Anti-Cancer Drugs, 1, pp. 133–141

The antineoplastic activity of trimethylamine carboxyboranes and related esters and amides in murine and human tumor cell lines

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Trimethylamine carboxyboranes including their esters and amides were shown to have antineoplastic activity *in vivo* against Ehrlich ascites carcinoma growth. The derivitization to the ester or amide did not necessarily improve activity. Cytotoxicity of the derivatives was observed against the growth of murine and human tumor cells. Selectivity was demonstrated by the boron derivatives in the human solid tumor screens. Almost all the compounds demonstrated cytotoxicity against single-cell suspension growths, eg Tmolt$_3$, L$_{1210}$, HeLa-S^3. Selection of two compounds to examine their mode of action in L$_{1210}$ lymphoid leukemia cells showed that the agents perferentially inhibited DNA synthesis followed by protein and RNA synthesis. The d(TTP) pools were markedly reduced because of inhibition of nucleotide kinase activity. The agents also inhibited regulatory enzymes in the *de novo* purine pathway and afforded DNA strand scission. These effects by the agents were probably additive to bring about tumor cell death.

Key words: Antineoplastic activity, trimethylamine carboxyboranes.

Introduction

A series of trimethylamine cyanoboranes has previously been shown to possess activity *in vivo* against the growth of Ehrlich ascites, Lewis lung carcinoma and B_{16} melanoma growth in mice and Walker 256 carcinosarcoma in rats.[1] Subsequently a metal complex, tetrakis-μ-(trimethylamineborane-carboxylato)-bis(trimethylamine-carboxyborane) di-copper(II)[2] and a number of di- and tripeptide derivatives of trimethylamine carboxyborane demonstrated activity against Ehrlich ascites growth.[3] Heterocyclic amine adducts of cyano- and carboxyboranes were active against murine and human tumor cell growth.[4]

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In vitro studies in Ehrlich ascites studies showed that a number of the trimethylamine cyanoboranes inhibited DNA and RNA synthesis but not protein synthesis.[5] Furthermore, Ehrlich nuclear DNA polymerase, PRPP amido transferase and dihydrofolate reductase activities were inhibited by these agents.

The amine carboxyboranes have demonstrated antineoplastic activity.[4] These agents have improved LD_{50} values of 200–1000 mg/kg ip in mice compared with the cyanoboranes. Additional acute toxicity studies in mice have shown no deleterious effects at five times their therapeutic dose based on histological and morphological examination of the major organs, clinical chemistry values and hematopoietic parameters.[5] Consequently, the current study expands the types of amine carboxyboranes including their esters and amides. Their mode of action as antineoplastic agents is examined in L_{1210} lymphoid leukemic cells for inhibition of nucleic acid and protein metabolism.

Source of compounds

All carboxyborane derivatives have previously been synthesized and their chemical and physical properties reported: ammonia carboxyborane **1**,[6] methylamine carboxyborane **2**,[6] dimethylamine carboxyborane **3**,[6] trimethylamine carboxyborane **4**,[5] ethylamine carboxyborane **5**,[7] di-*n*-propylamine carboxyborane **6**,[7] *N*,*N*,*N*′,*N*′-tetramethylethylenediamine *N*,*N*′-bis-carboxyborane **7**,[8] *N*,*N*-dimethylhexadecylamine carboxyborane **8**,[9] *N*,*N*-dimethyloctadecylamine carboxyborane **9**,[9] hydrazine monocarboxyborane **10**,[7] ammonia carbomethoxylborane **11**,[10] methylamine carbomethoxy-

borane **12**,[10] dimethylamine carbomethoxyborane **13**,[6] trimethylamine carbomethoxyborane **14**,[10] *n*-butylamine carbomethoxyborane **15**,[7] *N*,*N*-dimethylhexadecylamine carbomethoxyborane **16**,[9] *N*,*N*-dimethylhexadecylamine carboethoxyborane **17**,[9] trimethylamine carboethoxyborane **18**,[10] ammonia *N*-ethylcarbamoylborane **19**,[11] methylamine *N*-ethylcarbamoylborane **20**,[11] dimethylamine-*N*-ethyl carbamoylborane **21**,[11] trimethylamine *N*-ethylcarbamoylborane **22**,[11] trimethylamine *N*-phenylcarbamoylborane **23**,[7] trimethylamine *n*-propyl-carbamoylborane **24**,[7] trimethylamine *N*-butylcarbamoylborane **25**,[7] trimethylamine *N*-octylcarbamoylborane **26**,[7] tetramethylethylenediamine *N*,*N*-bis(*N*-ethylcarbamoylborane) **27**,[6,7] and trimethylamine (*N*-ethylcarbamoyl)methylborane **28**.[7]

