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The anti-neoplastic activity of ethylamine-carboxyborane and triphenylphosphine-carboxyborane in L-1210 lymphoid leukemia cells

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Studies on the mode of action of two boron-containing anti-neoplastic agents, ethylamine-carboxyborane and triphenylphosphine-carboxyborane, are reported. The major site of inhibition was in the pyrimidine *de novo* synthetic pathway at orotidine monophosphate decarboxylase activity. Additional sites which may facilitate the inhibition of cell growth were IMP dehydrogenase, thymidine kinase, TMP kinase and TDP kinase, m-RNA, r-RNA and t-RNA polymerase activities as well as topoisomerase II activity. The reduction in enzyme activities led to sufficient reduction of d(NTP) levels to suppress DNA synthesis and cell growth. DNA strand scission was evident in the presence of drug. Multiple modes of action are common with amine-carboxyboranes. Acute toxicity studies in mice showed that both agents were safe in their therapeutic range based on organ weights, histological tissue sections, clinical chemistry values and hematopoietic parameters.

Keywords: Boron, anti-neoplastic, leukemia, topoisomerase II, RNA polymerase, DNA polymerases, acute toxicity

INTRODUCTION

There is rapidly growing interest in the biological activity of boron compounds in animals, including humans. Three factors are primarily responsible for this increased interest. Boron compounds are currently being explored in preclinical and clinical studies for neutron-capture therapy for the treatment of cancer.[1] There is evidence that boron may be an essential micronutrient for animals, including humans.[2] Lastly, boron compounds have been found to possess potent pharmacological activity (e.g. anti-inflammatory, analgesic, hypolipidemic, etc.) in animal model studies.[3]

Research in boron chemistry in our laboratories has focused on the synthesis of boron (often isoelectronic and isosteric) analogs of biologically important molecules for use not only in boron neutron-capture therapy, but also as potential pharmaceuticals. Boron compounds, ranging from analogs of the α-amino-acids, peptides, neurotransmitters (e.g. acetylcholine) to boronated DNA, have been prepared and tested for their biological activity.[3,4] In view of this promising pharmacological activity, we have been carrying out studies on select compounds to establish further their mode of action and acute toxicity.

Previously two carboxyborane derivatives demonstrated potent antineoplastic activity in murine screens and cytotoxicity in murine and human cancer tissue culture lines.[5–7] Ethylamine-carboxyborane (**1**), $CH_3CH_2NH_2BH_2COOH$, demonstrated 96 % inhibition *in vivo* against the growth of Ehrlich ascites carcinoma at 8 mg kg^{-1} day^{-1} i.p. This compound was active in the tumor cell lines affording ED_{50} values of 3.87 μg cm^{-3} in the L-1210 and 3.12 μg cm^{-3} in the P388 murine screens. In the human tumor cell lines compound **1** showed ED_{50} values of 2.19 μg cm^{-3} in the colon adenocarcinoma, 1.63 μg cm^{-3} in the HeLa-S^3 and 3.67 μg cm^{-3} in the KB nasopharynx, and it was not active against bronchogenic lung, osteosarcoma or glioma growth.[6] Triphenylphosphine-carboxyborane (**2**), $(C_6H_5)_3PBH_2COOH$, also demonstrated good activity.[7] In the L-1210 screen an ED_{50} value of 2.97 μg cm^{-3} was obtained, in the $Tmolt_3$,

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2.89 μg cm^{-3}, in the colon adenocarcinoma, 2.76 μg cm^{-3}, and in glioma, 3.64 μg cm^{-3}.[7] The present study is an attempt to determine the mode of action as anti-neoplastic agents and to evaluate the agents' acute toxicity in mice.

EXPERIMENTAL

Source of materials

Compound **1**, $CH_3CH_2NH_2BH_2COOH$, was synthesized as outlined previously.[6] Compound **2**, $(C_6H_5)_3PBH_2COOH$, has been reported in the literature,[7,8] and was synthesized as such. The physical and chemical characteristics are identical to those reported.[6–8]

All radioisotopes were purchased from New England Nuclear (Boston, MA, USA) 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, USA).

Pharmacological methods

Cytotoxic activity

Compounds **1** and **2** were tested for cytotoxic activity by preparing a 1 mmol dm^{-3} solution of the drugs in 0.05 % Tween 80/H_2O by homogenization. The drug solutions were sterilized by passing them through an Acrodisc 45 μ. The following cell lines were maintained by the literature techniques: murine L-1210 lymphoid leukemia, P-388 lymphocytic leukemia, human $Tmolt_3$ acute lymphoblastic T cell leukemia, colorectal adenocarcinoma SW480, lung bronchogenic MB-9812, osteosarcoma TE418, KB epidermoid nasopharynx, HeLa-S^3 suspended cervical carcinoma and glioma EH 118MG. The protocol used to assess cytotoxicity was that of Geran *et al.*[9] Standards were determined in each cell line. Values are expressed for the drug's cytotoxicity as ED_{50} in μg cm^{-3}, i.e. the concentration which inhibits 50 % of the cell growth was determined by the Trypan Blue exclusion technique. A value of less than 4 μg cm^{-3} was required for significant activity of growth inhibition.[9] Solid tumor cytotoxicity was determined by the method of Liebovitz *et al.*[10]

