medium was inactivated with 10% perchloric acid containing 1% NaP-P and the acid-insoluble [^{3}H]RNA was collected on nitrocellulose filters and counted.[12] Formate incorporation into purines for 60 min at 37°C was determined by the method of Spassova *et al.*[36] with 0.5 μCl [^{14}C]formic acid (52 mCi/mmol). Purines were separated by silica gel TLC eluted with *n*-butanol:acetic acid:water (4:1:5). Using standards for guanine and adenine, the appropriate spots were scraped and counted. Inosinic acid dehydrogenase activity was determined by the method of Becker and Lohr[37] using 60 min incubation at 37°C with [8-^{14}C]inosine-5′-monophosphate (61 mCi/mmol). XMP was separated from IMP by TLC on PEI plates eluted with 0.5 M $(NH_4)_2SO_4$. The appropriate spot (standard, XMP) was scraped and counted. Thymidylate synthetase activity was determined in a supernatant (9000 × *g* for 10 min) fraction by the method of Kampf *et al.*[38] with [^{3}H]dUMP (11

Ci/mmol). The nucleotides were absorbed on charcoal, filtered on Whatman #1 filters and a sample of the aqueous filtrate was counted. *N*-ethylmaleimide, a known thiol alkylating agent, from 0 to 200 μM concentration, was utilized to assess inhibition of the enzyme activities.

Ribonucleoside diphosphate reductase activity was measured by a modification of the method of Moore and Hurlbert.[39] An aliquot of the 5000 × *g* supernatant was incubated for 60 min at 37°C with reaction medium containing 0.1 μCi ([5-^{3}H]CDP (16.2 Ci/mmol). The reaction was stopped by boiling; samples were incubated with calf intestine alkaline phosphatase, spotted on PEI plates, and eluted with ethanol/saturated sodium borate/ammonium acetate/EDTA. Plates were scraped at the R_f of the standard, deoxycytidine, and counted. Deoxyribonucleoside triphosphates were extracted with perchloric acid by the method of Bagnara and Finch.[40] After neutralization with 5 N KOH and 1 M KH_2PO_4 deoxyribonucleoside triphosphate pool levels were determined by the method of Hunting and Henderson.[41] The neutralized extract was incubated for 30 min at 37°C with the reaction medium containing calf thymus DNA, *E. coli* DNA polymerase I non-limiting amounts of three deoxyribonucleoside triphosphates which were not being assayed, including 0.04 mCi [^{3}H-methyl]dTTP (80 Ci/mmol) or [5-^{3}H]dCTP (15–30 Ci/mmol). The samples were spotted on Whatman #3 filters, which were rinsed in 5% trichloroacetic acid + 40 μM sodium pyrophosphate and in 95% ethanol, after which they were dried, and counted for radioactivity. Thymidine kinase, TMP kinase and TDP kinase activities were determined[12] using [^{3}H]thymidine incubated in the medium of Maley and Ochoa.[42] After extraction with ether, the aqueous layer was plated on PEI-F plates and eluted with 0.5 M formic acid:lithium chloride (1:1). The areas identical to the R_f of the standards of thymidine TMP and TDP were scraped and counted. Kinetic studies were conducted on select enzymes at three times the ED_{50} value of each drug for 15, 30, 45 and 60 min. The enzyme methods and extraction procedures were those previously described.

DNA interaction with drug

DNA strand scission studies were conducted in L-1210 cells incubated with 10 μCi [methyl-^{3}H]thymidine (84.0 Ci/mmol) for 24 h at three times the ED_{50} values of compounds I, II, V or IX. The lysed cells were placed on a 5–20% alkaline sucrose gradient and centrifuged at 25 000 rpm for 60 min at 20°C (Beckman rotor SW60). Fractions were taken, acidified and counted.[43–46] Compounds I, II, V and IX were incubated for 24 h at 37°C with isolated calf thymus DNA (Sigma) which had been further purified. DNA denaturation, DNA viscosity and DNA UV absorption studies were conducted as described by Zhoa *et al.*[47]

Results

In the cytotoxicity screens significant activity [$ED_{50} \leq 4$ μg/ml according to the NCI protocol (Geran *et al.*, 1972)] was demonstrated by the compounds against the growth of murine L-1210 lymphoid leukemia, human $Tmolt_3$ leukemia and HeLa-S^3 uterine carcinoma (Table 1) with a few exceptions. The sodium salts (XII–XV) demonstrated good activity against human SW-480 colorectal adenocarcinoma as did the copper (II) complex 1 and chromium complex 4. Compounds VI, VII, XV and XVI demonstrated significant activity against the growth of human KB nasopharynx tumor. Most of the compounds were not active against lung bronchogenic tumor with the exception of I and XII. Significant activity was demonstrated by compounds VI, X, XV and XVI against the growth of human osteosarcoma. Brain glioma tumor growth was reduced by I, XII, XV and XVI.

