Synthesis, Cytotoxicity, Hypolipidemic and Anti-inflammatory Activities of Amine–Boranes and Esters of Boron Analogues of Choline and Thiocholine

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Abstract □ Boron analogues of carbamoylcholine and thiocholine and esters of these analogues were prepared. These compounds were fairly stable toward hydrolysis and demonstrated moderate anti-inflammatory and hypolipidemic activities in mice. The hypolipidemic activity of the compounds at a dose of 8 mg/kg/day was equivalent in reducing lipid levels in serum to those of clofibrate at 150 mg/kg/day and lovastatin at 8 mg/kg/day. The compounds demonstrated significant cytotoxic activity against the growth of murine and human tumor cells; all were active against the growth of human HeLa-S^3 uterine suspended cells, and some were active against murine L_{1210} lymphoid leukemia, human $Tmolt_3$ leukemia cells, colorectal adenocarcinoma, KB nasopharynx, osteosarcoma, and glioma. These studies demonstrated that antimetabolite analogues of acetylcholine exhibit the same types of pharmacological activity as other boron-substituted betaine and amino acids. Furthermore, a strong positive correlation exists between hypolipidemic activity and cytotoxicity for these new choline derivatives, as has previously been demonstrated for other boron-containing amino acids, amides, esters, and peptides.

Table I—Structures of Compounds

Compound	Structure
1	NH_3BH_3
2	$(CH_3)_3CNH_2BH_3$
3	$(CH_3)_3NBH_3$
4	$(CH_3)_2NHBH_3$
5	$Na(CH_3)_2NBH_3$
6	$H_3BN(CH_3)_2CH_2CH_2OC(O)CH_3$
7	$H_3BN(CH_3)_2CH_2CH_2OC(O)N(CH_3)_2$
8	$H_3BN(CH_3)_2CH_2CH_2OC(O)C_6H_5$
9	$H_3BN(CH_3)_2CH_2CH_2SH$
10	$H_3BN(CH_3)_2CH_2CH_2SC(O)CH_2C_6H_5$
11	$[H_3BN(CH_3)_2CH_2CH_2SC(O)CH_2-]_2$
12	$[H_3BN(CH_3)_2CH_2CH_2SC(O)(CH_2)_3-]_2$
13	$[H_3BN(CH_3)_2CH_2CH_2OC(O)(CH_2)_3-]_2$

Continued investigation of carboxyboranes and cyanoboranes has demonstrated that some of these derivatives possess potent antineoplastic, hypolipidemic, and anti-inflammatory activities in rodents.[1] These derivatives include amine-, cyano-, and carboxyboranes;[2] di- and tripeptides of boron analogues of amino acids;[3] aminomethylphosphonate–cyanoborane adducts;[4] tricyclohexyl- and triphenylphosphine–borane;[5] metal complexes and salts of substituted hydroborates;[6] boron analogues of phosphonoacetates;[7] heterocyclic amine–carboxyboranes;[8] and hydropolyborates.[9]

We have now synthesized boron-containing carbamoylcholine and thiocholine and esters of the latter derivative as analogues of the neurotransmitter acetylcholine. Because these compounds are similar to the previous examples in that they are also Lewis base–borane adducts, the purpose of the present study was to investigate the effects of boron substitution directly onto the nitrogen atom so that these derivatives resemble acetylcholine. These compounds were evaluated for antitumor, anti-inflammatory, and hypolipidemic activities for comparison with other boron-substituted α-amino acids.

Experimental Section

Chemistry—The IR spectra were recorded on a Perkin-Elmer 297 spectrometer. The ^{1}H NMR spectra were obtained on either a Brucker NR80 or a Varian XL300 spectrometer. A JEOL FX90Q spectrometer was used to record the ^{11}B NMR spectra, and the ^{11}B chemical shifts are with respect to $BF_3 \cdot Et_2O$. Elemental analyses were performed by Galbraith Laboratories, Tennessee.

The following chemicals (see Table I for structures) were obtained commercially and were used without further purification unless stated otherwise: ammonia–borane (1), *t*-butylamine–borane (2), trimethylamine–borane (3), dimethylamine–borane (4), dimethylamine–borane, sodium salt (5), sodium borohydride, 2-(*N*,*N*-dimethylamino)ethanol, 2-(*N*,*N*-dimethylamino)ethanethiol hydrochloride, *N*,*N*-dimethylcarbamoyl chloride, acetyl chloride, phenylacetyl chloride, succinyl chloride, suberoyl chloride, tetraethylammonium hydroxide (solution in methanol), and borane complex of tetrahydrofuran (THF). (2-Acetoxyethyl)dimethylamine–borane[10] (6), (2-benzoyloxyethyl)dimethylamine–borane[10] (8), (2,2′(suberoyldioxy)diethyl)-bis(dimethylamine–borane)[10] (13), and tetraethylammonium borohydride[11] were prepared as described previously. Solvents were dried by routine methods and stored over a 4-Å molecular sieve.