Incorporation studies

Incorporation of labeled precursors into [^{3}H]DNA, [^{3}H]RNA and [^{3}H]protein for 10^6 L-1210 cells was determined by method of Liao *et al.*[11] The concentration response for inhibition of DNA, RNA and protein synthesis was determined at 25, 50 and 100 μmol dm^{-3} at 60 min. [^{14}C]Glycine (53.0 mCi mmol^{-1}) incorporation into purines was determined by the method of Cadman *et al.*[12] [^{14}C]Formate (53.0 mCi mmol^{-1}) incorporation into pyrimidines was determined by the method of Christopherson *et al.*[13]

Enzyme assays

Inhibition of various enzyme activities were carried out by first preparing the appropriate L-1210 cell homogenate or subcellular fraction, then adding the drug to be tested during the enzyme assay. For the concentration response studies, the inhibition of enzyme activity was determined at 25, 50 and 100 μmol dm^{-3} of **1** or **2** for 60-min incubations. DNA polymerase alpha activity was determined in a cytoplasmic extract isolated by the method of Eichler *et al.*[14] Nuclear DNA polymerase was determined by isolating nuclei.[15] The polymerase assay for both alpha and beta was that of Sawada *et al.*[16] with [^{3}H]TTP. Messenger, ribosomal and transfer-RNA polymerase enzymes were isolated with different concentrations of ammonium sulfate and the individual RNA polymerase activities were determined using [^{3}H]UTP.[17,18] Ribonucleotide reductase activity was measured with [^{14}C]CDP with and without dithioerythritol.[19] 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^{-1}) and the medium of Maley and Ochoa.[20] Carbamyl phosphate synthetase activity was determined by the method of Kalman *et al.*[21] and citrulline was determined colorimetrically.[22] Aspartate transcarbamylase activity was determined by the method of Kalman *et al.*[21] and carbamyl aspartate was determined colorimetrically.[23] Thymidylate synthetase activity was analyzed by the method of Kampf *et al.*[24] 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.*[25] phosphoribosyl pyrophosphate (PRPP) amidotransferase activity was determined by the

method of Spassova *et al.*[26] and IMP dehydrogenase activity was determined with [8-^{14}C]IMP (54 mCi mmol^{-1}) (Amersham, Arlington Heights, IL, USA) where XMP was separated on polyethyleneimine cellulose (PEI) plates (Fisher Scientific) by TLC.[27] Protein was determined for all of the enzymatic assays by the Lowry technique.[28]

Deoxyribonucleoside triphosphates were extracted by the method of Bagnara and Finch.[29] Deoxyribonucleoside triphosphates were determined by the method of Hunting and Henderson[30] with calf thymus DNA, *E. 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.

The effects of the agents on isolated DNA topoisomerase II activity was determined by the method of Miller *et al.*[31] Knotted DNA was prepared from bacteriophage P4 as outlined in the literature.[32] The enzyme, DNA topoisomerase II, was isolated from HeLa uterine carcinoma cells. The reaction medium contained 0.20 mol dm^{-3} Tris, pH 7.5, 0.4 mol dm^{-3} KCl, 0.04 mol dm^{-3} $MgCl_2$, 120 μg cm^{-3} BSA, 2.0 mol dm^{-3} EDTA, 4.0 mmol dm^{-3} DDT and 4 mmol dm^{-3} ATP. For the enzyme assay 2.5 μl of the reaction medium and 0.25 μl of knotted DNA were added and diluted to 8 μl with distilled water. The agents were added with 1.0 μl of enzyme for a final volume of 10 μl which was incubated for 60 min at 37 °C. Buffer (50 % w/v sucrose, 0.5 % w/v sodium dodecyl sulfate, 0.25 % w/v Bromophenol Blue and 0.25 % w/v sodium dodecyl sulfate, 0.25 % w/v Bromophenol Blue and 0.25 % xylene cyanol) (2.5 μl) was added to stop the reaction. Samples of the reaction, pure DNA and pure enzyme were placed on an agarose electrophoresis gel at 23 V. VP-16, etoposide, was used as an internal standard (100 μmol dm^{-3}). Inhibition of the activity is noted in the gel when topoisomerase II reduces the ability to unknot the knotted DNA. The inhibition of the activity appears as a smear of DNA in the gel as opposed to distinct separation of bands.