Cytotoxic activity

All newly synthesized amine carboxyboranes and their derivatives were tested for cytotoxicity by preparing a 1 mM solution in 0.05% Tween 80–H_2O by homogenization. The drug solution was sterilized using an acrodisc 45 μM. The following cell lines were maintained by literature techniques: murine L_{1210} lymphoid leukemia,[12] P388 lymphocytic leukemia,[12] human $Tmolt_3$ acute lymphoblastic T-cell leukemia,[13] colorectal adenocarcinoma SW480,[14] lung bronchogenic MB-9812,[15] osteosarcoma TE418,[16] KB epidermoid nasopharynx[10,17] HeLa-S^3 suspended cervical carcinoma[18] and glioma EH 118 MG.[19] The protocol used to assess cytotoxicity was that of Geran *et al.*[12] 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. Solid tumor cytotoxicity was determined by the method of Huang *et al.*[20] Ehrlich ascites carcinoma *in vivo* tumor screens were conducted in CF_1 male mice ($\sim$28 g) with test drugs at 8 mg/kg/day ip.[5] 6-Mercaptopurine was used as an internal standard.

Mode of Action Studies for Compounds **4** and **14**

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.

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.*[21] Drugs were present at 1, 2 and 3 times the concentration of their ED_{50} values in the L_{1210} tissue culture cells. The reaction mixtures were inactivated with cold 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.[22] Acid-insoluble precipitates from the RNA and protein experiments were collected on GF/B and Whatman No. 3 filters, respectively and washed with 10% trichloroacetic acid. The filter discs were placed in Scintiverse^R, dried and counted in 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[23] using the incubation medium of Sawada *et al.*[24] with [^{3}H-methyl]-dTTP (82.4 Ci/mmol), dCTP, dGTP and dATP. Incubation was for 60 min at 37°C. The acid-insoluble precipitate was collected on glass fiber discs and counted. m-, r- and t-RNA polymerase enzymes were separated by ammonium fractionation and the mRNA polymerase, tRNA polymerase and rRNA polymerase activities were determined using ^{3}H-UTP (23.2 Ci/mmol).[25] The reaction medium was inactivated with 10% perchloric acid containing 1% NaP-P and the ^{3}H-acid insoluble RNA was collected on nitrocellular filters and counted.[26] Formate incorporation into purines for 40 min at 37°C was determined by the method of Spassova *et al.*[27] with 0.5 μCi ^{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[28] using 30 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*** × 10 min) fraction by the method of Kampf *et al.*[29] with ^{3}H-dUMP (11 Ci/mmol). The nucleotides were absorbed on charcoal, filtered on Whatman No. 1 filters, and a sample of the aqueous filtrate was counted. *N*-ethylmaleimide, a known thiol

alkylating agent, with 0-100 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.[29,30] An aliquot of the 5000*g* supernatant was incubated for 30 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 scrapped at the R_f of the standard, deoxycytidine, and counted. Deoxyribonucleoside triphosphates were extracted with perchloric acid by the method of Bagnara and Finch.[31] 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.[32] The neutralized extract was incubated for 30 min at 37°C with the reaction medium containing calf thymus DNA, *Escherichia coli* DNA polymerase I, and non-limiting amounts of three deoxyribonucleoside triphosphates not being assayed, including 0.04 μCi [^{3}H-methyl]-dTTP (80 Ci/mmol) or [5-^{3}H]-dCTP (15–30 Ci/mmol). The samples were spotted on Whatman No. 3 filters, which were rinsed in 5% trichloroacetic acid–40 mM 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[33] using ^{3}H-thymidine incubated in the medium of Maley and Ochoa.[34] 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.

