Boron Betaine Analogs: Antitumor Activity and Effects on Ehrlich Ascites Tumor Cell Metabolism

I. H. HALL *x, C. O. STARNES *, B. F. SPIELVOGEL ‡x, P. WISIAN-NEILSON ‡, M. K. DAS ‡, and L. WOJNOWICH ‡

Received March 6, 1978, from the *Division of Medicinal Chemistry, School of Pharmacy, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514, and the ‡Paul M. Gross Chemical Laboratory, Duke University, Durham, NC 27706. Accepted for publication November 15, 1978.

Abstract □ Several newly synthesized boron betaine analogs had antitumor activity in Ehrlich ascites, Walker 256 ascites carcinosarcoma, and Lewis lung screens and marginal activity in the B-16 melanotic melanoma screen. *In vivo* testing demonstrated that trimethylamine-cyanoborane inhibited Ehrlich ascites cell DNA and protein syntheses as well as gene modulation by chromatin protein phosphorylation and methylation. Trimethylamine-cyanoborane increased cyclic-AMP levels. *In vitro* testing showed that nuclear DNA polymerase, thymidylate synthetase, *S*-adenosylmethyltransferase, nonhistone chromatin methylation, deoxyribonuclease, ribonuclease, and cathepsin were inhibited by the boron analogs. These compounds did not demonstrate high antitumor activity at the doses employed, but blockage of methyl transfer from *S*-adenosylmethionine was established as a feasible method for controlling cell proliferation.

Keyphrases □ Antineoplastic agents—betaine, boron analogs, metabolic effects, protein synthesis, nucleic acid synthesis, methyl transfer □ Betaine analogs—boron, antineoplastic activity, metabolic effects, protein synthesis, nucleic acid synthesis, methyl transfer □ Protein synthesis—effect of boron betaine analogs □ Methylation—effect of boron betaine analogs □ Nucleic acid synthesis—effect of boron betaine analogs

Blockage of methyl group transfer from choline *via* betaine to L-homocysteine would ultimately inhibit L-methionine and protein syntheses. Since *S*-adenosyl-L-methionine requires L-methionine as a precursor, its synthesis would be reduced, as would be the availability of the methyl transfer from *S*-adenosyl-L-methionine to transfer or ribosomal RNAs and to ribosomal, regulating enzyme, and chromatin protein amino acid residues (1–4). *S*-Adenosyl-L-methionine may play a minor role as the methyl donor cofactor to the enzyme thymidylate synthetase (1, 2). Cancer cells have more methylated chromatin protein and nucleotide bases, particularly transfer RNA, and higher methyl transferase activity than normal tissue.

Numerous reports cite the utilization of folate cofactor antimetabolites to block the methyl transfer to uracil for thymine synthesis. Few studies have investigated the blockage of methyl transfer from *S*-adenosyl-L-methionine since an initial attempt produced ethionine, the ethyl analog of L-methionine, which is a liver carcinogen. A boron analog series of possible betaine or choline antimetabolites was selected to test this premise.

A previous note reported the synthesis of isoelectronic and isostructural boron α-amino acid analogs (5). Trimethylamine-carboxyborane, the protonated boron analog of betaine $[(CH_3)_3N^+CH_2CO_2^-]$, its *N*-ethylamide, and a series of related compounds were prepared from trimethylamine-cyanoborane. Because of their structural similarity to betaine, these compounds will be referred to as boron betaine analogs. These boron analogs were evaluated for their antineoplastic activity in a number of tumor models.

EXPERIMENTAL[1]

Chemistry—*Reagents*—The following compounds were purchased: *tert*-butylamine borane[2] $[(CH_3)_3CNH_2{:}BH_3]$ (I), morpholine borane[2] $[O(CH_2CH_2)_2NHBH_3]$ (II), trimethylamine borane[3] $[(CH_3)_3N{:}BH_3]$ (III), and dimethylamine borane[3] $[(CH_3)_2NH{:}BH_3]$ (IV).

Trimethylamine-cyanoborane $[(CH_3)_3NBH_2CN]$ (*V*)—Compound V was synthesized in high yields (83%) by reacting sodium cyanoborohydride with trimethylamine hydrochloride in dry tetrahydrofuran (7). The product was purified by sublimation, mp 62° [lit. (8) mp 63°]; ^{1}H-NMR (C_6H_6): δ 2.25 (s, CH_3) ppm; BH_2 not observed.