The effects of compounds **1** and **2** on DNA strand scission was determined by the *in vitro* method of Suzuki *et al.*,[33] Pera *et al.*[34] and Woynarowski *et al.*[35] L-1210 lymphoid leukemia cells were incubated with 10 μCi [^{3}H-*methyl*]thymidine, 84.0 Ci mmol^{-1} for 24 h at 37 °C. After harvesting the L-1210 cells (10^7), the cells were centrifuged at 600 $g \times 10$ min in phosphate-buffered saline (PBS), washed and suspended in 1 cm^3 of PBS. Lysis buffer (0.5 cm^3; 0.5 mol dm^{-3} NaOH, 0.02 mol dm^{-3} EDTA, 0.01 % Triton X-100 and 2.5 % sucrose) was layered onto a 5–20 % alkaline–sucrose gradient (5 cm^3; 0.3 mol dm^{-3} NaOH, 0.7 mol dm^{-3} KCl and 0.01 mol dm^{-3} EDTA) followed by 0.2 cm^3 cell preparation. After incubating for 2.5 h at room temperature, the gradient was centrifuged at 12 000 rpm at 20 °C for 60 min (Beckman rotor SW60). Fractions (0.2 cm^3) were collected from the top of the gradient, neutralized with 0.2 cm^3 of 0.3 mol dm^{-3} HCl, and radioactivity was measured. Thermal calf thymus DNA denaturation studies and DNA viscosity studies were conducted after incubation of compounds **1** and **2** at 100 μmol dm^{-3} at 37 °C for 24 h.[36]

Mouse toxicity

LD_{50} acute toxicity was determined in CF_1 male mice (~28 g) i.p. using doses of 5 mg kg^{-1} to 1 g kg^{-1} as a single dose. The number of deaths in each group was noted over the next seven days.

For the mouse toxicity study, CF_1 male mice (~28 g) were dosed at 8, 16 and 40 mg kg^{-1} day^{-1} i.p. for seven days with compounds **1** and **2**. The food consumption was determined daily and water was *ad libitum*. The animals were maintained in 12-h light and dark cycles at 72 °F (22 °C).

Clinical chemistry

At the time of the sacrifice, the major organs were excised, trimmed of fat and weighed. Blood was obtained from the carotid and centrifuged at 3500 $g \times 10$ min to obtain the serum. Chemical or enzymatic assays were performed with Sigma Chemical kits on the following: urea nitrogen (BUN, No. 640), alanine aminotransferase (SGPT, No. 505), alkaline phosphatase (AP, No. 104), glucose (No. 510), lactic dehydrogenase (LDH, No. 500), creatine phosphokinase (CP-kinase, No. 661), and total and direct bilirubin (No. 605). Serum triglycerides were determined with a diagnostic kit from Boehringer Mannheim; serum cholesterol was determined by the method of Ness *et al.*[37] Albumin and total protein were determined by the method of Lowry *et al.*[28] Cholic acid and uric acid were determined as outlined by Tietz.[38]

Blood collection and parameters

Blood was obtained from the carotid, a drop was placed on glass slides and it was fixed in Wright's stain. Differential white blood cell counts, platelet counts and hematocrits were obtained for each mouse group sacrificed at the specified times.[39]

Histological section

The animals were killed by carbon dioxide asphyxiation. After all vital signs had ceased, a midline incision was made from the lower jaw to the inguinal area. Thymus, spleen, liver and kidney were excised and weighed, and representative tissue samples were fixed in 10 % buffered formalin, trimmed and sectioned at 6 μm in thickness and stained with hematoxylin and eosin.

Female fertility

CF_1 female mice (~30 g) were administered 8, 20 or 40 mg kg^{-1} day^{-1} for three weeks. While continuing dosing, the females were exposed to males (2:1) for another three weeks. The males were rotated every seven days to eliminate infertility. After three weeks the males were removed. The percentage of pregnancies, numbers of live births and deaths, and birth weights were noted. Four weeks after birth the pups' weight, percentage survival and sex were noted for each group.

Table 1 The effects of ethylamine-carboxyborane on L-1210 lymphoid leukemia nucleic acid metabolism