The effects of amine carboxyboranes **4** and **14** on DNA strand scission were determined by the *in vitro* methods of Suzuki *et al.*,[35] Pera *et al.*[36] and Woynarowski *et al.*[37] L_{1210} lymphoid leukemia cells were incubated with 10 μCi thymidine (methyl-^{3}H, 84.0 Ci/mmol) for 24 h at 37°C. After harvesting the L_{1210} cells (10^7) the cells were centrifuged at 600*g* × 10 min in 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 M KCl and 0.01 M EDTA) followed by 0.2 ml cell preparation. After incubating for 2.5 h at room temperature, the gradient was centrifuged at 12 000 rev/min at 20°C for 60 min (Beckman rotor SW 60). Fractions (0.2 ml) were collected from the top of the gradient, neutralized with 0.2 ml of 0.3 N HCl, and radioactivity was measured.

Thermal calf thymus DNA denaturation studies and DNA viscosity studies were conducted after incubation of **4** or **14** at 100 μM at 37°C for 24 h as outlined previously.[38]

Results

In the *in vivo* Ehrlich ascites carcinoma screen at 8 mg/kg/day ip, the acids in general were more active than the esters and amides (Table 1). For example, **2**, **3** and **5** resulted in 93%, 95% and 96% inhibition of carcinoma growth. Extension of the R group to longer aliphatic groups **6**, **8** and **9** led to a loss in antineoplastic activity. The methyl ester of **2**, compound **12**, retained good activity, i.e. 91%, but the methyl ester of **3**, compound **13**, fell to 71% inhibition. The methyl and ethyl esters of **8**, compounds **16** and **17**, fell in activity from 82% inhibition to 67% and 72% respectively. Placement of the ester group on compound **1**, to afford **11**, resulted in a reduction of inhibition from 77% to 69%.

The ethyl amide group when added to compounds **2** and **3** led to a loss of activity for **20** but was equivalent for compound **21**. Compound **29** resulted in good inhibition of Ehrlich ascites growth of 94% at 8 mg/kg/day.

The murine cytotoxicity assays (Table 2) showed some selectivity of the compounds, i.e. compounds **9**, **14** and **17** all demonstrated ED_{50} values below 2.0 μg/ml in the L_{1210} lymphoid leukemia screens. In the P_{388} lymphocytic leukemia screens in general the compounds demonstrated less activity but compounds **5**, **6**, **11**, **15** and **21** afford ED_{50} values less than 4 μg/ml, the cut-off for activity as designated by NCI.

In the human culture cell lines (Table 2) good activity was observed for **1**, **4**, **9**, **15**, **22**, **23**, **25** and **28** against the growth of $Tmolt_3$ leukemia cells with ED_{50} values <2 μg/ml. Growth of adenocarcinoma of colon was effectively reduced by **1**, **2**, **11**, **12**, **13**, **16**, **21** and **28**. HeLa-S^3 uterine growth was reduced by **5**, **15**, **17**, **19**, **23** and **27** ($ED_{50} \leq 2$ μg/ml). Most compounds except **7** and **10** demonstrated some activity against HeLa-S^3 growth. In the lung bronchogenic screen only compounds **12** and **26** demonstrated ED_{50} values <4 μg/ml. KB nasopharynx carcinoma growth was reduced by the agents **5**, **6**, **12**, **15**, **16**, **19**, **20**, **22**, **25** and **28**. Osteosarcoma bone growth was reduced

Table 1. The *in vivo* antineoplastic activity against Ehrlich ascites carcinoma growth of amine carboxyboranes and related derivatives at 8 mg/kg/day ip