(2-Dimethylcarbamyloxyethyl)dimethylamine—To a stirred solution of $Me_2NCH_2CH_2OH$ (17.83 g, 0.2 mol) in anhydrous diethyl ether (50 mL) at 0 °C, 50 mL of a solution of Me_2NCOCl (10.75 g, 0.1 mol) in anhydrous diethyl ether was added in a dropwise manner. After the addition was complete, the mixture was allowed to warm to room temperature and was stirred overnight. The white solid ($Me_2NCH_2CH_2OH \cdot HCl$) that formed as a by-product was filtered and washed with anhydrous diethyl ether [yield, 12.0 g (95.5%)]. The filtrate and washings were combined and concentrated, and the residue was distilled under reduced pressure to give the desired product as a clear, colorless liquid: bp 50 °C (0.43 mmHg); yield, 11.75 g (74%); ^{1}H NMR ($CDCl_3$): δ 2.13 (s, Me_2N), 2.42 (t, CH_2N), 2.75 (s, $Me_2NC(O)-$), and 4.02 (t, CH_2O); IR (neat): ν (CO), 1715 cm^{-1}.

(2-Dimethylcarbamyloxyethyl)dimethylamine–Borane (7)—To a stirred solution of (2-dimethylcarbamyloxyethyl)dimethylamine (7.72 g, 0.048 mol) in anhydrous THF was added 100 mL of 1 M $BF_3 \cdot$ THF under an atmosphere of N_2. The mixture was heated at reflux overnight and cooled, and the solvent was removed under reduced pressure. The residue was dissolved in CH_2Cl_2 (300 mL), washed with water (3 × 100 mL), dried over anhydrous $MgSO_4$, and concentrated to give a colorless oil. The oil was heated to 140 °C under reduced pressure (0.85 mmHg) to remove unreacted starting material (0.90 g) and other impurities. The residue, which solidified upon

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cooling, was dissolved in CH_2Cl_2, diluted with diethyl ether (35 mL), and cooled to −30 °C. The yellow impurities, which precipitated upon cooling, were filtered out, and the solvent was removed from the filtrate under reduced pressure to give 7 in a yield of 5.48 g (65.28%; 73.9% based on starting material consumed): mp 45–47 °C; ^{11}B NMR ($CDCl_3$): δ −9.47 (q, $^1J_{B,H}$ = 95 ± 5 Hz); ^1H NMR ($CDCl_3$): δ 2.66 (s, Me_2N), 2.92 (s, $Me_2NC(O)-$), 3.07 (t, CH_2N), and 4.49 (t, CH_2O); IR (nujol mull): ν(BH), 2390, 2325, and 2285 cm^{-1}; ν(CO), 1715 cm^{-1}.

Anal.—Calc. for $BC_7H_{19}N_2O_2$: B, 6.21; C, 48.31; H, 11.00; N, 16.09. Found: B, 6.18; C, 48.11; H, 11.16; N, 15.64.

(2-Mercaptoethyl)dimethylamine–Borane (9)—Sodium borohydride (13.35 g, 352.89 mmol) was added slowly to a suspension of dimethylaminoethanethiol hydrochloride (25.00 g, 176.47 mmol) in a mixture of anhydrous THF (500 mL) and anhydrous dichloromethane (100 mL) under an atmosphere of N_2. The mixture was stirred at room temperature for 1 h, heated at reflux for 3 days, and then cooled to room temperature; the solvent was removed under reduced pressure. The residue was diluted with CH_2Cl_2 (400 mL), and excess sodium borohydride was destroyed by slow addition of water. The organic layer was separated, and the aqueous layer was extracted with CH_2Cl_2. The combined extracts were washed once with water and dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure to give 9 in a yield of 18.20 g (86.64%). The crude product was crystallized from CH_2Cl_2–ether: mp 118–121 °C; ^{11}B NMR ($CDCl_3$): δ −10.11 (q, $^1J_{B,H}$ = 95 ± 2 Hz); ^1H NMR ($CDCl_3$): δ 2.53 (s, Me_2N), 3.00 (second order, CH_2CH_2), and 3.73 (s, SH); IR (nujol mull): ν(BH), 2385, 2355, 2315, and 2280 cm^{-1}.

Anal.—Calc. for $BC_4H_{14}NS$: B, 9.08; C, 40.36; H, 11.85; N, 11.77; S, 26.93. Found: B, 8.44; C, 40.45; H, 11.83; N, 11.77; S, 26.69.