Dimethylamine-cyanoborane $[(CH_3)_2NHBH_2CN]$ (*VI*)—Compound VI was synthesized similarly, except that dimethylamine hydrochloride was used and produced a 53% yield, mp 55–57° [lit. (9) mp 56–57°]; ^{1}H-NMR (CD_3CN): δ 2.0 (q, J_{BH} = 100 Hz, BH_2), 2.35 (t, J_{HNCH} = 6 Hz, CH_3), and 4.48 (s, NH_2) ppm.

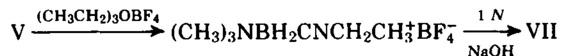
Anal.—Calc. for $C_3H_9BN_2$: C, 42.93; H, 10.81; B, 12.88; N, 33.38. Found: C, 43.11; H, 10.57; B, 12.66; N, 33.17.

*Trimethylamine-*N*-ethylamidoborane* $[(CH_3)_3NBH_2C(O)N(CH_2CH_3)H]$ (*VII*)—A solution of V (11.9 g, 0.12 mole) and $(CH_3CH_2)_3OBF_4$ (250 ml, 0.1 *N* in methylene chloride) was refluxed under nitrogen for 24 hr (Scheme I). The reaction mixture was cooled to 0°, and 1.0 *N* NaOH was added slowly with vigorous stirring until the solution was basic. After stirring for 1 hr at room temperature, the organic layer was separated and the aqueous layer was extracted three times with methylene chloride. The organic portions were combined and dried over magnesium sulfate, and the solvent was removed *in vacuo*.

The remaining viscous liquid was distilled *in vacuo* with minimum heating to give VII (13.1 g, 75% yield, bp 80°/0.15 torr); IR[4]: 3430 (m), 3350 (vs), 3010 (m), 2990 (s), 2950 (s), 2890 (m), 2420 (sh), 2370 (s, br), 2320 (m), 2290 (m), 2240 (w), 2200 (w), 1600 (s), 1480 (s), 1460 (s), 1435 (s), 1400 (m), 1375 (w), 1345 (w), 1295 (w), 1255 (s), 1140 (s), 1100 (s), 1050 (s), 1010 (w), 990 (s), 970 (m), 895 (w), 860 (s), 720 (w), and 670 (w) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 1.07 (t, *J* = 8 Hz, CH_2CH_3), 2.75 (s, CH_3N), 3.57 (m, CH_2CH_3), and 5.43 (broad s, NH) ppm; ^{13}C-NMR ($CDCl_3$): δ 15.3 (CH_3CH_2), 31.5 (CH_2CH_3), 51.8 (CH_3N), and 182.8 (CO) ppm; ^{11}B ($CDCl_3$, 50°): δ 7.4 (1:2:1 t, *J* = 90 Hz) ppm.

Trimethylamine-carboxyborane $[(CH_3)_3NBH_2COOH]$ (*VIII*)—Compound V (20.8 g, 0.214 mole) and triethyloxonium tetrafluoroborate (450 ml, 0.1 *N* in methylene chloride) were refluxed for 24 hr. The solvent and the reaction by-product, boron trifluoride etherate, were removed *in vacuo*, and water was added to the solid residue. After stirring at room temperature for 2–3 days, the solution was extracted with five 75-ml portions of methylene chloride. The organic extractions were combined and dried over magnesium sulfate, and the solvent was removed *in vacuo*.

The solid residue was recrystallized from hot water and dried under vacuum to give VIII (13.8 g, 55.5% yield, mp 131° dec.); IR[3]: 3050 (vs, br), 2950 (s), 2860 (s, sh), 2720 (m), 2620 (m), 2580 (m), 2380 (s), 2290 (w), 2210



$$V \xrightarrow{(CH_3CH_2)_3OBF_4} (CH_3)_3NBH_2CNCH_2CH_3^+BF_4^- \xrightarrow[NaOH]{1\ N} VII$$

Scheme I

[1] IR spectra were measured on a Perkin-Elmer model 297 spectrometer. Liquid samples were run neat between sodium chloride disks, and solid samples were run as potassium bromide pellets. Elemental analysis was performed by Galbraith Laboratories, Knoxville, Tenn. Triethyloxonium tetrafluoroborate was obtained commercially as 0.1 *N* solutions in methylene chloride or was prepared as described in the literature (6).
[2] Alfa Products.
[3] Aldrich Chemical.
[4] Legend: s = strong, m = medium, w = weak, sh = shoulder, br = broad, and v = very.