In the Ehrlich ascites carcinoma screen a dose of 8 mg/kg/day of compounds I, III, VI, XIII, XV and 6MP, administered ip demonstrated 99, 84, 96, 86 and 99% inhibition of tumor growth, respectively. Compounds IV, V, VII, IX, XI and XVI possessed moderate activity, inhibiting tumor growth by 79–81%. Compounds II, X, XII and XIV inhibited growth by 61, 67, 74 and 69%, respectively. Compounds VIII and XVII were essentially inactive, giving 32 and 25% inhibition.

Examination of the metabolic effects of compounds I, V and IX showed that these agents preferentially inhibited DNA synthesis of L-1210 lymphoid leukemia cells (Table 2). Compounds I and V at a concentration of three times their ED_{50} values reduced DNA synthesis by 50% at 60 min. Only compound IX produced a >75% reduction of DNA synthesis at twice its ED_{50} value. Compound II had the least effect on DNA synthesis in L-1210 cells, and also gave a 28% reduction of RNA synthesis and 34% reduction of protein synthesis. Moderate reductions of RNA synthesis and protein

Table 1. *In vitro* cytotoxicity of metal complexes and salts of substituted boranes and hydroborates. ED_{50} values in μg/ml

Compound	Murine L-1210 lymphoid	Human						
		$Tmolt_3$ low level	Colorectal	Uterine HeLa-S^3	Nasopharynx KB	Bronchogenic lung	Osteo-sarcoma	Glioma
I	3.14	2.02	2.00	2.21	5.33	3.72	4.53	2.19
II	2.53	2.96	5.85	2.55	7.11	7.98	7.58	6.63
III	4.36	4.14	5.79	3.40	4.60	7.89	8.20	5.59
IV	4.25	3.18	3.86	2.79	4.21	7.96	6.34	4.27
V	2.69	3.47	4.29	2.87	5.39	7.92	5.69	5.29
VI	3.46	2.82	8.06	2.13	2.91	7.25	2.91	8.02
VII	4.31	3.02	4.61	2.99	3.15	7.90	7.43	7.28
VIII	—	4.83	6.08	2.08	6.31	6.05	5.91	—
IX	2.59	4.72	6.54	2.10	7.89	7.98	5.11	7.73
X	3.88	5.14	4.83	2.18	6.57	8.04	0.82	7.63
XI	2.74	2.77	6.61	3.03	4.69	5.79	8.17	—
XII	2.33	3.32	3.25	1.46	7.27	2.85	4.49	2.88
XIII	1.67	3.68	3.85	2.56	6.56	5.65	5.93	—
XIV	4.62	3.30	2.59	2.35	4.94	7.03	5.03	9.46
XV	4.92	2.29	3.51	2.62	2.95	7.86	3.83	2.24
XVI	1.96	1.47	4.40	1.26	3.01	6.70	3.39	3.45
XVII	1.89	2.88	7.62	1.87	6.84	6.55	4.28	5.40
Standard agents for comparison								
5-FU	1.41	2.14	3.09	2.47	1.25	5.64	—	1.28
AraC	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	7.52	2.27

—Not determined.
ED_{50} = μg of drug required to cause 50% reduction of cell growth.

Table 2. The effects of boron compounds I, II, V and IX on the nucleic acid and protein synthesis of L-1210 lymphoid leukemia at 1, 2 and 3 × their respective ED_{50} values

		DNA synthesis	RNA synthesis	Protein synthesis
		Percent of control ($X \pm SD$)		
Control 0.05% Tween 80		100 ± 6[a]	100 ± 8[b]	100 ± 5[c]
Compound 1	1 ×	82 ± 5	74 ± 6*	75 ± 4*
	2 ×	78 ± 5*	62 ± 7*	72 ± 4*
	3 ×	20 ± 3*	56 ± 6*	75 ± 5*
Compound 2	1 ×	105 ± 8	104 ± 6	79 ± 7
	2 ×	89 ± 8	100 ± 7	84 ± 7
	3 ×	70 ± 6*	72 ± 6*	66 ± 5*
Compound 5	1 ×	97 ± 6	96 ± 9	91 ± 8
	2 ×	66 ± 7*	112 ± 10	82 ± 7
	3 ×	30 ± 3*	76 ± 5*	82 ± 8
Compound 9	1 ×	82 ± 7	93 ± 8	105 ± 8
	2 ×	22 ± 4*	94 ± 8	107 ± 6
	3 ×	9 ± 2*	88 ± 7	87 ± 7

[a] 26 162 dpms/10^6 cells/60 min.
[b] 4851 dpms/10^6 cells/60 min.
[c] 7164 dpms/10^6 cells/60 min.
* $p \leq 0.001$ Student's *t*-test.

synthesis in L-1210 cells were observed with the other three compounds. RNA synthesis in L-1210 cells was reduced by 44% and protein synthesis was reduced by 25% by compound I at a concentration of three times ED_{50} value. Compounds V and IX had less effect on RNA and protein synthesis of L-1210 cells.