(2-Thioacetoxyethyl)dimethylammonium Chloride—Dimethylaminoethanethiol hydrochloride (20.00 g, 141.17 mmol) was dissolved in anhydrous CH_2Cl_2 (400 mL) under an atmosphere of N_2 and cooled to 0 °C. To this solution, acetyl chloride (12.14 g, 154.70 mmol) was added in a dropwise manner with stirring. The mixture was stirred at room temperature for 45 min and then heated at reflux for 24 h. It was then cooled and filtered, and the solvent was removed under reduced pressure. The white solid obtained after the removal of solvent was crushed, washed with anhydrous ether, and dried under reduced pressure to yield 19.76 g (76.20%) of product: ^1H NMR ($CDCl_3$): δ 2.39 (s, CH_3), 2.97 (s, Me_2N), 3.32 (s, CH_2CH_2), and 11.84 (br s, NH^+).

(2-Thioacetoxyethyl)dimethylamine–Borane—To a solution of Et_4NBH_4 (2.93 g, 20.19 mmol) in anhydrous CH_2Cl_2 (50 mL) under an atmosphere of N_2, 50 mL of a solution of (2-thioacetoxyethyl)dimethylammonium chloride (2.48 g, 13.5 mmol) in anhydrous CH_2Cl_2 was added in a dropwise manner. The mixture was heated at reflux overnight. The solvent was removed under reduced pressure, and the residue was dissolved in ether. The ether solution was filtered and concentrated to give a clear, colorless oil with some white solid on the surface. This residue was dissolved in benzene, and the solid was carefully precipitated by adding petroleum ether. After removal of solid by filtration, the solvent was removed to give the pure product in a yield of 1.35 g (62.08%): ^{11}B NMR ($CDCl_3$): δ −9.94 (q, $^1J_{B,H}$ = 95 ± 2 Hz); ^1H NMR ($CDCl_3$): δ 2.37 (s, CH_3), 2.66 (s, NMe_2), 2.86 (m, CH_2S), and 3.22 (m, NCH_2); IR (neat): ν(BH), 2395, 2360, 2310, and 2280 cm^{-1}; ν(CO), 1695 cm^{-1}.

Anal.—Calc. for $BC_6H_{16}NOS$: B, 6.71; C, 44.74; H, 10.01; N, 8.70; S, 19.90. Found: B, 7.02; C, 44.12; H, 9.91; N, 8.73; S, 20.33.

(2-Phenylthioacetoxyethyl)dimethylammonium Chloride—Dimethylaminoethanethiol hydrochloride (20.00 g, 141.17 mmol) was dissolved in anhydrous CH_2Cl_2 (400 mL) under an atmosphere of N_2 and was cooled to 0 °C. To this solution, phenylacetyl chloride (24.01 g, 155.31 mmol) was added in a dropwise manner. The mixture was stirred at room temperature for 45 min, heated at reflux for 24 h, cooled, and filtered, and the solvent was removed under reduced pressure to give a yellow solid. The solid was crushed, washed with anhydrous ether, and dried under reduced pressure to yield 31.5 g (85.88%) of product: ^1H NMR(D_2O): δ 2.70 (s, NMe_2), 2.99 (second order, CH_2N and CH_2S), 3.76 (s, $CH_2C(O)-$), and 7.05–7.25 (m, aromatic).

(2-Phenylthioacetoxyethyl)dimethylamine–Borane (10)—To a solution of Et_4NBH_4 (4.05 g, 27.91 mmol) in anhydrous CH_2Cl_2 (60 mL) under an atmosphere of N_2, 60 mL of a solution of (2-phenylthioacetoxyethyl)dimethylammonium chloride (4.84 g, 18.63 mmol) in anhydrous CH_2Cl_2 was added in a dropwise manner. The mixture was heated at reflux overnight and cooled to room temperature, and the solvent was removed under reduced pressure. The residue was dissolved in anhydrous diethyl ether, and the solution was filtered to remove insoluble material and then concentrated under reduced pressure to give a clear, colorless oil in a yield of 3.82 g (86.46%): ^{11}B NMR ($CDCl_3$): δ −9.94 (q, $^1J_{B,H}$ = 100 ± 5 Hz); ^1H NMR ($CDCl_3$): δ 2.61 (s, NMe_2), 2.81 (m, SCH_2), 3.20 (m, NCH_2), 3.83 (s, $CH_2C(O)-$), and 7.20–7.38 (m, aromatic); IR (neat): ν(BH), 2390, 2325, and 2280 cm^{-1}; ν(CO), 1690 cm^{-1}.

