Hypolipidemic, Anti-obesity, Anti-inflammatory, Anti-osteoporotic, and Anti-neoplastic Properties of Amine Carboxyboranes

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The amine-carboxyborane derivatives were shown to be effective antineoplastic/cytotoxic agents with selective activity against single-cell and solid tumors derived from murine and human leukemias, lymphomas, sarcomas, and carcinomas. The agents inhibited DNA and RNA synthesis in preference to protein synthesis in L_{1210} lymphoid leukemia cells. Inosine-monophospate dehydrogenase apparently is a target site of the compounds; similar effects on phosphoribosyl-pyrophosphate amido transferase, orotidine-monophosphate decarboxylase, and both nucleoside and nucleotide kinases were observed. Deoxyribonucleotide pool levels were reduced in the cells; DNA strand scission was observed with the agents. In rodents, the amine carboxyboranes were potent hypolipidemic agents, lowering both serum cholesterol and triglyceride concentrations, in addition to lowering cholesterol content of very low-density lipoprotein and low-density lipoprotein (LDL) and elevating high-density lipoprotein (HDL) cholesterol concentrations. *De novo* regulatory enzymes involved in lipid synthesis were also inhibited (e.g., hypocholesterolemic 3-hydroxy-3-methyl-Coenzyme A reductase, acyl-Coenzyme A cholesterol acyltransferase, and *sn*-glycerol-3-phosphate acyltransferase). Concurrently, the agents modulated LDL and HDL receptor binding, internalization, and degradation, so that less cholesterol was delivered to the plaques and more broken down from esters and conducted to the liver for biliary excretion. Tissue lipids in the aorta wall of the rat were reduced and fewer atherosclerotic morphologic lesions were present in quail aortas after treatment with the agents. Cholesterol resorption from the rat intestine was reduced in the presence of drug. Genetic hyperlipidemic mice demonstrated the same types of reduction after treatment with the agents. The agents would effectively lower lipids in tissue based on the inhibition of regulatory enzymes in pigs. These findings should help improve domestic meat supplies from fowl and pigs. The amine-carboxyboranes were effective anti-inflammatory agents against septic shock, induced edema, pleurisy, and chronic arthritis at 2.5 to 8 mg/kg. Lysosomal and proteolytic enzyme activities were also inhibited. More significantly, the agents were dual inhibitors of prostaglandin cyclooxygenase and 5'-lipoxygenase activities. These compounds also affected cytokine release and white cell migration. Subsequent studies showed that the amine-carboxyboranes were potent anti-osteoporotic agents reducing calcium resorption as well as increasing calcium and proline incorporation into mouse pup calvaria and rat UMR-106 collagen. — Environ Health Perspect 102(Suppl 3):21–30 (1994)

Key words: amine carboxyboranes, antineoplastic, atherosclerosis, osteoporosis, inflammation

Introduction

The amine–borane derivatives were synthesized as amine-, cyano- and carboxyboranes (*1–4*); di- and tripeptides of boron analogues of amino acids (*5*); aminomethylphosphonate cyanoborane adducts (*6,7*); tricyclohexyl- and triphenylphosphineboranes (*8*); metal complexes and salts of substituted hydroborates (*9*); boron analogues of phosphonoacetates (*10*); heterocyclic amine-carboxyboranes (*11*); hydropolyborates (*12*), boron analogs of choline and thiocholine (*13*); and 2'-deoxynucleoside and nucleotide cyanoboranes (*14–16*). All of these derivatives demonstrated a variety of pharmacologic activities. This discussion will concentrate on the amine carboxyboranes and their esters as representative therapeutic agents of the entire boron group of chemicals.

Initially, the amine-carboxyborane derivatives were shown to be nontoxic in CF_1 male mice when administered at 20, 50, or 100 mg/kg/day, intraperitoneally (ip), for 7 days (*17*). Variables measured were survival, total body weight, daily food consumption, individual body weight, clinical chemistry values, hematopoietic values, and histologic evaluations of major organs. LD_{50} values (dose required for 50% deaths) of these compounds were between 1 and 2 g/kg, ip, in mice.