	Compounds	*In vivo* Ehrlich ascites carcinoma growth inhibition (%)
1	NH_3BH_2COOH	77
2	$CH_3NH_2BH_2COOH$	93
3	$(CH_3)_2NBH_2COOH$	95
4	$(CH_3)NBH_2COOH$	82
5	$CH_3CH_2NH_2BH_2COOH$	96
6	$(n\text{-}C_3H_7)_2NHBH_2COOH$	77
7	$[CH_2N(CH_3)_2BH_2COOH]_2$	87
8	$C_{16}H_{33}N(CH_3)_2BH_2COOH$	82
9	$C_{18}H_{37}N(CH_3)_2BH_2COOH$	85
10	$H_2NNH_2BH_2COOH$	77
11	$H_3NBH_2COOCH_3$	69
12	$CH_3NH_2BH_2COOCH_3$	91
13	$(CH_3)_2NHBH_2COOCH_3$	71
14	$(CH_3)_3NBH_2COOCH_3$	91
15	$n\text{-}CH_3CH_2CH_2CH_2NH_2BH_2COOCH_3$	85
16	$C_{16}H_{33}N(CH_3)_2BH_2COOCH_3$	67
17	$C_{16}H_{33}N(CH_3)_2COOC_2H_5$	72
18	$(CH_3)_3NBH_2COOCH_2CH_3$	86
19	$H_3NBH_2C(O)N(H)C_2H_5$	82
20	$CH_3NH_2BH_2C(O)N(H)C_2H_5$	74
21	$(CH_3)_2NHBH_2C(O)N(H)C_2H_5$	91
22	$(CH_3)_3NBH_2C(O)N(H)C_2H_5$	69
23	$(CH_3)_3NBH_2C(O)N(H)C_6H_5$	39
24	$(CH_3)_3NBH_2C(O)N(H)(n\text{-}CH_2CH_2CH_3)$	76
25	$(CH_3)_3NBH_2C(O)N(H)(n\text{-}CH_2CH_2CH_2CH_3)$	32
26	$(CH_3)_3NBH_2C(O)N(H)\text{-}(n\text{-}(CH_2)_7CH_3)$	74
27	$[CH_2N(CH_3)_2BH_2C(O)N(H)C_2H_5]_2$	94
28	$(CH_3)_3NBH(CH_3)C(O)NHCH_2CH_3$	88
	6 Mercaptopurine	99

N (Number of animals/group) = 6.

by **6**, **7**, **8**, **9**, **10**, **12**, **13**, **17**, **19**, **23**, **26** and **27**. Brain glioma growth was effectively reduced by **1**, **3**, **4**, **7**, **8**, **10**, **11**, **12**, **13**, **17**, **18**, **20** and **23**.

Compounds **4** and **14** were selected to examine their effects on L_{1210} cell nucleic acid and protein metabolism at 1, 2 and 3 times their ED_{50} values since these compounds demonstrated moderate and significant inhibition, respectively, of growth in this cell line (Table 3). L_{1210} DNA, RNA and protein synthesis were inhibited by **4** and **14**. DNA synthesis at 3 times the ED_{50} values was reduced by a greater magnitude after 60 min incubation than RNA or protein synthesis which had not even achieved 50% inhibition by the agents at the time.

Examination of the d(NTP)s pool after 60 min incubation at 3 times the ED_{50} value demonstrated marked reductions of dTTP pool levels by both agents, i.e. to 15% of control values. Reductions in dCTPs pool were also significant at 60 min for both drugs. The reduction in deoxyribonucleotide levels would account for the observed reduction of DNA synthesis and cell death after incubation with the agents at 3 times the ED_{50} value.

Examination of enzyme activities after 60 min incubation showed that L_{1210} nuclear DNA polymerase, mRNA polymerase and rRNA polymerase activities were not inhibited by **4** and **14** but rather they were stimulated in activity (Table 4). tRNA polymerase activity was inhibited at 1, 2 and 3 times the ED_{50} values of the agents which would contribute to the observed inhibition of protein synthesis produced by the drugs. Both agents inhibited nucleoside kinase activities in the following order: TMP kinase, thymidine kinase and TDP kinase activities with TDP kinase activity being inhibited greater than 75% by both agents at 2 times their ED_{50} values. Thymidylate synthetase, carbomyl phosphate synthetase and aspartate amido transferase activities were not significantly inhibited by the agents. PRPP amido transferase, IMP

Table 2. The cytotoxicity of amine carboxyboranes against the growth of murine and human tissue culture lines