Anal.—Calc. for $BC_{12}H_{20}NOS$: B, 4.56; C, 60.77; H, 8.50; N, 5.91; S, 13.52. Found: B, 4.62; C, 59.40; H, 7.74; N, 5.66; S, 13.35.

(2-Thiosuccinyloxydiethyl)-bis(dimethylamine) Dihydrochloride—Commercial dimethylaminoethanethiol hydrochloride was dissolved in anhydrous CH_2Cl_2; the solution was filtered to remove insoluble materials and concentrated to give pure dimethylaminoethanethiol hydrochloride. A portion of this solid (3.00 g, 21.18 mmol) was dissolved in anhydrous CH_2Cl_2 (150 mL) under an atmosphere of N_2 and was cooled to 0 °C. A solution of succinyl chloride (1.81 g, 11.68 mmol) in anhydrous CH_2Cl_2 (20 mL) was added in a dropwise manner. The mixture was heated at reflux for 24 h, cooled, and filtered. The white solid was washed with anhydrous CH_2Cl_2 and was dried under reduced pressure to yield 2.62 g, (67.72%) of product: ^1H NMR (D_2O): δ 2.84 (s, Me_2N), 2.98 (s, $CH_2C(O)$), and 3.21 (second order, NCH_2CH_2).

(2-Thiosuccinyloxydiethyl)-bis(dimethylamine)diborane (11)—To a solution of Et_4NBH_4 (1.76 g, 12.13 mmol) in anhydrous CH_2Cl_2 (100 mL) under an atmosphere of N_2 was slowly added (2-thiosuccinyloxydiethyl)-bis(dimethylamine) dihydrochloride (1.50 g, 4.11 mmol). The mixture was heated at reflux overnight, cooled to room temperature, and washed with water (5 × 50 mL; washings were performed until an impurity having an ethyl group was completely removed, as checked by ^1H NMR). The organic layer was dried over anhydrous Na_2SO_4, and the solvent was removed under reduced pressure. The white solid was washed twice with anhydrous ether (50 mL), filtered, and dried under reduced pressure to yield 0.80 g (60.87%) of product: mp 100–103 °C; ^{11}B NMR ($CDCl_3$): δ −10.15 (q, $^1J_{B,H}$ = 94 ± 3 Hz); ^1H NMR ($CDCl_3$): δ 2.65 (s, NMe_2), 2.85 (m, CH_2S), 2.95 (s, $CH_2C(O)-$), and 3.26 (m, CH_2N); IR (nujol mull): ν(BH), 2365, 2345, 2320, and 2275 cm^{-1}; ν(CO), 1690 cm^{-1}.

Anal.—Calc. for $B_2C_{12}H_{30}N_2O_2S_2$: B, 6.75, C, 45.03; H, 9.45; N, 8.75; S, 20.03. Found: B, 7.51; C, 44.65; H, 8.86; N, 8.82; S, 20.71.

(2-Thiosuberoyloxydiethyl)-bis(dimethylamine) Dihydrochloride—Purified dimethylaminoethanethiol hydrochloride (vide supra; 3.00 g, 21.18 mmol) was dissolved in anhydrous CH_2Cl_2 (150 mL) under an atmosphere of N_2 and cooled to 0 °C. A solution of suberoyl chloride (2.46 g, 11.66 mmol) in anhydrous CH_2Cl_2 (20 mL) was added in a dropwise manner. The mixture was heated at reflux for 24 h and cooled, and solvent was removed under reduced pressure. The residue was washed with ether and filtered and stored under an atmosphere of N_2. The yield was 4.36 g (98.6%): ^1H NMR ($CDCl_3$): δ 1.34 (br s, CH_2), 1.66 (br s, CH_2), 2.60 (t, $CH_2C(O)$), 2.94 (s, NMe_2), 3.24 and 3.33 (br s, CH_2S and CH_2N), and 12.09 (br s, N^+H).

(2-Thiosuberoyloxydiethyl)-bis(dimethylamine)diborane (12)—To a solution of Et_4NBH_4 (2.06 g, 14.20 mmol) in anhydrous CH_2Cl_2 (100 mL) under an atmosphere of N_2 was slowly added (2-thiosuberoyloxydiethyl)-bis(dimethylamine) dihydrochloride (2.00 g, 5.32 mmol). The mixture was heated at reflux overnight, cooled to room temperature, and washed with water (5 × 100 mL and 3 × 50 mL; washings were performed until an impurity having an ethyl group was completely removed, as determined by ^1H NMR). The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The white solid was washed with anhydrous ether (3 × 30 mL), filtered, and dried under reduced pressure to yield 0.60 g (33.61%) of product: mp 78–80 °C; ^{11}B NMR ($CDCl_3$): δ −9.98 (q, $^1J_{B,H}$ = 96 ± 3 Hz); ^1H NMR ($CDCl_3$): δ 1.34 (br s, CH_2), 1.66 (br t, CH_2), 2.57 (t, $CH_2C(O)-$), 2.66 (s, NMe_2), 2.85 (m, CH_2S), and 3.21 (m, CH_2N).