L-1210 dihydrofolate reductase, PRPP amidotransferase, IMP dehydrogenase, thymidine kinase and thymidine diphosphate kinase activities were all significantly inhibited in the presence of I, II, V and IX at three times their ED_{50} values (Table 3). Figures 1–4 demonstrate that the inhibition of the activity of these enzymes was time dependent over a 60 min period. Dihydrofolate reductase activity was suppressed quickly by these boron agents, inhibition approaching 50% by 45 min. TDP kinase activity was suppressed rapidly by compound I. The agents had the least effect on IMP dehydrogenase activity over the 60 min period.

Other studies demonstrated that the inhibition of these enzymes followed a concentration-dependent pattern (Table 4). It should be noted that a concentration of three times the ED_{50} value of the agents was required to afford ≥50% inhibition of

Table 3. The effects of boron on L-1210 lymphoid leukemia nucleic acid metabolism after incubation for 60 min

Enzyme activity	Percent of control ($X \pm$ SD) 3 × ED_{50} values of compounds				
	Control	Compound			
		I	II	V	IX
DNA synthesis	100 ± 6[a]	20 ± 3	70 ± 6*	30 ± 3*	9 ± 2*
DNA polymerase α	100 ± 11[b]	303 ± 15*	94 ± 7	111 ± 8	80 ± 11
mRNA polymerase	100 ± 8[c]	296 ± 10*	89 ± 5	222 ± 7*	280 ± 9*
rRNA polymerase	100 ± 9[d]	171 ± 6*	129 ± 5*	182 ± 6*	275 ± 7*
tRNA polymerase	100 ± 8[e]	23 ± 4*	74 ± 5*	59 ± 4*	14 ± 5*
Ribonucleotide reductase	100 ± 6[f]	69 ± 6*	71 ± 5*	62 ± 5*	97 ± 6*
Dihydrofolate reductase	100 ± 5[g]	33 ± 4*	22 ± 3*	13 ± 3*	19 ± 2*
PRPP amidotransferase	100 ± 5[h]	31 ± 3*	21 ± 4*	9 ± 3*	31 ± 4*
IMP dehydrogenase	100 ± 6[i]	64 ± 4*	67 ± 6*	53 ± 4*	55 ± 5*
Carbamyl phosphate synthetase	100 ± 9[j]	116 ± 12	91 ± 8	90 ± 8	84 ± 7
Aspartate amidotransferase	100 ± 8[k]	109 ± 9	111 ± 9	110 ± 7	99 ± 8
Thymidylate synthetase	100 ± 4[l]	110 ± 6	109 ± 5	115 ± 7	111 ± 6
Thymidine kinase	100 ± 8[m]	60 ± 7*	42 ± 4*	68 ± 6*	48 ± 5*
TMP kinase	100 ± 7[n]	98 ± 7	86 ± 6	81 ± 8	79 ± 6*
TDP kinase	100 ± 6[o]	17 ± 4*	64 ± 5*	43 ± 5*	48 ± 4*
dATP levels	100 ± 7[p]	74 ± 7*	32 ± 5*	36 ± 6*	57 ± 5*
dCTP levels	100 ± 6[q]	31 ± 8*	5 ± 4*	12 ± 2*	5 ± 3*
dGTP levels	100 ± 7[r]	43 ± 6*	66d ± 6*	59 ± 5*	62 ± 6*
dTTP levels	100 ± 8[s]	7 ± 3*	5 ± 2*	16 ± 3*	7 ± 3*