Compound	Cytotoxicity ED_{50} (μg/ml)								
	Murine tumors		Human tumors						
	L_{1210}	P_{388}	$Tmolt_3$	Colon	HeLa-S^3	Lung	KB	Osteosarcoma	Glioma
1	2.83	5.10	1.78	1.86	2.30	5.46	4.33	4.08	1.46
2	4.01	14.61	4.72	0.835	3.92	4.35	4.32	5.21	6.74
3	2.72	12.86	5.76	2.25	2.67	4.89	4.59	5.27	2.62
4	3.96	10.22	1.79	3.97	2.94	6.85	5.02	7.85	2.33
5	3.87	3.12	4.71	2.19	1.63	7.25	3.67	5.45	6.96
6	2.37	3.80	4.91	3.67	2.25	8.34	2.47	3.03	6.07
7	4.59	16.67	3.56	4.24	4.83	4.89	6.67	3.54	2.87
8	4.80	13.51	3.63	2.25	2.50	7.43	7.89	2.47	2.89
9	1.74	4.49	1.41	6.60	2.25	9.60	4.05	3.12	7.84
10	3.50	5.26	3.09	7.82	8.37	7.07	4.30	2.78	3.10
11	3.56	3.18	2.59	1.83	2.21	5.15	5.34	4.53	0.923
12	3.08	7.94	3.69	1.68	2.02	3.18	3.49	3.71	1.64
13	5.48	10.11	4.70	1.82	2.70	4.60	4.46	2.15	1.56
14	1.02	11.11	2.30	5.87	2.73	4.24	8.45	5.13	5.01
15	3.62	3.45	1.41	5.30	1.78	4.35	3.26	4.62	4.05
16	3.6	12.5	2.22	1.62	2.17	7.46	3.85	6.47	8.69
17	1.1	11.9	2.08	3.26	1.75	7.62	5.07	3.54	1.40
18	3.34	—	3.25	3.27	2.68	6.85	4.74	4.73	2.05
19	2.83	5.10	5.38	7.02	1.50	7.95	2.64	2.65	4.04
20	11.00	11.90	2.95	8.50	2.13	8.16	3.54	6.52	1.94
21	2.00	3.41	3.96	1.00	2.40	6.37	7.54	5.17	7.95
22	2.90	10.16	1.45	2.28	2.73	7.32	2.32	7.84	7.55
23	3.31	4.02	0.739	4.50	1.38	5.25	4.21	3.30	2.96
24	3.96	4.67	2.35	2.12	2.13	7.32	4.15	4.67	4.57
25	5.51	6.75	1.91	3.38	2.22	4.69	3.80	5.69	3.62
26	4.43	NA	2.75	4.77	2.25	3.08	4.05	2.29	3.72
27	5.89	5.35	2.02	2.05	2.07	5.71	7.02	3.30	5.00
28	7.15	6.30	1.82	0.954	2.07	—	3.80	—	5.35
Standards									
5FU	1.41	3.72	2.14	3.09	2.47	5.69	1.25	—	1.28
araC	2.76	4.23	2.67	3.42	2.13	4.60	2.84	—	1.88
Hydroxyurea	2.67	—	3.18	4.74	1.96	7.37	5.29	7.59	2.27
VP-16	0.64	1.16	—	—	—	—	—	—	—

dehydrogenase and dihydrofolate reductase activities were inhibited in a concentration-dependent manner by at least 50% at 3 times the ED_{50} values after 60 min. Ribonucleotide reductase activity was inhibited only moderately by around 40% at 3 times the ED_{50} values.

Kinetic studies at 3 times the ED_{50} values of the two agents showed that TDP kinase activity was inhibited 40–50% by 15 min (Table 5). IMP dehydrogenase and PRPP amido transferase enzyme activities were not inhibited by 50% until 45 min of incubation and dihydrofolate reductase and thymidine kinase required 60 min incubation to achieve at least 50% inhibition by the agents. The inhibition of DNA synthesis lagged behind the inhibition of some enzyme activities and probably reflects the net inhibition of a number of these enzymes' activities by the agents.

Incubation of compound **4** or **14** at 3 times the ED_{50} values with calf thymus DNA demonstrated no indications of UV absorption hyperchromic shifts. Thermal denaturation studies after 24 h incubation demonstrated a change in T_m from 63°C (control) to 71°C with compound **14** and 72°C with compound **4**. DNA viscosity studies after 24 h incubation showed a control of 3 min 12 s whereas drug **14** showed 3 min 9.4 s and drug **4** showed 3 min 7.3 s. When **14** and **4** were incubated for 24 h with L_{1210} cells, DNA strand scission studies showed a shift of the DNA in the gradient indicative of smaller DNA fragments induced by the drugs (Figure 1). DNA fragmentation induced

Table 3. The effects of compounds **4** and **14** on DNA, RNA and protein synthesis of L_{1210} lymphoid leukemia cells after 60 min incubations