	Percentage of control				
($N=6$)	Control	10 (μmol dm^{-3})	25 (μmol dm^{-3})	50 (μmol dm^{-3})	100 (μmol dm^{-3})
DNA synthesis	100 ± 6[a]	77 ± 4*	67 ± 5*	56 ± 5*	52 ± 4*
RNA synthesis	100 ± 5[b]	137 ± 6	65 ± 5*	65 ± 6*	36 ± 4*
Protein synthesis	100 ± 6[c]	97 ± 7	96 ± 6	88 ± 7	72 ± 5*
Purine incorporation	100 ± 8[d]	92 ± 6*	60 ± 8*	26 ± 7	65 ± 5*
Pyrimidine incorporation	100 ± 7[e]	128 ± 7	34 ± 5*	25 ± 4*	21 ± 3*
PRPP amidotransferase	100 ± 6[f]	105 ± 6	99 ± 5	98 ± 5	96 ± 4
IMP dehydrogenase	100 ± 7[g]	75 ± 5*	63 ± 6*	60 ± 7*	59 ± 5*
Carbamyl phosphate	100 ± 7[h]	82 ± 6	81 ± 7	78 ± 6*	77 ± 6*
Aspartate transcarbamylase	100 ± 5[i]	362 ± 8*	290 ± 11*	270 ± 9*	160 ± 7*
OMP decarboxylase	100 ± 6[j]	85 ± 6	69 ± 7*	58 ± 5*	57 ± 4*
Thymidylate synthetase	100 ± 6[k]	101 ± 5	95 ± 7	94 ± 6	90 ± 6
DNA polymerase (beta)	100 ± 5[l]	155 ± 8	107 ± 7	105 ± 4	101 ± 5
m-RNA polymerase	100 ± 5[m]	110 ± 7	61 ± 5*	49 ± 4*	42 ± 5*
r-RNA polymerase	100 ± 4[n]	175 ± 8*	69 ± 8*	45 ± 7	27 ± 4*
t-RNA polymerase	100 ± 6[o]	57 ± 5*	57 ± 6*	45 ± 4*	36 ± 5*
Thymidine kinase	100 ± 7[p]	80 ± 6	79 ± 5*	70 ± 5*	60 ± 6*
TMP kinase	100 ± 6[q]	97 ± 7	87 ± 6	84 ± 6	68 ± 5*
TDP kinase	100 ± 4[r]	96 ± 6	92 ± 7	81 ± 5	74 ± 6*
Ribonucleoside reductase	100 ± 7[s]	76 ± 5*	56 ± 5*	20 ± 3*	19 ± 3*
Dihydrofolate reductase	100 ± 6[t]	102 ± 6	102 ± 7	104 ± 5	107 ± 7
d (ATP)	100 ± 4[u]				117 ± 8
d(GTP)	100 ± 6[v]				71 ± 5*
d(CTP)	100 ± 5[w]				69 ± 6*
d(TTP)	100 ± 6[x]				62 ± 4*

[a] 7719 dpm.
[b] 1014 dpm.
[c] 17492 dpm.
[d] 28614 dpm.
[e] 19758 dpm.
[f] 19575 dpm.
[g] 0.273 mol citrulline.
[h] 0.0878 O.D. units.
[i] 0.807 mol *N*-carbamyl aspartate.
[j] 57387 dpm.
[k] 77616 dpm.
[l] 9019 dpm.
[m] 1343 dpm.
[n] 325 dpm.
[o] 400 dpm.
[p] 1371 dpm.
[q] 1179 dpm.
[r] 1891 dpm.
[s] 48780 dpm.
[t] 0.114 O.D. units
[u] 17.07 pmol.
[v] 13.58 pmol.
[w] 33.60 pmol.
[x] 31.04 pmol.
*$P<0.001$ (Student's t).

Table 2 The effects of triphenylphosphine-carboxyborane on L1210 lymphoid leukemia nucleic acid metabolism

	Percentage of control				
($N=6$)	Control	10 (μmol dm^{-3})	25 (μmol dm^{-3})	50 (μmol dm^{-3})	100 (μmol dm^{-3})
DNA synthesis	100 ± 6[a]	84 ± 6	69 ± 5*	54 ± 6*	49 ± 4*
RNA synthesis	100 ± 5[b]	68 ± 5	64 ± 5	49 ± 4*	44 ± 4*
Protein synthesis	100 ± 6[c]	148 ± 7	112 ± 6	99 ± 5	88 ± 6
Purine synthesis	100 ± 8[d]	264 ± 8	210 ± 9	129 ± 7	81 ± 6*
Pyrimidine synthesis	100 ± 7[e]	85 ± 6	70 ± 5*	38 ± 4*	31 ± 4*
PRPP amidotransferase	100 ± 6[f]	109 ± 7	107 ± 6	100 ± 5	95 ± 6
IMP dehydrogenase	100 ± 7[g]	79 ± 6*	71 ± 5*	62 ± 6*	55 ± 4*
Carbamyl phosphate	100 ± 5[h]	107 ± 5	102 ± 5	101 ± 6	95 ± 5
Aspartate transcarbamylase	100 ± 7[i]	119 ± 6	118 ± 7	109 ± 6	97 ± 7
OMP decarboxylase	100 ± 6[j]	90 ± 8	64 ± 5*	54 ± 6*	46 ± 4*
Thymidylate synthetase	100 ± 6[k]	122 ± 7	103 ± 6	82 ± 6	78 ± 4*
DNA polymerase (beta)	100 ± 5[l]	91 ± 6	85 ± 5	85 ± 6	82 ± 5
m-RNA polymerase	100 ± 5[m]	85 ± 6	40 ± 3*	35 ± 3*	34 ± 4*
r-RNA polymerase	100 ± 4[n]	79 ± 6	39 ± 3*	27 ± 3*	17 ± 3*
t-RNA polymerase	100 ± 6[o]	45 ± 5*	35 ± 4*	34 ± 4*	22 ± 3*
Thymidine kinase	100 ± 6[p]	99 ± 7	95 ± 6	66 ± 5*	64 ± 4*
TMP kinase	100 ± 6[q]	118 ± 8	92 ± 7	82 ± 6	70 ± 5*
TDP kinase	100 ± 4[r]	114 ± 9	101 ± 8	89 ± 7	78 ± 6*
Ribonucleoside reductase	100 ± 7[s]	138 ± 8	110 ± 7	74 ± 5*	34 ± 4*
Dihydrofolate reductase	100 ± 6[t]	106 ± 7	103 ± 6	100 ± 5	98 ± 6
d(ATP)	100 ± 4[u]				97 ± 6
d(GTP)	100 ± 6[v]				44 ± 4*
d(CTP)	100 ± 5[w]				75 ± 5*
d(TTP)	100 ± 6[x]				53 ± 5*