[a] 26 152 dpms/10^6 cells/ml.
[b] 47 804 dpms [^{3}H]dTTP incorporated/h/mg of protein.
[c] 1502 dpms [^{3}H]UTP incorporated/h/mg of protein.
[d] 4239 dpms [^{3}H]UTP incorporated/h/mg of protein.
[e] 6400 dpms [^{3}H]UTP incorporated/h/mg of protein.
[f] 2744 dpm [^{3}H]dCDP formed/h/mg of protein.
[g] 0.868 v.OD. units/h/mg of protein.
[h] 0.936 v.OD. units/h/mg of protein.
[i] 76 058 dpms [^{3}H] XMP formed/h/mg of protein.
[j] 0.392 mmol citrulline formed/h/mg of protein.
[k] 1.064 mmol N-carbamyl-aspartate formed/h/mg of protein.
[l] 18 463 dpms ^3H_2O formed/h/10^6 cells.
[m] 0.867 v.OD_{340}/h/mg of protein.
[n] 0.825 v.OD_{340}/h/mg of protein.
[o] 0.121 v.OD_{340}/h/mg of protein.
[p] 6.17 pmol dATP/10^6 cells.
[q] 6.87 pmol dCTP/10^6 cells.
[r] 5.27 pmol dGTP/10^6 cells.
[s] 6.94 pmol dTTP/10^6 cells.

enzyme activity in 60 min. Other enzymes which demonstrated reduction in activity after incubation with the boron derivatives at this concentration included tRNA polymerase and ribonucleotide reductase (Table 3). However, the magnitude of reduction was significantly less than that observed for the previous enzymes. The d(NTP) pools were all markedly reduced after incubation with the drugs at levels of three times their ED_{50} values for 60 min (Table 3). The deoxypyrimidine triphosphate pools were affected more severely than the deoxypurine phosphate pools after 60 min incubation. Compounds I, II, V and IX interacted with isolated calf thymus after 24 h incubation at three times their ED_{50} values. All of the agents caused DNA strand scission (Figure 5). The binding to DNA was evident in the UV studies with the drugs causing hyperchromic shifts to the right, i.e. to a high UV wavelength. Thermal denaturation of DNA was also altered after incubation with the drugs. The Tm_{50} for the control DNA was 82°C: for compounds I, II, V and IX Tm_{50} values were 63, 68, 72 and 78°C, respectively. DNA viscosity studies showed that less time was required for DNA incubated with drugs to pass through the reservoirs: control = 3.53 min; I = 3.33 min,

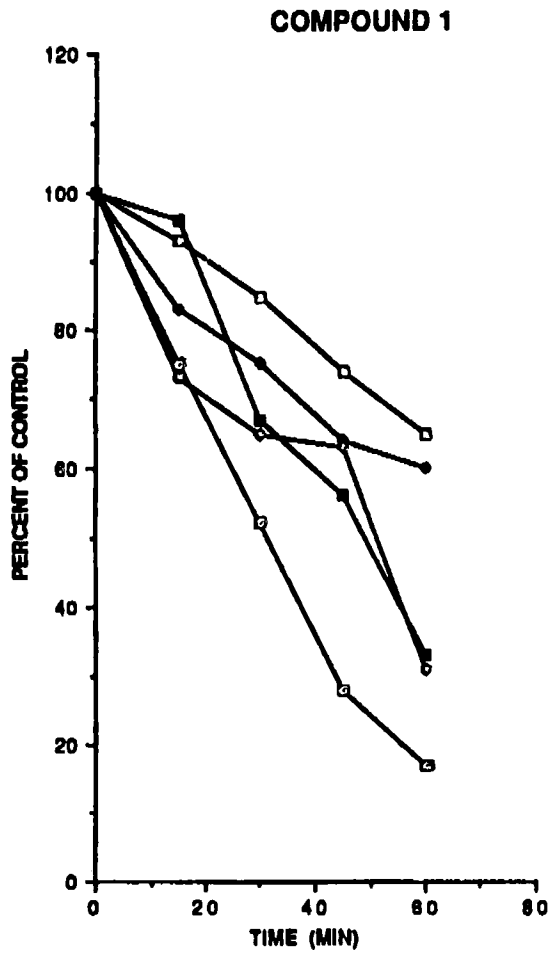

Figure 1. The effect of three times the ED_{50} of compound I on L-1210 cell enzyme activities. (⊡) Thymidine diphosphate kinase activity; (◆) thymidine kinase activity; (□) IMP dehydrogenase activity; (◇) PRPP amidotransferase activity; (■) dihydrofolate reductase activity. The assays were conducted as described in Methods for 15, 30, 45 and 60 min ($n = 6$). Standard deviations were less than 3% for all values.