Then we examined these compounds for antineoplastic activity (*9*). Because these amine carboxyboranes were similar in structure and size to endogenous intermediary substrates, they could be functioning as antimetabolites (e.g., of amino acids or betaine). Once they are taken up into cells, a second mechanism of action is neutron capture. An identified organ or foci of tissues can be irradiated with neutrons, whereupon the boron atom will capture a neutron and decay to emit an alpha particle. This process causes local tissue damage and cell death. *In vivo* these boron derivatives have demonstrated strong activity against Ehrlich ascites carcinoma growth, Lewis lung carcinoma, and B_{16} melanoma. Marginal activity against P_{388} lymphocytic and L_{1210} lymphoid leukemia cells was also demonstrated. Selected compounds (Table 1) were tested for cytotoxicity against murine and human tissue culture cells. These compounds were active against the growth of single tumor cells (e.g., L_{1210}, $Tmolt_3$, and HeLa-S[3]; Table 1). Selectivity

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Table 1. Effects of amine boranes on murine and human tissue cell growth[a].

	ED_{50} value (μg/ml)								
	Murine			human					
Compound	L_{1210}	P_{388}	$Tmolt_3$	SW480 colon	Lung-bronchogenic	HeLa-S^3 uterine	Glioma	KB naso-pharyngeal	Osteo-sarcoma
$(C_6H_5)_3PBH_2COOH$	2.97	—	2.89	2.76	6.57	—	3.64	—	—
$C_{16}H_{33}N(CH_3)_2BH_2COOH$	4.80	13.51	3.63	2.25	7.43	2.50	2.89	7.89	2.47
$C_{18}H_{37}N(CH_3)_2BH_2COOH$	1.74	4.49	1.41	6.60	9.60	2.25	7.84	4.05	3.12
$(CH_3)_2NHBH_2COOCH$	5.48	10.11	4.70	1.82	4.00	2.70	1.56	4.46	2.15
$(CH_3)_3NBH_2COOCH_3$	1.02	11.11	2.30	5.87	4.24	2.73	5.01	8.45	5.13
$CH_3NH_2BH_2COOH$	4.01	14.61	4.72	0.84	4.35	3.92	6.74	4.32	5.21
$CH_3CH_2NH_2BH_2COOH$	3.87	3.12	4.71	2.19	7.25	1.63	6.96	3.67	5.45
$(CH_3)_2NHBH_2COOH$	2.72	12.86	5.76	2.25	4.89	2.67	2.62	4.59	5.27
5FU	1.41	3.72	2.14	3.09	3.69	2.47	1.28	1.25	—
Ara C	2.76	4.23	2.67	3.42	4.60	2.13	1.88	2.84	—
Hydrox-urea	2.67	—	3.18	4.74	7.37	1.96	2.27	5.29	7.59
VP 16	0.64	1.16	—	—	—	—	—	—	—

[a]Cytotoxicity of specific amine boranes (first 8 listed) was determined in tissue culture cells grown by literature techniques. ED_{50} (effective dose, 50%) values were determined for L_{1210}, P_{388}, $Tmolt_3$, and HeLa-S^3 tumor cells by counting single cells in a hemocytometer using trypan blue exclusion (n=5). Solid tumor cell growth was determined with crystal violet/MeOH in 96 well plates that were read with a Molecular Devices Softmax at 580 nm. Significant ED_{50} values were less than 4 μg/ml.

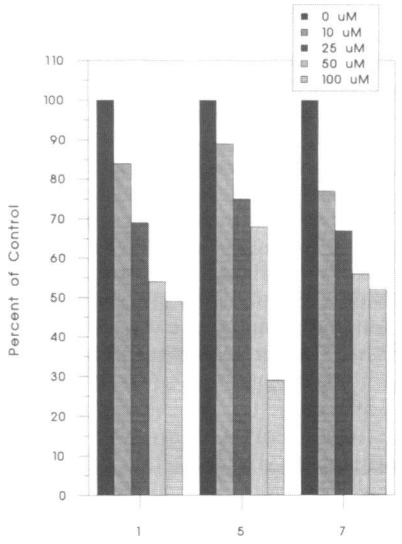


Figure 1. DNA synthesis in L_{1210} cells. Effects of compounds 1, 5, and 7 at 0, 10, 25, 50, and 100 μM after 60-min incubations on 10^6 L_{1210} lymphoid leukemia cells. ^{3}H-thymidine incorporation into DNA.