Anal.—Calc. for $B_2C_{16}H_{38}N_2O_2S_2$: B, 5.74; C, 51.08; H, 10.18; N, 7.45; S, 17.04. Found: B, 6.17; C, 51.03; H, 9.93; N, 7.36; S, 17.28.

Biological Screening—All compounds were suspended in the vehicle by homogenization (10×). Drug solutions were sterilized by passing through a 45-μM acrodisc.

Antineoplastic Activity: Ehrlich Ascites Carcinoma Tumor Screen—Male mice (CF_1; ~25 g) were inoculated intraperitoneally (ip) with 2 × 10^6 Ehrlich ascites carcinoma cells in isotonic sterile saline on day zero. On days 1–9, drugs suspended in 0.05% polysorbate 80 (Tween 80):H_2O were administered at 8 mg/kg/day. On day

10, the mice were sacrificed, and the ascites fluid from the peritoneal cavity was collected. The volume and ascrit (packed-cell volume) were determined for each animal, and the percent inhibition of tumor growth was calculated.[12]

Cytotoxicity Assays for Murine and Human Tissue Culture Cell Lines—The following cell lines were maintained by literature techniques:[8,13] murine L_{1210} lymphoid leukemia, P_{388} lymphocytic leukemia, human $Tmolt_3$ acute lymphoblastic T cell tumor, colorectal adenocarcinoma SW480, lung bronchogenic MB-9812, osteosarcoma TE418, KB nasopharynx, HeLa-S^3 suspended cervical carcinoma, and brain glioma EH118MG. On day zero, 10^4 tissue culture cells were mixed with sterile drugs at concentrations of 1 to 100 μg/mL (1 mM stock solution in 0.05% polysorbate 80:H_2O) and growth medium to a final volume of 1 mL. Cells were counted on day 3, because growth was still logarithmic at this time. The cells were counted with a hemocytometer and the trypan blue exclusion technique. The ED_{50} values were calculated from a semilogarithmic plot as representing the concentration of drugs required to kill 50% of the tumor cells by day 3.

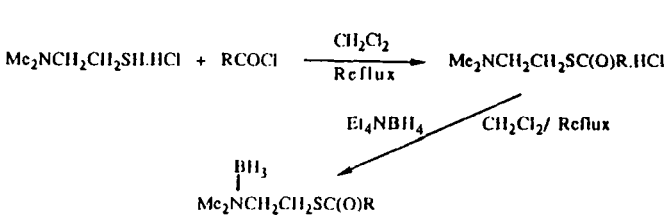
Anti-inflammatory Activity—Male mice (CF_1; ~25 g) were administered test drugs at 8 mg/kg (ip) in 0.05% polysorbate 80:H_2O 3 h and again 30 min before the injection of 0.2 mL of 1% carrageenan in 0.9% saline into the plantar surface of the right hind foot. Saline was injected into the left hind foot to serve as a base line. After 3 h, both feet were excised at the tibiotarsal (ankle) joint, according to the modified method of Winter.[14,15] The control mice afforded an increase in paw weight of 78 ± 3 mg.

Hypolipidemic Activity—Male mice (CF_1; 28 g) were administered drug suspended in 1% carboxymethylcellulose at 8 mg/kg/day (ip) for 16 days. On days 9 and 16, the mice were bled by tail vein bleeding into capillary tubes. The serum was obtained by centrifugation at 3000 × *g* for 3 min. The total cholesterol in serum was determined by the Liebermann–Burchard reaction,[16] and the triglyceride in serum was determined by a commercial kit (Bio-Dynamics/bmc triglyceride kit).

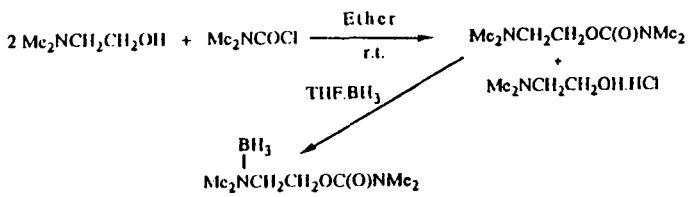
Results and Discussion

Chemistry—Boron analogues of carbamoylcholine and thiocholine and esters of the latter analogue were prepared by methods similar to those used previously[10] for the synthesis of boron analogues of choline and its esters. Thus, dimethylaminoethanethiol hydrochloride was reacted with the appropriate acid chloride under slightly harsher conditions than those used previously for choline ester analogues to give the corresponding ester hydrochlorides (Scheme I). Reaction with Et_4NBH_4 resulted in the formation of the corresponding borane adducts. For 9, sodium borohydride was used instead. Yields of various products varied from a low of 33% for 12 to a high of 86% for 9 and 10.