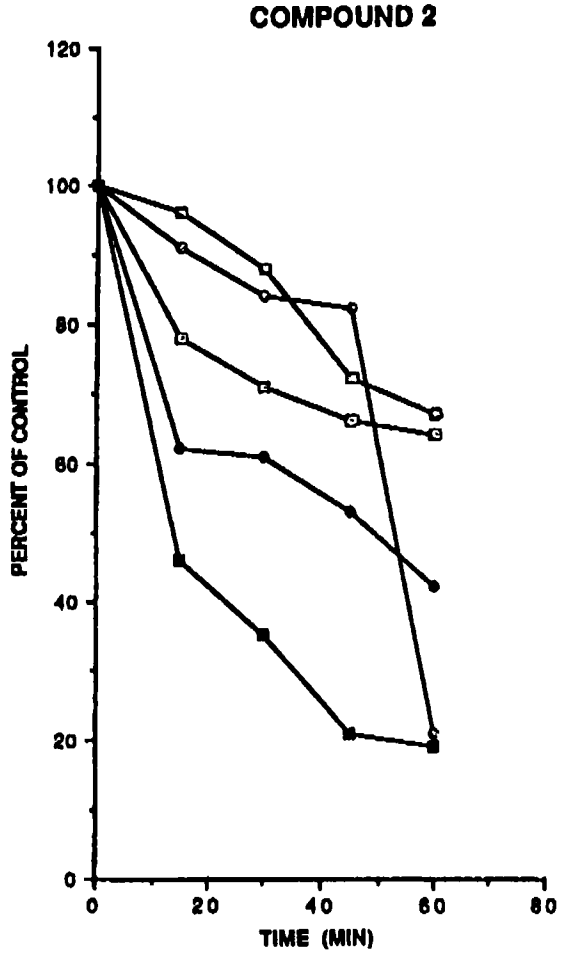

Figure 2. The effect of three times the ED_{50} of compound II on L-1210 cell enzyme activities. (⊡) Thymidine diphosphate kinase activity; (◆) thymidine kinase activity; (□) IMP dehydrogenase activity; (◇) PRPP amidotransferase activity; (■) dihydrofolate reductase activity. The assays were conducted as described in Methods for 15, 30, 45 and 60 min ($n = 6$). Standard deviations were less than 3% for all values.

II = 2.75 min; V = 2.73 min and IX = 2.89 min ($n = 3$), an observation which would be consistent with DNA strand scission in that smaller molecules move through the viscometer reservoirs at a faster rate.

Discussion

The metal complexes of trimethylamine cyano- and carboxyboranes and the substituted borane and hydroborate salts were observed to be cytotoxic in murine and human tumor cell lines. The agents appeared to preferentially block the growth of tumor cells which were single cell suspensions, e.g. L-1210, $Tmolt_3$ and Hela-S^3. Selected agents demonstrated good activity against the growth of monolayers of cells derived from solid human tumors, such as compound I against colon, lung, and glioma; compound XVI against KB, osteosarcoma and glioma; and compound XV against KB, colon, glioma and osteosarcoma. Compound XII was active against lung bronchogenic growth and VI and X demonstrated potent activity against osteosarcoma growth, indicating some selectivity for specific agents against certain tumor growth. Compounds I and XVI were highly potent with a wide spectrum of *in vitro* cytotoxicity. Compound I

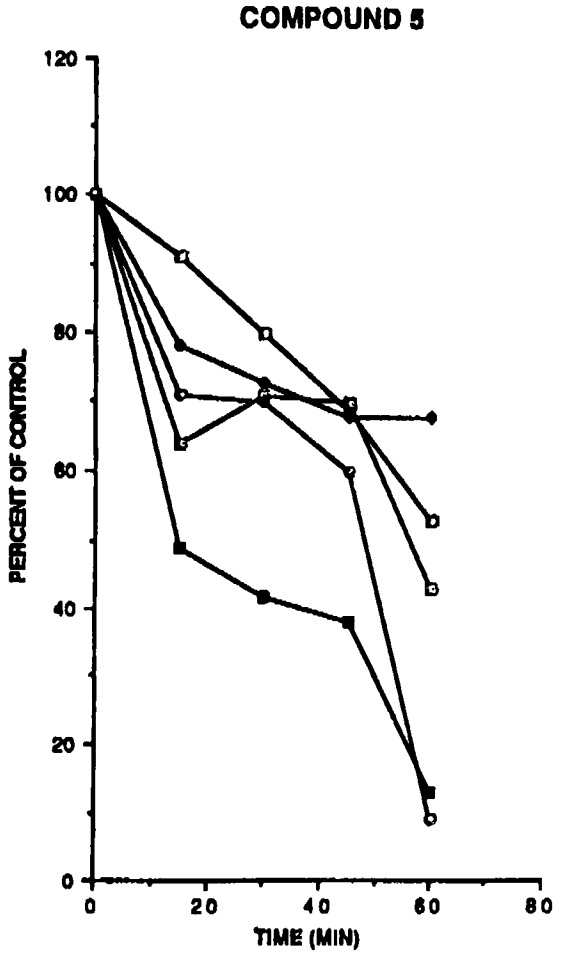

Figure 3. The effect of three times the ED_{50} of compound V on L-1210 cell enzyme activities. (⊡) Thymidine diphosphate kinase activity; (◆) thymidine kinase activity; (□) IMP dehydrogenase activity; (◇) PRPP amidotransferase activity; (■) dihydrofolate reductase activity. The assays were conducted as described in Methods for 15, 30, 45 and 60 min ($n = 6$). Standard deviations were less than 3% for all values.