For footnotes, refer to Table 1.

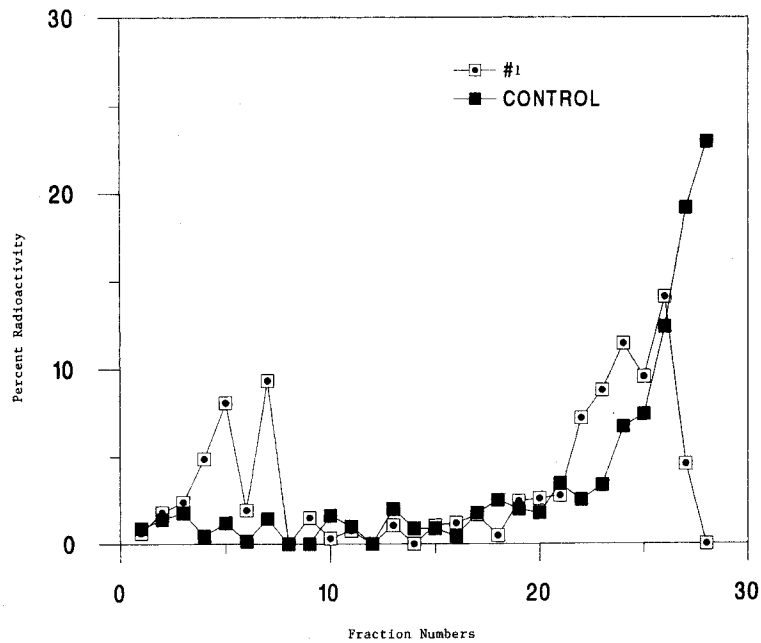

Figure 1 DNA strand scission by compound **1**.

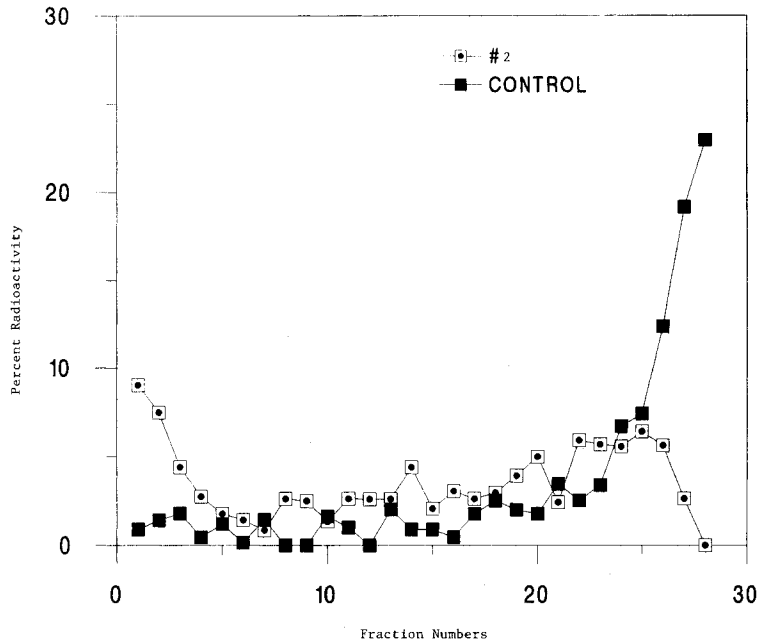

Figure 2 DNA strand scission by compound **2**.

Statistics

The mean and standard deviation are designated by the letter *N*. The probable level of significance (*P*) between test samples and control samples was determined by Student's *t* test with raw data.