The boron analogue of carbamoylcholine (1) was prepared in a slightly different manner. Reaction of 2-*N*,*N*-dimethylethanolamine with carbamoyl chloride, in either a 1:1 or 2:1 ratio, resulted in the formation of free (2-carbamyloxyethyl)dimethylamine and 2-*N*,*N*-dimethylethanolamine hydrochloride (Scheme II). Complexation of free amine with THF–BH_3 gave the carbamoylcholine analogue in ~65% yield. The formation of free amine under these conditions is similar to that observed upon reaction of 2-*N*,*N*-dimethylethanolamine with phenoxyacetyl chloride[10] but is different from that observed with various other acid chlorides.[10] Reasons for this abnormal behavior are as yet unclear. Because the acyl



Scheme I



Scheme II

groups are too far removed to have much effect on the basicity of amino group via inductive effects, the differences in basicity must, therefore, be related to the differences in conformation.

Stability in Water—Hydrolysis of the BH_3 groups of thiocholine analogues and their esters was followed by ^{11}B NMR. Because of the insolubility of these compounds in D_2O, a mixture of D_2O and THF-d^8 was used as solvent. The ^{11}B NMR spectra showed that the BH_3 group in these compounds is fairly stable toward hydrolysis. Thus, after ~27 h at room temperature, 80–90% of the BH_3 groups remained unaltered. After ~100 h, the hydrolysis was ~30–35%, and after ~190 h, the hydrolysis varied from 35 to 45%. The hydrolytic stability of the BH_3 groups of choline analogues has been reported previously.[10] These compounds are slightly more stable toward hydrolysis of B–H bonds than the thiocholine analogues.

Pharmacology—Cytotoxic activity was demonstrated by all 13 compounds in the murine and human tissue cultured cell lines (Table II). In the murine leukemias, 1, 4, and 12 significantly inhibited P_{388} leukemia growth. The amine–carboxyboranes,[2] amine–cyanoboranes,[4] di- and tripeptides with boron,[3] phosphonoacetate boron analogues,[7] and heterocyclic amine–boranes were generally inactive in this screen. The tricyclohexyl- and triphenylphosphine–borane derivatives were more active than other classes of boron analogues in the P_{388} screen.[5]

Compounds 1–4, 7, and 9–13 significantly reduced the growth of L_{1210} lymphoid leukemia. Their activity in the L_{1210} screen was comparable with those of selected amine–carboxyboranes,[2] amine–cyanoboranes,[4] heterocyclic amine–boranes,[8] tricyclohexyl- and triphenylphosphine–boranes,[5] and metal complexes and boron-substituted salts.[9] All of the derivatives mentioned previously were more active in the L_{1210} screen than the di- and tripeptide boron derivatives.[3]

Human colon carcinoma growth was reduced by 5–13; $Tmolt_3$ leukemia growth by 1 and 5–13; HeLa-S^3 uterine carcinoma growth by 1–13; KB nasopharynx growth by 1, 5–9, and 13; osteosarcoma growth by 1, 3, 5–9, 12, and 13; and brain glioma growth by 1, 10, and 12. The activity of 5, 7, 9, 10, and 11 against colon adenocarcinoma growth is of particular interest because few commerical agents possess activity against human colon adenocarcinoma growth. With regard to colon cancer, the heterocyclic amine–boranes,[8] metal complexes of boron,[9] phosphite–boranes,[7] tricyclohexyl- and triphenylphosphine–boranes,[5] amine–carboxyboranes,[2] and amine–cyanoboranes[4] were not as active as the choline and thiocholine boron esters in inhibiting colon cell growth. In addition, boron metal complexes,[9] amine–carboxyboranes,[2] and amine–cyanoboranes[4] were also not as active as the thiocholine and choline boron esters in the KB nasopharynx and osteosarcoma screens. The tricyclohexyl- and triphenylphosphine–boranes[5] and phosphite–boranes[7] were not as active in the KB screen, but demonstrated activity comparable to that of the thiocholine and choline boron esters against osteosarcoma growth. The heterocyclic amine–boranes,[8] amine–cyanoboranes,[4] amine–carboxyboranes,[2] boron analogues of phosphonoacetates,[7] and the tricyclohexyl- and triphenylphosphine–boranes[5] were more active than the thio-