0022-3549/79/0600-0685$01.00/0
© 1979, American Pharmaceutical Association

Table I—Boron Analog Toxicity and Antitumor Activity in Rodents

Compound	Ehrlich Ascites, 20 mg/kg/day, CF_1 Male Mice, % inhibition	Walker 256, 2.5 mg/kg/day, Sprague–Dawley Rats, T/C	P-388 Leukemia, 20 mg/kg/day, DBA/2 Mice, T/C	B-16 Melanoma, 20 mg/kg/day, C_{57} BL/6 Mice, T/C	Lewis Lung, 20 mg/kg/day, C_{57} BL/6 Mice, T/C	LD_{50}, CF_1 Male Mice, mg/kg
I	Toxic	137	Toxic	Toxic	Toxic	16
II	37	162	117	108	139	475
III	83	145	113	141	87	740
IV	96	156	111	90	178	200
V	98	202	109	143	144	70
VI	81	103	86	113	91	39
VII	69	178	132	145	174	320
VIII	82	174	98	134	144	1800
IX	53	125	116	117	154	250
X	32	105	113	92	Toxic	100
Phenylalanine mustard	99	317	—	—	—	—
Fluorouracil	—	—	186	164	—	—
Cyclophosphamide	—	—	—	—	140	—
Control[a]	100	100	100	100	100	—
Activity required for significant activity	80	125	125	140	140	—

[a] 0.05% Tween 80–water.

(w), 2165 (w), 2100 (w), 1650 (s, br), 1480 (sh), 1465 (s), 1405 (w), 1360 (w), 1245 (s), 1125 (s), 1065 (s), 1020 (m), 980 (m), 975 (m), 915 (m), 865 (s), 810 (m), 725 (w), and 665 (m) cm^{-1}; ^1H-NMR (D_2O): δ 2.72 (s, CH_3N) and 4.65 (s, HDO) ppm.

Anal.—Calc. for $C_4H_{12}BNO_2$: C, 41.08; H, 10.34; B, 9.24; N, 11.98. Found: C, 41.14; H, 10.45; B, 9.23; N, 11.85.

Trimethylamine-iodoborane [$(CH_3)_3NBH_2I$] (*IX*)—This compound was prepared using the literature method (10) and was sublimed before use, mp 73° [lit. (11, 12) mp 73°]; ^1H-NMR (CD_2Cl_2): δ 2.82 (s, CH_3) ppm.

Hexakis(ammonia-cyanoborane)sodium Iodide {$[Na(NH_3BH_2CN)_6]I$} (*X*)—Compound X was synthesized from ammonia, sodium cyanide, and IX and was recrystallized from ether (13).

Anal.—Calc. for $C_6H_{30}B_6IN_{12}Na$: C, 14.86; H, 6.24; B, 13.38; N, 34.66. Found: C, 15.05; H, 6.37; B, 12.71; N, 34.91.

Pharmacological Screens—Toxicity studies were carried out in CF_1 male mice (~30 g) using a literature method (14).

Antitumor screening was carried out at doses that preliminary screening for V demonstrated to be nontoxic. In the Ehrlich ascites screen, 10^6 tumor cells were implanted intraperitoneally into CF_1 male mice (~30 g). Test compounds were suspended in 0.05% polysorbate 80–water and homogenized. Each compound was injected at 20 mg/kg/day ip. On the 8th day, the mice were sacrificed. The total ascites tumor volume and packed tumor cells (ascrit) volume were determined to calculate the percent inhibition (15). Mercaptopurine and melphalan were used as positive controls for the screen.

In the Walker 256 carcinosarcoma screen, 10^6 tumor cells were implanted intraperitoneally into Sprague–Dawley male rats (~80 g). Test compounds were administered at 2.5 mg/kg/day ip, and the day of death was recorded. The T/C values were calculated according to the NIH protocol (16). Melphalan was used as a positive standard.

Screening was carried out in DBA/2 male mice (~25 g) for the P-388 lymphocytic leukemia model. On Day 0, 10^6 cells were implanted intraperitoneally and test compounds were administered at 20 mg/kg/day. The T/C values were calculated from the group survival times (16). Fluorouracil was used as a positive standard.