RESULTS

Compounds **1** and **2** in L-1210 cells were shown to inhibit DNA and RNA synthesis in a concentration-dependent manner achieving approximately 50 % suppression or better at 100 μmol dm^{-3} (Tables 1 and 2). Protein synthesis was not affected by the same magnitude, with only 12–28 % inhibition after 60 min. Glycine incorporation into purines was affected at 100 μmol dm^{-3} by both compounds with 19–35 % reduction, but formate incorporation into pyrimidines was reduced more significantly by the agents at 100 μmol dm^{-3} from 69–79 % reduction in 60 min. In the purine pathway, the site at IMP dehydrogenase was reduced in activity by both agents 41–45 %, but the other regulatory site in the pathway PRPP–amidotransferase was not affected by the compounds. In the pyrimidine pathway, carbamyl phosphate synthetase was not affected by either drug, but aspartate transcarbamylase activity was stimulated by compound **1**. Nevertheless, OMP decarboxylase activity was inhibited significantly by both agents. The inhibition of this enzyme was of sufficient magnitude to account for the observed inhibition of DNA synthesis in L_{1210} cells after 60 min. Thymidylate synthetase activity was only marginally inhibited by compound **2**. Ribonucleoside reductase activity was inhibited by both agents. Compound **1** caused greater than 80 % inhibition of ribonucleoside reductase activity whereas compound **2** resulted in only 66 % after 60 min at 100 μmol dm^{-3}.

Other biochemical parameters which were affected by the carboxyboranes were the polymerases and the nucleoside kinases. Compound **1** did not affect DNA polymerase activity; however mRNA, rRNA and tRNA polymerase activities were significantly reduced below 50 % after 60 min. Thymidine kinase activity was reduced 40 % by compound **1** with TMP and TDP kinase activities following a similar pattern with slightly less inhibition at 60 min. Compound **2** was also effective in inhibiting polymerase activities. Actually DNA polymerase activity was suppressed 18 %. The RNA polymerases were inhibited in a concentration-dependent manner with rRNA and tRNA polymerase activities being inhibited more than 80 % at 100 μmol dm^{-3}. Thymidine, TMP

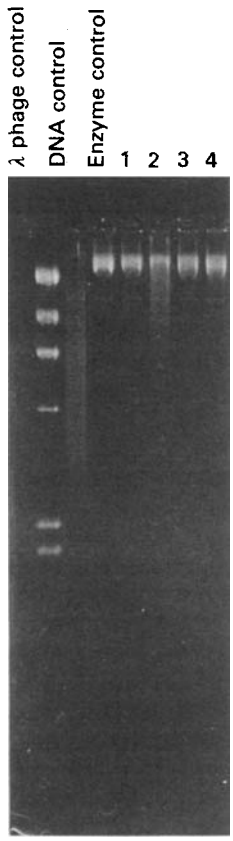

Figure 3 P4 phage DNA unknotting assay: lane 1, unknotted phage DNA control; lane 2, P4 phage DNA control; lane 3, HeLa topoisomerase II control. Drugs:
1, $(CH_3)_3NBH_2C(O)NHCH(CH_2C_6H_5)C(O)OCH_3$;
2, $CH_3CH_2NH_2BH_2COOH$;
3, $(CH_3)_2N(BH_3)CH_2CH_2OC(O)C_6H_5$;
4, $(C_6H_5)_3PBH_2COOH$.

and TDP kinase activities were all inhibited 22–36 % by compound **2**.

Deoxyribonucleotide pool levels were also examined after a 60-min incubation with the agents at 100 μmol dm^{-3} concentration. The d-GTP levels were reduced after treatment with both agents, whereas d-ATP levels were within normal limits. The d-CTP and d-TTP levels in L_{1210} cells were reduced significantly by both agents.

Studies with calf thymus DNA showed that after incubation of drugs **1** and **2** at 100 μmol dm^{-3} for 24 h, the DNA viscosity of the control was 294.1 s, whereas that of drug **1** was 319 s and that of drug **2** was 320 s, i.e. the time required to pass through the reservoirs. In the thermal denaturation studies the DNA melting temperature (T_m) values for calf thymus DNA did not change after drug incubation, nor were any changes observed in the ultraviolet absorption of DNA. L-1210 DNA strand scission studies showed that smaller-molecular-weight DNA appeared in the gradient after incubating for 24 h (Figs 1 and 2). *In vitro* topoisomerase II activity from HeLa uterine cancer cells was inhibited by compound **1** at 100 μmol dm^{-3} but not by compound **2** (Fig. 3). Compound **2** required a much higher concentration (200 μmol dm^{-3}) to achieve the same level of inhibition.