		Percent of control		
		DNA synthesis	RNA synthesis	Protein synthesis
Compound **4**	Control	100 ± 5[a]	100 ± 7[b]	100 ± 6[c]
	1 × ED_{50} value	60 ± 6[d]	99 ± 8	64 ± 5
	2 × ED_{50} value	53 ± 4[d]	75 ± 7[d]	56 ± 5[d]
	3 × ED_{50} value	35 ± 3[d]	56 ± 5[d]	54 ± 5[d]
Compound **14**	Control	100 ± 5[a]	100 ± 7[b]	100 ± 6[c]
	1 × ED_{50} value	75 ± 6[d]	91 ± 6	75 ± 6[d]
	2 × ED_{50} value	68 ± 5[d]	81 ± 6	56 ± 5[d]
	3 × ED_{50} value	29 ± 4[d]	71 ± 5[d]	55 ± 5[d]

$N = 6$.
[a] 118 342 dpm/10^6 cells.
[b] 2512 dpm/10^6 cells.
[c] 4203 dpm/10^6 cells.
[d] $p \leq 0.001$.

Table 4. The effects of compounds **4** and **14** on the *in vitro* enzyme activities involving DNA synthesis in L_{1210} lymphoid leukemia cells after 60 min incubation

Enzyme assay	Control	Per cent of control					
		Compound **4**			Compound **14**		
		1 ×	2 × ED_{50} values	3 ×	1 ×	2 × ED_{50} values	3 ×
DNA polymerase nuclear	100 ± 6[a]	147 ± 8[s]	193 ± 9[s]	212 ± 8[s]	141 ± 5	172 ± 6	211 ± 10[s]
mRNA polymerase	100 ± 7[b]	186 ± 7[s]	380 ± 11[s]	248 ± 10[s]	321 ± 12	405 ± 11[s]	691 ± 9[s]
rRNA polymerase	100 ± 7[c]	179 ± 8[s]	184 ± 7[s]	186 ± 8[s]	170 ± 9[s]	203 ± 8[s]	238 ± 8[s]
tRNA polymerase	100 ± 6[d]	63 ± 5[s]	34 ± 4[s]	22 ± 3[s]	54 ± 5[s]	28 ± 4[s]	19 ± 3[s]
Thymidine kinase	100 ± 7[e]	71 ± 6[s]	49 ± 6[s]	48 ± 5[s]	82 ± 6[s]	52 ± 4[s]	47 ± 5[s]
TMP kinase	100 ± 5[f]	91 ± 6	81 ± 5[s]	78 ± 6[s]	188 ± 7	85 ± 5	79 ± 5[s]
TDP kinase	100 ± 6[g]	52 ± 5[s]	24 ± 4[s]	17 ± 3[s]	49 ± 6[s]	24 ± 5[s]	22 ± 3[s]
Thymidylate synthetase	100 ± 8[h]	92 ± 7	111 ± 9	110 ± 8	98 ± 7	113 ± 9	114 ± 10
Carbomyl phosphate synthetase	100 ± 6[i]	107 ± 7	110 ± 6	114 ± 7	121 ± 7	122	129 ± 6
Aspartate amido transferase	100 ± 6[j]	94 ± 5	92 ± 5	89 ± 6	101 ± 7	96 ± 5	89 ± 6
PRPP amido transferase	100 ± 5[k]	75 ± 5[s]	31 ± 4[s]	27 ± 4[s]	74 ± 5[s]	27 ± 4[s]	12 ± 3[s]
IMP dehydrogenase	100 ± 7[l]	125 ± 7	68 ± 6[s]	48 ± 5[s]	110 ± 7	73 ± 6[s]	49 ± 5[s]
Dihydrofolate reductase	100 ± 6[m]	92 ± 7	77 ± 6[s]	19 ± 3[s]	102 ± 6	74 ± 5[s]	17 ± 3[s]
Ribonucleotide reductase	100 ± 7[n]	97 ± 6	84 ± 7	60 ± 7	82 ± 7	75 ± 6	66 ± 6
d(NTP)s pool levels							
dATP	100 ± 6[o]	—	—	94 ± 5	—	—	71 ± 6[s]
dGTP	100 ± 5[p]	—	—	93 ± 6	—	—	30 ± 5[s]
dCTP	100 ± 5[q]	—	—	71 ± 5[s]	—	—	60 ± 4[s]
dTTP	100 ± 6[r]	—	—	15 ± 3[s]	—	—	15 ± 3[s]