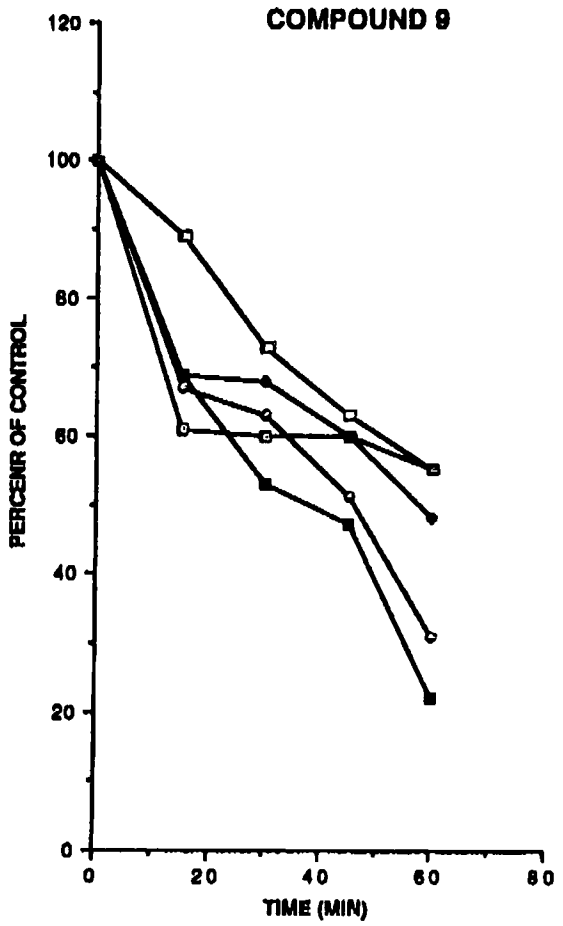

Figure 4. The effect of three times the ED_{50} of compound IX on L-1210 cell enzyme activities. (⊡) Thymidine diphosphate kinase activity; (◆) thymidine kinase activity; (□) IMP dehydrogenase activity; (◇) PRPP amidotransferase activity; (■) dihydrofolate reductase activity. The assays were conducted as described in Methods for 15, 30, 45 and 60 min ($n = 6$). Standard deviations were less than 3% for all values.

was also very active in an *in vivo* Ehrlich ascites carcinoma screen while compound XVI was moderately active.

Examination of the effects of selected derivatives on nucleic acid and protein metabolism showed that DNA synthesis of L-1210 lymphoid leukemic cells was inhibited whereas only moderate inhibition of RNA and protein synthesis was observed at a dose of three times the respective ED_{50} values of I, II, V and IX. The inhibition of nucleic acid synthesis was not related to the inhibition by the drug of DNA polymerse α, mRNA polymerase or rRNA polymerase activities. tRNA polymerase activity was inhibited by 26–86% by these compounds at three times their ED_{50} values, which may explain the observed decrease in protein synthesis.

One of the major effects of the boron derivatives appeared to be on the metabolic enzymes in the purine pathway. Dihydrofolate reductase, the enzyme required for one carbon transfer in the purine pathway and the two rate-limiting enzymes of the pathway, PRPP amidotransferase and IMP dehydrogenase, were inhibited in a concentration-dependent manner. The inhibition of dihydrofolate reductase and PRPP amidotransferase activities by the agent is sufficient to account for the observed inhibition of DNA synthesis as well as the cytotoxicity of the agents. The inhibition by the

Table 4. The effects of boron derivatives on L-1210 enzymes required for DNA synthesis with increasing concentrations at 60 min