The acute toxicity studies in mice demonstrated that the LD_{50} values in CF_1 male mice for both agents were >500 mg kg^{-1} i.p. At 8, 16 or 40 mg kg^{-1} for seven days, all of the mice survived with no significant change in daily food consumption or total body weight (Tables 3 and 4). Individual organ weights showed minor alterations with treatment, e.g. compound **1** caused a slight increase in kidney weight at 40 mg kg^{-1} and a decrease in small intestine and stomach at 8 and 16 mg kg^{-1}. Compound **2** caused a decrease in stomach weight at all doses and small and large intestine showed reductions after treatment. The hematocrits were all in a range considered to be normal, as were the platelet estimates. There was no indication that the drugs caused rouleux formation of the red blood cells (rbc) at any dose employed. The differential white cell count showed a slight increase in the percentage of lymphocytes and a reduction of polymorphoneutrophils (PMNs) as the dose of the agent increased. The minor white blood cells showed some modulation but these effects exhibited no significant trend. The clinical chemistry values showed some alterations, e.g. total serum protein was elevated with both agents, but albumin showed minor modulation. Serum glucose levels were reduced with both agents in a dose-dependent manner. Compound **1** lowered serum glutamic pyruric transaminase (SGPT), lipase, blood urea nitrogen (BUN), acid phosphatase and direct bilirubin levels with slight elevations of CP-kinase and triglyceride levels. Compound **2** caused a decrease in BUN, triglycerides, and CP-kinase levels, with elevations in SGPT, direct bilirubin, acid phosphatase and uric acid levels. Histological sections of the liver, kidney and spleen at all three doses employed were all normal. The agents were examined for their effects on fertility in female CF_1 mice. At the higher doses (20 and 40 mg kg^{-1} day^{-1}) there was a reduction in the percentage of pregnancies. The

number of fetuses/litter for compound **1** was similar to the control group, as was the birth weight. The survival of the pups and their weight gain was normal with compound **1**. Compound **2** caused a decrease in the number of fetuses/litter as well as the number surviving (Table 5).

DISCUSSION

These two carboxyborane derivatives demonstrated similar anti-neoplastic and cytotoxic activity to that expressed by other amine-carboxyboranes in murine and human cancer cells.[5–7] The current derivatives were more effective in inhibiting RNA synthesis and less active in inhibiting protein synthesis in L-1210 leukemic cells than other amine carboxyboranes. For example, the marked inhibition of m-RNA and r-RNA polymerase activity by compounds **1** and **2** has never been observed for other amine-carboxyboranes. The inhibition of t-RNA polymerase activity was previously observed, as has the inability of the

Table 3 Acute toxicity of ethylamine-carboxyborane in CF_1 male mice i.p.

($N=5$)	Control	8 mg kg^{-1}	16 mg kg^{-1}	40 mg kg^{-1}
Increase in total body wt				
from day 0	105.2	97.5	98.6	100.58
Food consumption, g day^{-1}	7.21	6.09	6.84	6.92
Survival	5/5	5/5	5/5	5/5
Hematocrit, %	45.07	51.25	48.51	48.51
Platelet estimate ($\times 10^4$)	19.3	19.1	19.2	19.4
Differential white cell count, %				
Lymphocytes	60.5	62.5	68.5	66.5
PMNs	37.0	34.5	27.0	30.0
Basophils	1.50	2.0	3.0	2.0
Eosinophils	0.50	0	0	0
Monocytes	0.50	1.0	1.0	1.50
Organ weight, % total weight				
Brain	0.4021	0.4089	0.3732	0.3902
Heart	0.1862	0.1753	0.1840	0.1824
Lung	0.2426	0.2451	0.2433	0.2349
Thymus	0.0487	0.0438	0.0627	0.0444
Liver	2.0963	1.9135	2.2106	2.4646
Kidney	0.6478	0.6557	0.6654	0.7628
Spleen	0.1251	0.1081	0.1328	0.1607
Stomach	1.2398	0.6682	0.7729	1.1121
Small intestine	1.8126	1.6586	1.7525	1.8938
Large intestine	1.0353	1.0413	1.3789	1.3478
Reproduction	1.1200	1.1757	1.2658	1.2041
Clinical chemistry				
Total protein	100	165	162	172
Albumin	100	116	119	126
Glucose	100	81	79	67
SGPT	100	73	40	57
BUN	100	117	70	83
Triglyceride	100	109	107	121
Cholesterol	100	102	96	108
Lipase	100	85	92	75
Lactate dehydrogenase (LDH)	100	98	89	118
CP kinase	100	120	139	134
Acid phosphatase	100	21	32	30
Direct bilirubin	100	48	24	52
Uric acid	100	83	95	89
Bile acids	100	107	108	101

Table 4 The acute toxicity of triphenylphosphine-carboxyborane in CF_1 male mice i.p.