$N = 6$.
[a] 9628 dpm (^{3}H-TTP) incorporated into DNA/h/mg nuclear protein.
[b] 2018 dpm (^{3}H-TTP) incorporated into mRNA/h/g protein.
[c] 1998 dpm (^{3}H-TTP) incorporated into rRNA/h/g protein.
[d] 12855 dpm (^{3}H-TTP) incorporated into tRNA/h/g protein.
[e] 0.256 ΔOD units/h/mg of protein.
[f] 0.260 ΔOD units/h/mg of protein.
[g] 0.085 ΔOD units/h/mg of protein.
[h] 15 309 dpm ^3H_2O formed/h/mg of protein.
[i] 0.134 mol citrulline formed/h/mg of protein.
[j] 0.695 mol *N*-carbamyl aspartate formed/h/mg of protein.
[k] 0.476 ΔOD units/h/mg of protein.
[l] 76 058 dmp ^{3}H-XMP formed/h/mg of protein.
[m] 0.085 ΔOD units/h/mg of protein.
[n] 532 dpm ^{3}H-dCDP formed/h/mg of protein.
[o] 32.39 pmol/10^6 cells.
[p] 23.88 pmol/10^6 cells.
[q] 86.29 pmol/10^6 cells.
[r] 22.04 pmol/10^6 cells.
[s] $p \leq 0.001$.

Table 5. The effects of compounds **4** and **14** on L_{1210} lymphoid leukemia cell enyme activities at three times their ED_{50} values over time

Enzyme assay[a]	Control	Per cent of control							
		Compound **4**				Compound **14**			
		15 min	30 min	45 min	60 min	15 min	30 min	45 min	60 min
Dihydrofolate reductase	100 ± 6	83 ± 5	82 ± 6	77 ± 5[b]	19 ± 4[b]	80 ± 6	77 ± 5[b]	61 ± 4	19 ± 3[b]
Thymidine kinase	100 ± 7	68 ± 6	67 ± 6	63 ± 5	48 ± 4[b]	54 ± 5[b]	53 ± 5[b]	53 ± 4[b]	47 ± 4[b]
TDP kinase	100 ± 6	54 ± 5[b]	49 ± 4[b]	24 ± 3[b]	17 ± 4[b]	50 ± 4[b]	48 ± 5[b]	45 ± 4[b]	22 ± 3[b]
PRPP amido transferase	100 ± 5	73 ± 6[b]	70 ± 5[b]	54 ± 5[b]	27 ± 4[b]	73 ± 6[b]	69 ± 5[b]	53 ± 5[b]	12 ± 2[b]
IMP dehydrogenase	100 ± 7	82 ± 6	56 ± 6[b]	50 ± 5[b]	48 ± 5[b]	77 ± 5	62 ± 5[b]	54 ± 5[b]	49 ± 5[b]
DNA synthesis	100 ± 5	73 ± 5[b]	62 ± 6[b]	43 ± 4[b]	35 ± 3[b]	73 ± 6[b]	59 ± 5[b]	45 ± 5	29 ± 3[b]

$N = 6$.
[a] See Table 4 for control values.
[b] $p \leq 0.001$ Student's *t*-test.