In the melanotic melanoma B-16 screens, 1 g of tumor was homogenized in 10 ml of Hanke's balanced salt medium, and 0.5 ml of homogenate was implanted subcutaneously in the inguinal region of C_{57}BL/6 male mice. Test compounds were administered at 20 mg/kg/day ip. The T/C values were calculated (16). Cyclophosphamide was used as a positive control.

In the Lewis lung screen, 2×10^6 tumor cells were implanted intramuscularly in the hindlegs of C_{57}BL/6 male mice. Test compounds were administered at 20 mg/kg/day ip, and T/C values were calculated. Cyclophosphamide was used as an internal standard.

The glioma 26 and 261 and ependymoblastoma brain tumor screens were carried out in a manner analogous to the B-16 screen. Cyclophosphamide and melphalan were the positive controls.

Biochemical Assays—Male CF_1 mice (~30 g) were implanted intraperitoneally on Day 0 with 10^6 Ehrlich ascites tumor cells. For the *in vivo* studies, animals were treated intraperitoneally with 0.25 mg of test compounds on Days 7, 8, and 9. Animals were sacrificed on Day 10, and the ascites fluid was collected from the peritoneal cavity. The number of tumor cells per milliliter and the 0.4% trypan blue uptake were determined with a hemocytometer (17).

In vitro enzymatic and oxidative phosphorylation studies were performed on untreated 10-day Ehrlich ascites cells. Thymidine incorporation into DNA was determined (18). One hour prior to sacrifice, 10 μCi of [methyl-^{14}C]-thymidine (54 mCi/mmole) was injected intraperitoneally. The DNA was isolated, and the ^{14}C content was determined in 0.4% 2,5-diphenyloxazole and 0.01% 1,4-bis[2-(5-phenyloxazolyl)]benzene in a solvent of two parts of toluene and one part of octoxynol, corrected for quenching. DNA concentration was determined by UV spectrophotometry at 260 nm using a calf thymus DNA standard.

Uridine incorporation into RNA was determined in an analogous manner utilizing 10 μCi of 5-^{3}H-uridine (24.2 Ci/mmole). RNA was extracted (19). Leucine incorporation into protein was determined (20) using 1 μCi of 1-^{14}C-leucine (56.9 mCi/mmole). The boron analog effect on 1-^{14}C-acetic acid (57.8 mCi/mmole) incorporation into Ehrlich ascites cell cholesterol was measured also (21, 22). Histone phosphorylation and methylation were determined by injecting intraperitoneally 10 μCi of (γ-^{32}P)-ATP (7.97 Ci/mmole) or [methyl-^{14}C]-choline chloride (49 mCi/mmole) 1 hr prior to sacrifice. The nuclei were isolated (23), and the histone chromatin protein was extracted (24).

Nonhistone chromatin phosphorylation and methylation were determined on isolated nuclei (25) utilizing 2 mmoles of γ-^{32}P-ATP or [methyl-^{14}C]-choline chloride. Chromatin protein was collected on nitrocellulose membrane filters (25). Cyclic-AMP levels were determined by a radioimmunoassay (26) using ^{3}H(G)-cyclic-AMP (39.8 Ci/mmole).

Table II—Trimethylamine-cyanoborane (V) Effects on *In Vivo* Ehrlich Ascites Tumor Metabolism

In Vivo (n = 6)	Percent Control: Control[a]	Percent Control: Treated, 0.25 mg on Days 7, 8, and 9
^{14}C-Thymidine incorporation into DNA	100 ± 10	48 ± 4[b]
^{3}H-Uridine incorporation into RNA	100 ± 24	100 ± 29
^{14}C-Leucine incorporation into protein	100 ± 27	10 ± 2[b]
^{14}C-Acetic acid incorporation into cholesterol	100 ± 4	75 ± 6[b]
[methyl-^{14}C]-Choline incorporation into RNA	100 ± 11	51 ± 13[b]
[methyl-^{14}C]-Choline incorporation into protein	100 ± 15	42 ± 10[b]
[methyl-^{14}C]-Choline incorporation into nonhistones	100 ± 2	44 ± 5[b]
[methyl-^{14}C]-Choline incorporation into histones	100 ± 16	132 ± 9[c]
^{32}P-γ-ATP incorporation into nonhistones	100 ± 3	41 ± 7[b]
^{32}P-γ-ATP incorporation into histones	100 ± 8	53 ± 4[b]
Cyclic-AMP level	100 ± 5	269 ± 8[b]
Number of tumor cells per milliliter	100 ± 19	33 ± 14[b]

[a] 0.05% Tween 80. [b] p = 0.001. [c] p = 0.005.