Table II—Cytotoxicity (ED_{50}, μg/mL) of Compounds[a]

Compound[b]	Murine Tumor		Human Tumor						
	P_{388}	L_{1210}	Colon	$Tmolt_3$	HeLa-S^3	KB	Lung	Osteosarcoma	Glioma
1	3.45	1.78	8.07	1.27	2.25	1.24	5.25	3.47	2.48
2	6.34	3.08	5.65	4.36	3.69	7.01	7.081	7.63	4.87
3	4.83	3.55	5.13	4.07	2.91	4.66	6.28	3.43	5.51
4	3.08	3.13	5.07	4.17	2.73	5.71	4.24	5.13	5.06
5	6.52	7.38	1.73	3.78	1.65	1.61	4.88	0.68	4.36
6	10.35	4.61	3.43	3.09	3.50	3.53	6.52	1.52	4.85
7	10.34	3.19	1.70	1.68	3.00	1.46	7.92	3.49	8.74
8	7.82	5.00	3.36	1.94	2.00	3.42	7.49	3.49	3.59
9	4.17	2.71	1.34	3.39	1.62	2.65	6.42	1.19	6.00
10	4.71	2.43	1.19	2.82	1.71	4.13	7.45	—[c]	3.06
11	4.60	2.60	1.19	3.71	1.69	5.24	5.42	6.25	4.45
12	3.20	2.00	4.42	2.82	1.87	6.94	7.04	2.76	2.76
13	7.62	1.87	3.20	2.15	2.13	1.19	6.52	2.32	6.98
Standards									
5-FU[d]	3.72	1.41	3.09	2.14	2.47	1.25	5.64	—	1.28
Ara C[e]	4.06	2.76	3.42	2.67	2.13	2.84	4.60	—	1.88
Hydroxyurea	—	2.67	4.74	3.18	1.96	5.29	7.37	7.54	2.27

[a] Values below 4 μg/mL are considered significantly active by the NCI protocol (ref 13). [b] n = 5. [c] —, Not determined. [d] 5-Fluorouracil. [e] Cytosine arabinoside.

choline and choline boron esters, as well as the metal complexes of boron compounds, against glioma growth. All of the boron chemical classes of drugs demonstrated similar excellent activity against HeLa-S^3 uterine carcinoma growth, and none of the boron chemical classes of compounds, including the new thiocholine and choline boron esters, were active against lung bronchogenic tumor growth. Thus, an activity pattern similar to that of other boron derivatives[2-9] has been observed.

Probably, substituted functional moieties of the individual boron derivatives afford some difference in the respective tissue culture screens for cell uptake and inhibition of growth. Structure modification to optimize the activity of thiocholine and choline boron esters that have demonstrated activity against osteosarcoma and glioma growth may, in the future, offer some promise in clinical use because these tumor sites in the body act as sanctuaries. Studies have shown that boron analogues are taken up and distributed to all tissues of the body.[17] Once in the cell, boron can be subject to neutron capture irradiation,[17] which would be a second mode of action in killing tumor cells.

Compounds 1 and 2 were toxic to mice at 10 mg/kg, with multiple doses of the drug, thus limiting their pharmacological use. The simple ammonia–borane 1, containing no ester or choline functions, afforded good activity in the $Tmolt_3$, KB nasopharynx, glioma, and HeLa-S^3 screens. However, the choline and thiocholine boron esters 7, 9, 10, and 11 demonstrated improved activity against colon adenocarcinoma growth compared with the smaller boron derivatives. Compound 5 is the sodium salt of dimethylamine–borane, and yet it demonstrated a broad spectrum of cytotoxicity against a number of tumor cell lines. This compound was generally more active than 4 against human tumor growth.

Among the choline and thiocholine boron esters, only 13 demonstrated effective in vivo activity (i.e., 86% inhibition of growth) against Ehrlich ascites carcinoma growth at 8 mg/