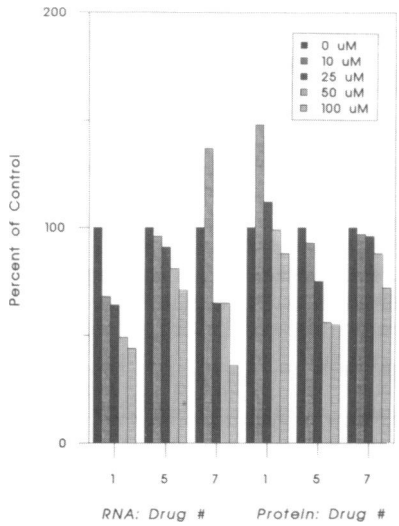


Figure 2. RNA and protein synthesis in L_{1210} cells. Effects of compounds 1, 5, and 7 at 0, 10, 25, 50, and 100 μM after 60 min incubations on 10^6 L_{1210} lymphoid leukemia cells. ^{3}H-Uridine incorporation into RNA and ^{3}H-leucine incorporation into protein.

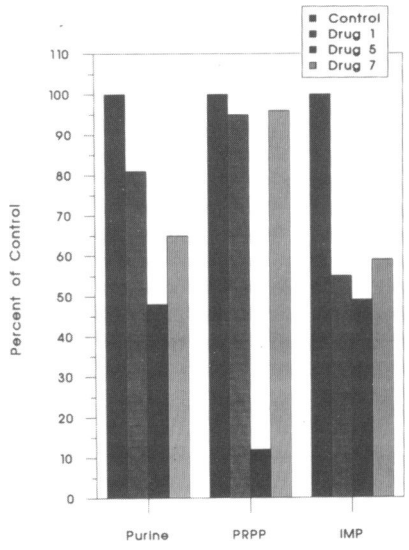


Figure 3. Purine synthesis in L_{1210} cells. Effects of compounds 1, 5, and 7 at 100 μM after 60-min incubations on L_{1210} lymphoid leukemia cells. *De novo* purine synthesis as ^{14}C-glycine incorporation, phosphoribosyl pyrophosphate, PRPP amido transferase, and inosine monophosphate, IMP dehydrogenase activities.

was demonstrated by the compounds against the growth of cells derived from solid tumors [e.g., SW480 adenocarcinoma of the colon, glioma, or osteosarcoma (*9*)]. Modification of structures, including a heterocyclic ring (*11*) or a 2′-deoxyribonucleoside (*14,15*), enhanced the antineoplastic activity.

The L_{1210} cell line was selected to investigate the mode of action for these derivatives because its properties are well established as a laboratory tumor cell model. DNA and RNA syntheses were inhibited in a concentration dependent manner (Figures 1, 2) and protein synthesis was sometimes marginally inhibited. The *de novo* nucleic acid synthesis of purines was reduced by the agents specifically at phosphoribosyl pyrophosphate (PRPP)-amido transferase and inosine monophosphate (IMP) dehydrogenase enzyme sites, the regulatory sites in the pathway (Figure 3). Apparently, the latter enzyme is a major target of the amine-cyanoborane derivatives. Pyrimidine *de novo* synthesis was also inhibited by compounds 1 and 7 (Figure 4). Orotidine monophosphate (OMP) decarboxylase was the major site at which the agents functioned as inhibitors. Nucleoside and nucleotide kinase activities were also inhibited (Figure 5). Ribonucleoside reductase, dihydrofolate reductase, DNA polymerase α and topoisomerase II activities were inhibited by specific boron agents, but not by all of the agents. Inhibition was dependent on the substituted moieties of the basic structure of the amine-carboxyboranes. This class of boron derivatives reduced the deoxyribonucleotide

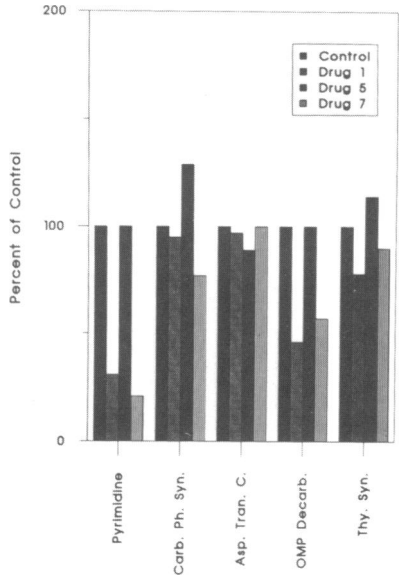


Figure 4. Pyrimidine synthesis in L_{1210} cells. Effects of compounds 1, 5, and 7 at 100 μM after 60-min incubations on L_{1210} lymphoid leukemia cells. *De novo* pyrimidine synthesis as ^{14}C-formate incorporation, carbamyl phosphate synthetase, aspartate transcarbamylase, orotidine monophosphate, OMP decarboxylase, and thymidylate synthetase activities.