Table III—*In Vitro* Boron Analog Effects on 10-Day Ehrlich Ascites Tumor Enzymatic Activities

	Percent Control ($n = 6$)						
Compound (6 μg)	DNA Polymerase Activity[a]	Thymidylate Synthetase Activity[b]	S-Adenosyl-L-methionine Methyltransferase Activity[c]	Methylation of Nonhistone Protein[d]	DNase Activity[e]	RNase Activity[f]	Cathepsin Activity[g]
I	75 ± 6[h]	44 ± 7[h]	81 ± 4[i]	76 ± 4[h]	7 ± 3[h]	72 ± 14[i]	80 ± 12
II	65 ± 9[h]	85 ± 2[h]	75 ± 12[i]	53 ± 6[h]	28 ± 8[h]	51 ± 16[h]	53 ± 18[j]
III	51 ± 8[h]	62 ± 2[h]	68 ± 10[h]	77 ± 8[h]	10 ± 4[h]	68 ± 12[i]	44 ± 19[h]
IV	71 ± 6[h]	68 ± 5[h]	66 ± 7[h]	78 ± 10[j]	19 ± 6[h]	70 ± 17[i]	33 ± 15[h]
V	46 ± 3[h]	48 ± 15[h]	49 ± 5[h]	44 ± 5[h]	14 ± 5[h]	47 ± 12[h]	68 ± 13[j]
VII	40 ± 8[h]	66 ± 2[h]	75 ± 9[i]	95 ± 10	47 ± 11[h]	98 ± 17	19 ± 8[h]
VIII	37 ± 6[h]	79 ± 2[h]	39 ± 19[h]	47 ± 5[h]	25 ± 9[h]	60 ± 9[h]	64 ± 20[i]
IX	53 ± 7[h]	—	—	—	—	—	—
X	54 ± 4[h]	—	—	—	—	—	—
Iodoacetate	41 ± 4	—	—	—	—	—	—
Fluorouracil	—	39 ± 7[h]	99 ± 13	—	—	—	—
Control[k]	100 ± 15	100 ± 3	100 ± 15	100 ± 5	100 ± 8	100 ± 19	100 ± 17

Control values for 10-day Ehrlich ascites tumor cells were as follows: [a] 7561 dpm/mg of protein. [b] 103,328 dpm/mg of protein. [c] 3268 dpm/mg of protein. [d] 8000 dpm/mg of chromatin protein. [e] 35 μg of DNA hydrolyzed/mg of protein/30 min. [f] 24 μg of RNA hydrolyzed/mg of protein/30 min. [g] 0.90 mg of protein hydrolyzed/mg of protein/30 min. [h] $p = 0.001$. [i] $p = 0.025$. [j] $p = 0.005$. [k] 0.05% Tween 80–water.

In vitro DNA polymerase activity was determined on washed (three times) isolated nuclei (23). The incubation system was that of Sawada *et al.* (27), except that 2-^{14}C-deoxythymidylate triphosphate (45 mCi/mmole) was used. The insoluble nucleic acids were collected on glass fiber GF/F by vacuum suction. Deoxythymidylate kinase and deoxythymidylate diphosphate kinase activities were determined by a method (28) based on the disappearance of reduced nicotinamide adenosine dinucleotide at 340 nm for 20 min. *In vitro* thymidylate synthetase activity was assayed by a method (29) utilizing a postmitochondrial supernate (9000×g for 10 min) and 5 μCi of 5′-^{3}H-deoxyuridine monophosphate (11 Ci/mmole). *S*-Adenosyl-L-methionine methyltransferase activity was determined (30) using *S*-[methyl-^{14}C]-adenosyl-L-methionine (53 mCi/mmole).

The effects of the boron analogs on the tumor cell hydrolytic enzymes were also determined. Deoxyribonuclease activity, at pH 5.0, was measured by a modification of the deDuve method (31). Ribonuclease and cathepsin activities, at pH 5.0, were determined by the method of Cho-Chung and Gullino (32). *In vitro* effects of boron analogs on Ehrlich ascites tumor cell oxidative phosphorylation processes were measured (33) in the presence and absence of adenosine diphosphate, utilizing succinate or α-ketoglutarate as the substrate. Protein was determined by the Lowry method (34).