	Percent of control ($X \pm$ SD)				
	Control[a]	Compound			
		I	II	V	IX
Dihydrofolate reductase					
$1 \times ED_{50}$	100 ± 7	112 ± 6	$65 \pm 6^*$	$62 \pm 7^*$	81 ± 7
$2 \times ED_{50}$	100 ± 6	107 ± 5	$19 \pm 3^*$	$67 \pm 5^*$	$71 \pm 6^*$
$3 \times ED_{50}$	100 ± 5	$33 \pm 4^*$	$21 \pm 4^*$	$13 \pm 3^*$	$19 \pm 2^*$
PRPP amidotransferase					
$1 \times ED_{50}$	100 ± 6	$66 \pm 6^*$	84 ± 5	$66 \pm 6^*$	$69 \pm 7^*$
$2 \times ED_{50}$	100 ± 5	$49 \pm 6^*$	$80 \pm 6^*$	$62 \pm 5^*$	$67 \pm 7^*$
$3 \times ED_{50}$	100 ± 3	$31 \pm 3^*$	$21 \pm 4^*$	$9 \pm 3^*$	$31 \pm 4^*$
IMP dehydrogenase					
$1 \times ED_{50}$	100 ± 7	99 ± 6	109 ± 5	90 ± 5	107 ± 6
$2 \times ED_{50}$	100 ± 6	$68 \pm 5^*$	102 ± 7	$60 \pm 6^*$	$73 \pm 5^*$
$3 \times ED_{50}$	100 ± 6	$65 \pm 4^*$	$67 \pm 6^*$	$53 \pm 4^*$	$55 \pm 5^*$
Thymidine kinase					
$1 \times ED_{50}$	100 ± 6	$74 \pm 6^*$	88 ± 7	83 ± 7	$79 \pm 3^*$
$2 \times ED_{50}$	100 ± 7	$73 \pm 6^*$	$73 \pm 7^*$	$75 \pm 5^*$	$56 \pm 6^*$
$3 \times ED_{50}$	100 ± 6	$60 \pm 7^*$	$42 \pm 5^*$	$43 \pm 6^*$	$48 \pm 5^*$
Thymidine diphosphate kinase					
$1 \times ED_{50}$	100 ± 6	$74 \pm 6^*$	88 ± 7	83 ± 7	$79 \pm 3^*$
$2 \times ED_{50}$	100 ± 6	$41 \pm 5^*$	$72 \pm 6^*$	$64 \pm 5^*$	$62 \pm 6^*$
$3 \times ED_{50}$	100 ± 6	17 ± 4	$64 \pm 5^*$	43 ± 5	$48 \pm 7^*$

[a] See Table 3 for control values.
* $p \leq 0.001$, $n = 6$.

agents of the nucleotide kinases such as thymidine kinase and TDP kinase, were also of a magnitude to account for the DNA synthesis inhibition. It was interesting to note that the boron derivatives did not inhibit the rate-limiting enzymes of the pyrimidine pathway, yet levels of the deoxyribonucleotides dCTP and dTTP, were significantly reduced in the presence of the agent. This was probably partly due to the inhibition of the nucleotide kinase activities by the boron derivatives. In addition, the d(NTP) levels were probably reduced by the boron agent's effects on ribonucleotide reductase activities, the enzyme responsible for the conversion of ribonucleotides to deoxyribonucleotides necessary for DNA synthesis.

The effects of the boron derivatives on metabolic enzymes were not the only observable effects. Compounds I, II, V and IX caused DNA strand scission, although it should be pointed out that the observed fragments of DNA in the gradient may have arisen from drug-induced failure of the newly formed low molecular weight DNA to be incorporated into larger molecular weight DNA. The evidence supported the thesis that the drug binds to purified DNA. However, it is not known whether this interaction between drug and DNA is intercalation or simply non-specific binding of the drug. The facts that Tm_{50} values and viscosity were altered, that the drug caused a hyperchromic shift in the UV absorption of DNA and that the DNA was fragmented into smaller molecules, suggests that specific binding of the drug occurred but this did not result in cross-linking of the two strands of DNA. The hyperchromic shift exposed more chromophores for absorption shifts and the decrease in viscosity is consistent with the drug causing DNA strand scission. Further studies to clarify the mode of action of these metal complexes are necessary. Whereas these studies suggest *in vivo* efficacy of the drug, further *in vivo* testing in tumor models is warranted.