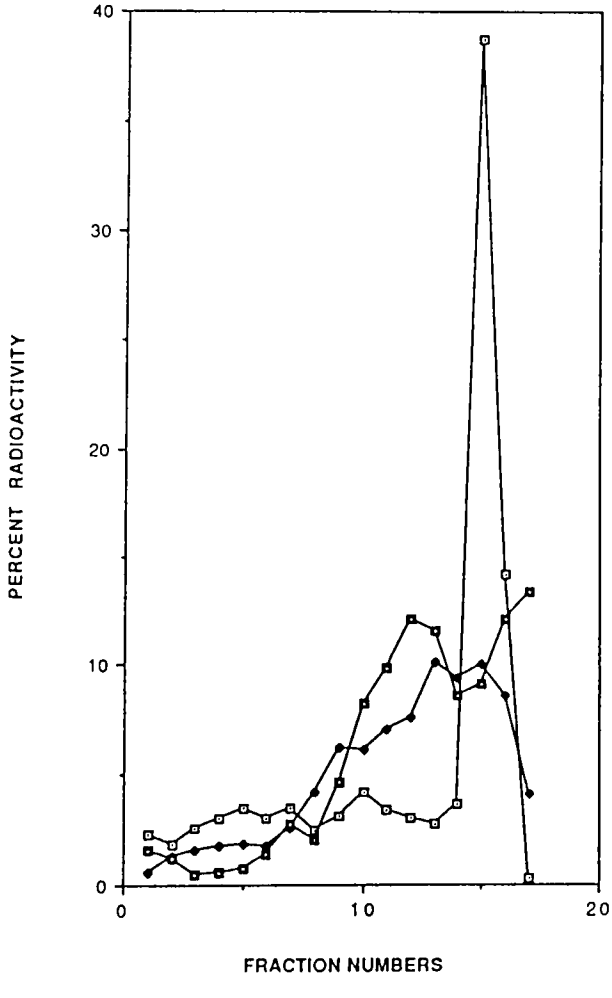

Figure 1. Results of DNA strand scission experiment: (⊡) control; (◆) compound **4**; (□) compound **14**.

by the drugs would be consistent with the observed decrease in DNA viscosity observed after 24 h incubation with the drug.

Discussion

A number of the amine carboxyborane derivatives demonstrated antineoplastic or cytotoxic activity. A certain degree of selectivity was observed for a variety of chemical agents so that all the compounds were not active in all the cytotoxic screens. The agents in general demonstrated the best inhibitory activity against the growth of L_{1210} lymphoid leukemia, HeLa-S[3] uterine carcinoma and $Tmolt_3$ leukemia. Less activity in general was demonstrated against the solid tumors, but it should be noted that select compounds demonstrated marked activity in the colon adenosarcoma, glioma, and osteosarcoma screens. Less activity was observed for the agents against the growth of KB nasopharynx and lung bronchogenic tumors.

The mode of action of compounds **4** and **14** in the L_{1210} lymphoid leukemia cells showed that DNA, RNA and protein synthesis were inhibited after 60 min at 1, 2 and 3 times their corresponding ED_{50} values. DNA synthesis was inhibited preferentially by the agents as a result of the inhibition at the following sites: thymidine kinase, TDP kinase, PRPP amido transferase, IMP dehydrogenase and dihydrofolate reductase. Moderate inhibition of TMP kinase and ribonucleoside reductase activities by the agents was noted by 60 min at 3 times the ED_{50} values. The inhibition of the activities of these enzymes was generally concentration dependent with 3 times the ED_{50} values affording the maximum inhibition. TDP

kinase activity was significantly inhibited at 1 times the ED_{50} value. Obviously suppression of this enzyme would lead to the low dTTP pools observed after 60 min incubation of **4** and **14** at 3 times the ED_{50} values. This action of the drugs alone would account for the observed DNA synthesis inhibition and cell death.

In the kinetic studies at 3 times the ED_{50} values of the agents the activity of the enzyme TDP kinase was inhibited around 50% by 15 min incubation. This was followed closely by the inhibition of thymidine kinase activity by the agent. The inhibition of dihydrofolate reductase, PRPP amido transferase and IMP dehydrogenase required at least 45 min incubation to observe 50% inhibition. Certainly the drug's effect on blocking regulatory enzymes of the purine pathway, e.g. IMP dehydrogenase, PRPP amido transferase and dihydrofolate reductase, will contribute to the overall inhibition of DNA and RNA synthesis which was observed after incubation of the agents in L_{1210} cells. It should be noted that, when calf thymus DNA template, d(NTP)s or r(NTP)s were added in sufficient quantity for the DNA and RNA polymerase assays, the drugs did not affect the polymerase activities, indicating that the blocks were not at this level. Instead the polymerase activities were actually stimulated after incubation with drugs which probably involved some type of feedback regulation owing to a block by the drugs in the purine pathway. The DNA strand scission with the evidence of lower molecular weight DNA in the gradient would also interfere with DNA synthesis. There was some good evidence that DNA intercalation and security binding of the agents occurred in that DNA viscosity was reduced after incubation with **4** and **14** indicating again the presence of small molecular weight DNA; DNA thermal denaturation studies showed that T_m values were elevated from control 63°C to 71–72°C indicative of DNA binding but not cross-linking of the strands by the agents. Thus, these agents appear to have multiple modes of action to cause inhibition of tumor growth, yet previous acute toxicity studies have demonstrated that compound **14** was safe to use therapeutically in rodents at 100 mg/kg/day and less.

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(Received 11 November 1990; accepted 18 November 1990)