All *in vitro* tests were carried out at 6 μmoles of test compounds/ml of assay mixture. *In vitro* UV binding studies were conducted with test compounds (0.2 μg/ml) and DNA (38 μg/ml) or deoxyguanosine monophosphate (38 μg/ml) in 0.1 *M* phosphate buffer, pH 7.2, over a range of 200–340 nm for 24 hr (22). Data in Tables II and III are the means of control and standard deviation percentages ($\bar{x} \pm SD$). The probable significance level (p) was determined by the Student t test (35).

RESULTS

Compounds III–V and VIII significantly inhibited Ehrlich ascites tumor growth at 1 mg/kg/day. Compounds I–V, VII, and VIII were significantly active (T/C ≧ 125) in the Walker 256 carcinosarcoma survival screen at a relatively low dose, 2.5 mg/kg/day. In the P-388 lymphocytic leukemia screen at 20 mg/kg/day, only VII had significant activity. In the B-16 melanoma screen, III, V, and VII demonstrated significant activity (T/C ≧ 140). In the Lewis lung survival system, IV, V, VII, IX, and X demonstrated activity at 20 mg/kg/day. Compound V was inactive in the brain tumor screens, glioma 26 and 261 and ependymoblastoma (Table I).

Boron analog effects on several biochemical events necessary for rapid cell proliferation were examined. ^{14}C-Thymidine incorporation into DNA for 10-day Ehrlich ascites cells was 280,629 dpm/mg of DNA. DNA synthesis was inhibited 52% by V, 45% by VII, and 60% by VIII (Table II). ^{3}H-Uridine incorporation into Ehrlich ascites cell RNA was unaffected by boron analogs. The UV spectral studies with V, VII, and VIII and DNA or deoxyguanosine monophosphate demonstrated no binding between drugs and nucleotides, indicating that the DNA template was intact for transcription.

^{14}C-Leucine incorporation into protein for the 10-day control Ehrlich ascites cells was 6238 dpm/mg of protein and was inhibited 90% after V administration. Cholesterol synthesis for newly synthesized cell membranes for the control 10-day tumor was 132,809 dpm/mg of protein. Cholesterol synthesis was inhibited 25% by V, 48% by VII, and 62% by VIII. The methyl transfer from choline to transfer and ribosomal RNA for the control animals was 1086 dpm/mg of RNA, which was inhibited 49% by treatment with V.

Protein methylation by the choline donor for the 10-day Ehrlich ascites cells was 1225 dpm/mg of protein. Compound V inhibited protein methylation 58%. Nonhistone chromatin protein methylation for the control was 8118 dpm/mg of chromatin protein; treatment with V resulted in 56% inhibition. Histone methylation was 40,976 dpm/mg of chromatin protein for the control, whereas administration of V caused a 32% elevation. Phosphorylation of the regulatory proteins controlling gene activity was 9803 dpm/mg of nonhistone chromatin protein and 2954 dpm/mg of histone chromatin protein. Treatment with V resulted in a 59 and a 65% reduction, respectively, of chromatin protein phosphorylation.

The cyclic-AMP level for 10-day Ehrlich ascites cells was 3.65 pmoles/10^6 cells, which was elevated 169% by V administration. The number of tumor cells per milliliter on Day 10 was 223 × 10^6, which was suppressed 67% by treatment with V. The boron analogs were also tested for their *in vitro* effects on specific enzymes of 10-day Ehrlich ascites cells (Table III). Nuclei DNA polymerase activities were suppressed by all compounds tested. Compounds V, VII, and VIII possessed the best inhibitory activity: 54, 60, and 63% inhibition, respectively. Thymidylate mono- and diphosphate kinase activities were not affected by the boron analogs. Thymidylate synthetase activity was inhibited significantly by I and V and marginally by III, IV, and VIII. *S*-Adenosyl-L-methionine transferase activity was inhibited 51% by V and 61% by VIII with marginal inhibition by II–IV and VII. Nonhistone methylation dependent on *S*-adenosyl-L-methionine-methyltransferase activity was inhibited significantly by V (56%) and VIII (53%), with marginal inhibition by I–IV.

Treatment with the boron analogs reduced the activity of deoxyribonuclease, ribonuclease and cathepsin. Deoxyribonuclease activity was affected the most drastically. Compound I reduced activity 93%, II 72%, III 90%, IV 81%, V 86%, and VIII 75%. Ribonuclease activity was inhibited significantly by V (53%) and II (49%) with marginal inhibition by I, III, IV, and VIII. Cathepsin activity was reduced 47% by II, 56% by III, 67% by IV, 32% by V and VIII, and 81% by VII. Basal and adenosine diphosphate-stimulated respiration of 10-day Ehrlich ascites cells was unaffected by V, VII, and VIII at 6 μmoles.