Cis-diammine dichloroplatinum (II)[cDDP] and related derivatives $Pt(mal)(NH_3)_2$, $PtCl_2(dach)$ and Pt(mal)(dach) were shown to be cytotoxic against P-388 lymphocytic leukemia cell growth, with ED_{50} of 2.4, 11.0, 10.5, and 13.6, respectively. Their ED_{50} values L-1210 against lymphoid leukemia cells varied between 1.1 and 9.4 μg/ml, demonstrating a

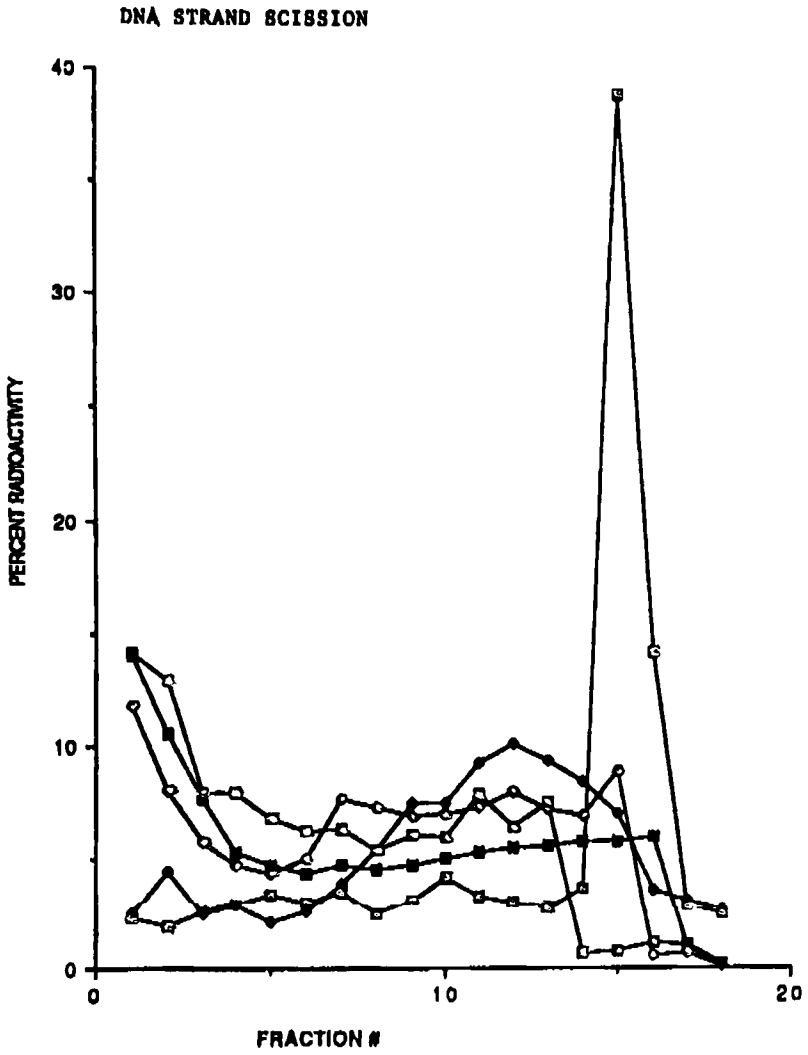

Figure 5. The effect of metal complexes on L-1210 DNA strand scission after 24 h at three times their ED_{50} value. Percentage radioactivity in each fraction 0 = top and 20 = bottom of sucrose gradient. $n = 4$. (⊡) Control; (◆) compound 1; (□) compound 2; (◇) compound 5; (■) compound 9.

similar parallel activity for these derivatives. All of the cisplatin derivatives afford T/C% > 200 in the P-388 *in vivo* screen.

Cisplatin (cDDP) and related derivatives were examined previously for a number of these same metabolic changes in P-388 lymphocytic leukemia cells as the boron derivatives. They were shown to be potent inhibitors of P-388 DNA synthesis but not RNA and protein synthesis. The cisplatin drugs caused DNA strand scission followed by DNA cross-linking.[48] The d(NTP) pools were elevated after treatment with cisplatin derivatives. These characteristics were quite different from those of the boron derivatives, which demonstrated no cross-linking of DNA and reduction of d(NTP)s pools in L-1210 cells. *In vivo* cisplatin derivatives reduced the enzyme activities of thymidine kinase, TMP kinase, carbamyl phosphate synthetase and aspartate amidotransferase in P-388 cells with moderate inhibition of DNA polymerase α. However, when these same cisplatin derivatives were examined in cultured P-388 cells, these enzymes were not markedly affected, with the exception of DNA polymerase, suggesting that a metabolite or breakdown product was responsible for the observed enzyme inhibition.[49] The DNA strand scission of cultured P-388 cells also demonstrated differences between the chloride and malonate

leading groups.[49] The boron derivatives inhibit L-1210 thymidine kinase and TDP kinase activities to a similar extent as cisplatin derivatives, but the metal complexes and salts of boron also inhibited IMP dehydrogenase, PRPP amidotransferase and dihydrofolate reductase activities, and actually stimulated DNA polymerse α activity. L-1210 DNA strand scission only was found with the boron derivatives. Thus the mechanisms of action on biochemical parameters of the two classes of metal containing antineoplastic agents are different.

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