($N=5$)	Control	8 mg kg^{-1}	16 mg kg^{-1}	40 mg kg^{-1}
Increase in total body wt				
from day 0	104.1	97.4	100.3	101.9
Food consumption, g day^{-1}	6.32	5.00	6.46	6.04
Survival	5/5	5/5	5/5	5/5
Hematocrit, %	49.73	43.13	45.33	46.81
Platelet estimate ($\times 10^4$)	19.2	19.0	19.4	19.1
Differential white cell count, %				
Lymphocytes	60.5	61.0	70.0	77.0
PMNs	37.0	35.0	29.5	21.0
Basophils	1.50	2.0	1.0	1.0
Eosinophils	0.50	0	0	0.50
Monocytes	0.50	1.75	0.50	0.50
Organ weights, % total weight				
Brain	0.3740	0.3792	0.3998	0.3773
Heart	0.1752	0.1609	0.1786	0.1465
Lung	0.2413	0.2228	0.2586	0.2092
Thymus	0.0590	0.0301	0.0473	0.0613
Liver	1.8549	1.8114	2.0324	1.8711
Kidney	0.5931	0.5770	0.6604	0.5662
Spleen	0.1441	0.1097	0.1532	0.1966
Stomach	1.1555	0.6671	0.8413	0.8769
Small intestine	1.7298	1.3476	1.6026	1.4829
Large intestine	1.3657	1.3133	1.1270	1.0833
Reproduction	1.1235	1.1521	1.0412	0.9517
Clinical chemistry				
Total protein	100	116	123	129
Albumin	100	101	92	85
Glucose	100	106	72	49
SGPT	100	142	181	159
BUN	100	79	46	76
Triglycerides	100	65	62	64
Cholesterol	100	100	99	107
Lipase	100	86	137	158
LDH	100	79	89	114
CP kinase	100	78	83	43
Acid phosphatase	100	62	149	91
Direct bilirubin	100	92	83	133
Uric acid	100	88	98	121
Bile acids	100	86	107	90

amine-carboxyboranes to inhibit the activities of DNA polymerases, alpha and beta. Whereas the inhibition of nucleoside kinase has been observed previously, the inhibition of regular steps in the pyrimidine pathway was not evident with the earlier derivatives.[5-7] OMP decarboxylase activity inhibition was of sufficient magnitude to explain the observed inhibition of *de novo* pyrimidine synthesis as well as DNA synthesis of L-1210 cells. The inhibition of dihydrofolate reductase activity has been noted with amine-carboxyboranes in tumor cell, in both tissue culture and *in vivo* tumors. Inhibition of this enzyme activity would reduce the one-carbon transfer for both pyrimidine and purine synthesis. Yet it was not observed with compounds **1** and **2**. The suppression of regular enzymes in the purine pathway has been noted at PRPP amidotransferase and IMP dehydrogenase in L_{1210} cells by other boron derivatives. Compounds **1** and **2** only inhibited IMP dehydrogenase activity. The inhibition by the agents in the pyrimidine and purine pathways

Table 5 The effect of carboxyboranes on CF_1 female mice fertility

	Control	8 mg kg^{-1}	20 mg kg^{-1}	40 mg kg^{-1}
Compound **1**				
Pregnancies, %	100	83	67	67
No. of viable fetuses/litter	9.33	9.6	9.0	9.5
Weight at birth, g	1.60	1.636	1.586	1.571
Viability of pups at week 3				
No. of viable pups/litter	9.2	9.6	9.0	7.25
Weight of pups	12.33	14.39	14.38	14.12
Males/litter, %	54.5	39.8	49.2	50.0
Compound **2**				
Pregnancies, %	100	83	100	83
No. of viable fetuses	9.33	6.4	10.0	4.6
Weight at birth, g	1.60	1.72	1.75	1.75
Viability of pups at week 3				
No. of viable pups	9.20	5.8	9.0	2.8
Weight of pups	12.33	15.56	13.86	15.2
Males/litter, %	54.5	40.0	44.8	43.4

would account for the observed reduction in the deoxyribonucleotide pool levels after 60 min of incubation. Another enzyme which would affect the pool levels is ribonucleoside reductase, which was inhibited significantly by compounds **1** and **2**. The reduction of deoxyribonucleotide pools and probably ribonucleotide pools would explain the significant reduction of DNA and RNA synthesis of L-1210 cells after 60 min. Another site where the agents may be having marginal effects involved the DNA molecule itself. The studies with calf thymus DNA showed no effects of drug intercalation betweeen the bases, i.e. T_m values were normal; however, the viscosity was increased with drug incubation, suggesting some type of DNA–drug interaction other than intercalation, which is supported by the inhibition of topoisomerase II activity and the fragmentation of L-1210 DNA to a smaller molecular-weight size.

The acute toxicity studies in mice demonstrated that both agents were safe at their therapeutic doses. None of the changes observed in organ weight was of significant magnitude to be of concern. There were slight changes in the differential white cell count and rbc at higher doses of these agents but these alterations were not significant with regard to anti-neoplastic agents. The increase in total protein with an increase in hematocrit may reflect dehydration after treatment with the agents which is manageable in the clinic. The lower glucose levels may require clinical intervention although these were within normal limits at the therapeutic dose of the agents. Whereas some of the other clinical values were significantly changed from the control values, they were not of a magnitude to suggest that tissue damage or toxicity was present in the animals. There were reductions in the percentage of pregnancies in mice after treatment with the agents. Compound **2** resulted in a reduction in the number of fetuses and their survival. Again, these observations are common with anti-neoplastic agents in that they tend to be teratogenic, fetal-toxic and carcinogenic in nature. The LD_{50} values of both agents was in the range to indicate that the agents possessed a safe therapeutic index.

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