DISCUSSION

The major boron analog effects were on Ehrlich ascites tumor cell protein and DNA syntheses. Protein synthesis inhibition may be the direct result of the α-amino boron analog on the translocation process or by blocking L-methionine synthesis. Enzymes required for DNA synthesis are synthesized in the early stages of cell proliferation (32). DNA polymerase activity was inhibited significantly whereas thymidylate kinases were unaffected by the boron analogs. Elevated cyclic-AMP levels resulting from boron analog administration are associated with cessation of cholesterol synthesis (36), reduced cellular proliferation, and reduction of H-1 histone phosphorylation, an event necessary for template activity (37).

Other protein kinases that are modulated by cyclic nucleotide levels

are those that phosphorylate nonhistone chromatin proteins which regulate transcription and differentiation. Compound V effectively blocks nonhistone and histone phosphorylation. The necessary phosphorus is derived from the γ-P of ATP, but Ehrlich ascites cell oxidative phosphorylation was not inhibited by the boron analogs. Lysosomal enzymatic activity is high prior to the mitotic apparatus formation, and membrane surface proteolytic activity is increased in cells undergoing rapid proliferation and metastasis (38). Elevated cyclic-AMP levels are associated with lysosomal membrane stabilization and blockage of the hydrolytic enzyme release (39).

Deoxyribonuclease activity was inhibited significantly by the boron analogs. Cathepsin proteolytic activity was inhibited moderately. *S*-Adenosyl-L-methionine transferase activity as well as the *S*-adenosyl-L-methionine-dependent methyl group transfer from choline was inhibited by the boron analogs. Protein and RNA methylation was suppressed significantly by boron analog treatment (1–4). Chromatin methylation is elevated in Novikoff hepatoma (40) and Hela cells (41). Cultured pancreatic anlase cells require *S*-adenosyl-L-methionine for proliferation and differentiation. Conversely, histone methylation by protein methylase III is highest late in the S and G_2 phases, indicating a cessation of replication (42). Boron analogs slightly increased histone methylation. Thymidylate synthetase activity also was inhibited by these agents, apparently producing cell death through thymine deprivation.

Generally, the degree of methyltransferase inhibition followed the antineoplastic activity demonstrated by these boron analogs in the Ehrlich ascites screen, with V having the highest activity in both areas. Boron betaine analogs are potent inhibitors of the protein synthesis and methyl transfer necessary for rapidly proliferating cells.

REFERENCES

(1) G. L. Cantoni, *Annu. Rev. Biochem.*, **44**, 435 (1975).

(2) G. R. Shepherd, J. M. Hardin, and B. J. Norland, *Arch. Biochem. Biophys.*, **143**, 1 (1971).

(3) M. Friedkin, *Adv. Enzymol.*, **28**, 235 (1973).

(4) W. K. Paik and S. Kim, *ibid.*, **42**, 227 (1975).

(5) B. F. Spielvogel, L. Wojnowich, M. K. Das, A. T. McPhail, and K. D. Hargrave, *J. Am. Chem. Soc.*, **98**, 5702 (1976).

(6) H. Meerwein, *Org. Synth.*, **46**, 113 (1966).

(7) B. F. Spielvogel, M. K. Das, and P. Wisian-Neilson, *Inorg. Chem.*, **17**, 2327 (1978).

(8) S. S. Uppal and H. C. Kelly, *J. Chem. Soc., Chem. Commun.*, **1970**, 1619.

(9) O. T. Beachley, Jr., and B. Washburn, *Inorg. Chem.*, **14**, 120 (1975).

(10) A. T. McPhail, K. D. Onan, B. F. Spielvogel, and P. Wisian-Neilson, *J. Chem. Res.*, **5**, 205 (1978).

(11) W. H. Myers, G. E. Ryschkewitsch, M. A. Mathur, and R. W. King, *Inorg. Chem.*, **14**, 2874 (1975).

(12) M. P. Brown, R. W. Heseltine, P. A. Smith, and P. J. Walker, *J. Chem. Soc. A*, **1970**, 410.