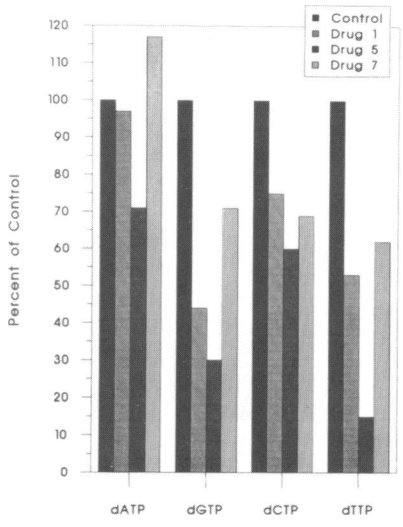


Figure 6. d(NTP) pools in L_{1210} cells. Effects of compounds 1, 5, and 7 at 100 μM after 60 min incubations on L_{1210} lymphoid leukemia cells. Deoxyribonucleotide, d(NTP) pools, including deoxyriboadenosine triphosphate, d(ATP), deoxyriboguanosine triphosphate, d(GTP), deoxyribocytosine triphosphate, d(CTP), and deoxyribothymidine triphosphate, d(TTP) determinations.

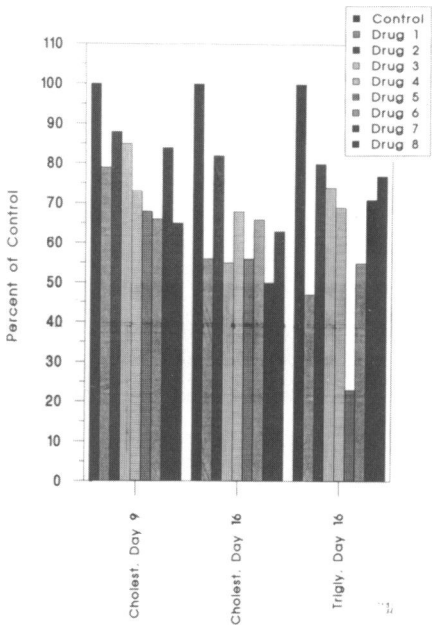


Figure 8. Mouse serum lipid levels. Effects of compounds 1 to 8 at 20 mg/kg/day, ip on CF_1 mouse serum cholesterol and triglyceride concentrations 9 and 16.

[d(NTP)] pools after incubation for 60 min at 100 μM (Figure 6). L_{1210} deoxyriboguanosine triphosphate, deoxyribocytosine triphosphate, and deoxyribothymidine triphosphate pools were reduced, whereas deoxyriboadenosine triphosphate pools were not as markedly affected. These reductions alone would account for the inhibition of DNA synthesis and cell growth. The inhibition of *de novo* purine synthesis by the agents is also reflected in the reduction of the d(NTP) pool levels. Further studies with calf thymus DNA *in vitro* indicated that these compounds did not act as intercalator binding between the bases of DNA or crosslinks between the two strands of DNA. However, these amine cyanoboranes did cause DNA strand scission in L_{1210} cells after 24-hr incubation at 100 μM (Figure 7).

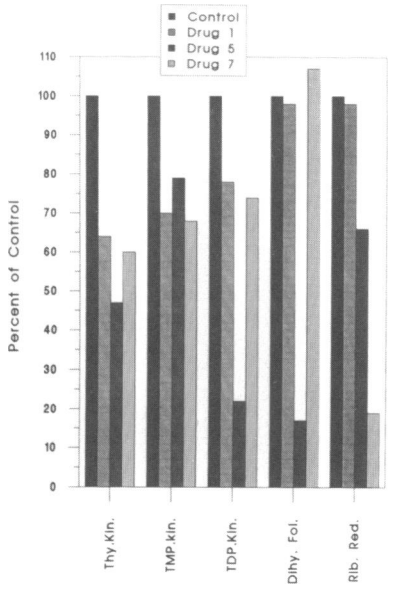


Figure 5. Enzyme activities in L_{1210} cells. Effects of compounds 1, 5, and 7 at 100 μM after 60-min incubations on L_{1210} lymphoid leukemia cells. Thymidine kinase, TMP kinase, TDP kinase, dihydrofolate reductase, and ribonucleoside reductase activities.