Table III—In Vivo Pharmacological Activities of Compounds in CF_1 Male Mice

Compound	Antineoplastic Activity in Ehrlich Ascites Carcinoma		Anti-inflammatory Activity		Hypolipidemic Activity, % of Control			
					Dose, mg/kg/day	Serum Cholesterol		Serum Triglyceride on Day 16
	Dose, mg/kg/day	Inhibition, %	Dose, mg/kg/day	Inhibition, %		Day 9	Day 16	
1	8	Toxic	10	—[a]	10	Toxic	—	—
2	20	Toxic	10	59 ± 6[b]	10	Toxic	—	—
3	20	83[b]	10	35 ± 3[b]	20	91 ± 7	70 ± 3[b]	85 ± 5
4	20	96[b]	10	34 ± 4[b]	20	71 ± 4[b]	58 ± 5[b]	88 ± 11
5	20	81[b]	8	24 ± 3	20	74 ± 4[b]	74 ± 5[b]	59 ± 5[b]
6	8	67	8	15 ± 3	8	72 ± 6[b]	65 ± 6[b]	72 ± 4[b]
7	8	40	8	39 ± 5[b]	8	78 ± 7	81 ± 6	84 ± 7
8	8	68	8	29 ± 3	8	74 ± 5[b]	71 ± 5[b]	87 ± 6
9	8	43	8	28 ± 4	8	103 ± 6	77 ± 7[b]	89 ± 7
10	8	52	8	26 ± 2	8	93 ± 6	78 ± 4[b]	81 ± 5
11	8	67	8	22 ± 3	8	83 ± 6	65 ± 4[b]	87 ± 7
12	8	41	8	28 ± 4	8	85 ± 47	68 ± 5[b]	83 ± 5
13	8	86[b]	8	11 ± 3	8	82 ± 6	75 ± 6[b]	62 ± 4[b]
Standards								
6MP[c]	0.5	99	—	—	—	—	—	—
Phenylbutazone	—	—	50	47 ± 4	—	—	—	—
Indomethacin	—	—	10	78 ± 5	—	—	—	—
Clofibrate	—	—	—	—	150	88 ± 4	87 ± 5	75 ± 5
Lovastatin	—	—	—	—	8	85 ± 4	82 ± 5	86 ± 7

[a] —, Not determined. [b] $p \leq 0.01$; *t* test. [c] 6-Mercaptopurine.

kg/day (ip). Compounds 3–5 afforded 83, 96, and 81% inhibition, respectively, at 20 mg/kg/day, and 6-mercaptopurine afforded 99% inhibition at 0.5 mg/kg/day (Table III). In contrast, 6, 8, and 11 afforded 67, 68, and 67% inhibition of growth, respectively, at 8 mg/kg/day. The remaining compounds afforded <50% inhibition at 8 mg/kg (ip) and were essentially ineffective. The activity of the new compounds in vivo was comparable with that of the amine–cyanoboranes[4] and di- and tripeptide boranes.[3] In general, the heterocyclic amine–boranes[8] and the amine–carboxyboranes[2] were more active in vivo than the thiocholine and choline boron esters.

In vivo anti-inflammatory activity at 8 mg/kg (ip) was also evident with certain compounds (Table III). Compound 2 gave 59% inhibition, 3, 4, and 7 gave 34–40% inhibition, 5 and 8–12 gave 22–29% inhibition, and the remaining compounds were inactive as anti-inflammatory agents. The anti-inflammatory activity at 8 mg/kg was not as effective as that of other reported derivatives (e.g., amine–cyanoboranes[18] and the amine–carboxyboranes[18]) but was more effective compared with that of the trihexyl- and triphenylphosphine–borane derivatives,[5] di- and tripeptide boron derivatives,[3] and phosphonoacetate boron derivatives (unpublished results).

All of the compounds (at 8 mg/kg/day) demonstrated the ability to lower cholesterol levels in serum of mice by day 16 (Table III). Compounds 4, 6, 11, and 12 afforded ≥32% reduction of cholesterol levels in serum, an activity better than that of clofibrate at 150 mg/kg/day. However, only 5 and 13 afforded ≥35% reduction of triglyceride levels in serum, and 6 afforded a 28% reduction. The hypolipidemic activities for the choline and thiocholine boron esters 6–13, at 8 mg/kg/day, were markedly reduced, with an average reduction of cholesterol levels in serum of 28% and of triglyceride levels in serum of 20%. The amine–carboxyboranes and their esters and amides,[1] tricyclohexyl- and triphenylphosphine–boranes,[5] heterocyclic amine–boranes,[19] phosphonoacetate and cyanoborane adducts of aminomethylphosphonates analogues,[20] and di- and tripeptide boranes[3] were far superior hypolipidemic agents at this dose in rodents when compared with the thiocholine and choline boron esters.

Conclusions

Boron derivatives of carbamoylcholine and thiocholine and esters of the latter analogue demonstrated pharmacological activities similar to those observed for related boron compounds. In general, the anti-inflammatory and hypolipidemic activities were not as potent as those of some boron derivatives studied in the past. The cytotoxicity demonstrated by selected compounds against the growth of human and solid tumors was significant. These thiocholine and choline boron esters may have therapeutic use in the future as antineoplastic agents. Further investigation of structure–activity relationships and characterization of biological activity are required.

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