(13) B. F. Spielvogel, P. Wisian-Neilson, A. T. McPhail, and K. D. Hargrave, *J. Chem. Soc. Dalton*, **1976**, 353.

(14) J. T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exp. Ther.*, **96**, 99 (1949).

(15) C. Piantadosi, C. S. Kim, and J. L. Irvin, *J. Pharm. Sci.*, **58**, 821 (1969).

(16) R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schmasdon, and B. J. Abbott, *Cancer Chem. Rep., Part 3*, **3**(2) (1972).

(17) I. H. Hall, K. H. Lee, E. C. Mar, C. O. Starnes, and T. G. Waddell, *J. Med. Chem.*, **20**, 333 (1977).

(18) C. B. Chae, J. L. Irvine, and C. Piantadosi, *Proc. Am. Assoc. Cancer Res.*, **9**, 44 (1968).

(19) R. G. Wilson, R. H. Bodner, and G. E. MacHorter, *Biochim. Biophys. Acta*, **378**, 260 (1975).

(20) A. C. Sartorelli, *Biochem. Biophys. Res. Commun.*, **27**, 26 (1967).

(21) G. T. Haven, J. R. Frzemien, and T. T. Nquyea, *Res. Commun. Chem. Pathol. Pharmacol.*, **6**, 253 (1973).

(22) F. Wada, K. Hirata, and Y. Sakamoto, *J. Biol. Chem.*, **65**, 171 (1969).

(23) W. C. Hymer and E. L. Kuff, *J. Histochem. Cytochem.*, **12**, 359 (1964).

(24) A. Raineri, R. C. Simsimau, and R. K. Boutwell, *Cancer Res.*, **33**, 134 (1973).

(25) Y. M. Kish and L. J. Kleinsmith, *Methods Enzymol.*, **40**, 201 (1975).

(26) A. C. Gilman, *Proc. Natl. Acad. Sci., USA*, **67**, 305 (1970). Calbiochem. cAmp assay kit.

(27) H. Sawada, K. Tatsumi, M. Sasada, S. Shirakawa, T. Nakumura, and G. Wakisaka, *Cancer Res.*, **33**, 41 (1974).

(28) F. Maley and S. Ochoa, *J. Biol. Chem.*, **233**, 1538 (1958).

(29) A. Kampf, R. L. Barfknecht, P. J. Shaffer, O. Osaki, and M. P. Mertes, *J. Med. Chem.*, **19**, 903 (1976).

(30) R. T. Borchardt, J. A. Huber, and Y. S. Wu, *ibid.*, **19**, 1094 (1976).

(31) I. H. Hall, K. S. Ishaq, and C. Piantadosi, *J. Pharm. Sci.*, **63**, 625 (1974).

(32) Y. S. Cho-Chung and P. M. Gullino, *J. Biol. Chem.*, **248**, 4743 (1973).

(33) I. H. Hall, K. H. Lee, and S. A. ElGebaly, *J. Pharm. Sci.*, **67**, 552 (1978).

(34) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randell, *J. Biol. Chem.*, **193**, 265 (1975).

(35) G. W. Snedecor, in "Statistical Methods," Iowa State College Press, Ames, Iowa, 1956, section 2.

(36) L. A. Bricker and G. S. Levy, *J. Biol. Chem.*, **247**, 4914 (1972).

(37) C. S. Rubin and O. M. Roseu, *Annu. Rev. Biochem.*, **44**, 831 (1975).

(38) H. P. Schnebli, in "Proteases and Proteases Inhibitors in Neoplasia in Proteinase Inhibition," H. Fritz, H. Tschnesche, L. J. Green, and E. Truscheit, Eds., Springer-Verlag, New York, N.Y., 1974, p. 615.

(39) H. Bourne, L. Lichenstein, K. Melmom, C. Henney, E. Weinstein, and C. Shearer, *Science*, **184**, 19 (1974).

(40) W. K. Paik, H. W. Lee, and H. P. Morris, *Cancer Res.*, **32**, 37 (1972).

(41) H. W. Lee, W. K. Paik, and T. W. Borum, *J. Biol. Chem.*, **248**, 4194 (1973).

(42) I. Parsa, N. H. Marsh, and P. J. Fitzgerald, *J. Exp. Res.*, **73**, 49 (1972).

ACKNOWLEDGMENTS

Supported by grants from the University of North Carolina, the American Cancer Society (IN-15R), and the U.S. Army (DAAG29-77-G-0099).