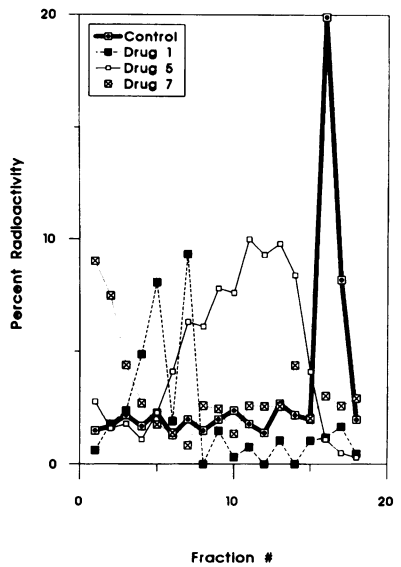


Figure 7. DNA strand scission. Effects of compounds 1, 5, and 7 at 100 μM after 24-hr L_{1210} lymphoid leukemia DNA strand scission.

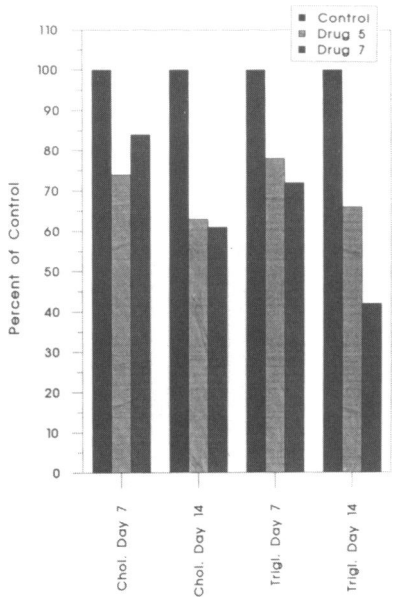


Figure 9. Rat serum lipids. Effects of compounds 5 and 7 at 8 mg/kg/day, orally on Sprague-Dawley rat serum cholesterol and triglyceride concentrations on days 7 and 14.

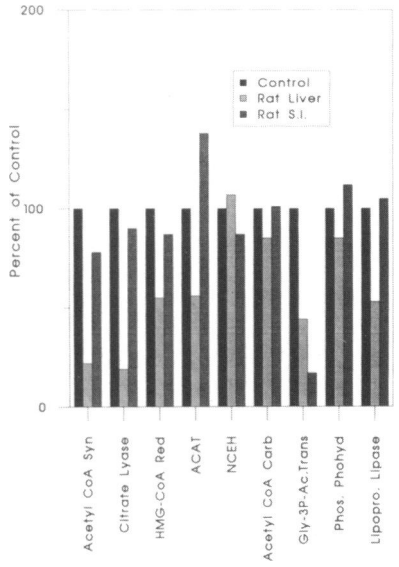


Figure 10. Rat enzyme activities *in vivo*. Effects of compound 5 at 8 mg/kg/day, orally on Sprague-Dawley rat liver *de novo* enzyme activites on day 14.

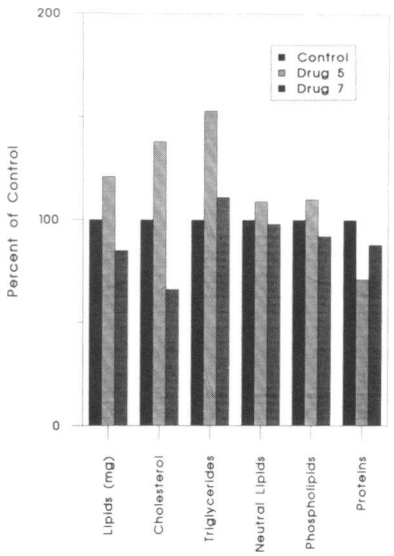


Figure 12. DNA strand scission. Effects of compounds 5 and 7 at 8 mg/kg/day, orally after 14 days on Sprague-Dawley rats. Aorta wall high density lipid concentrations.

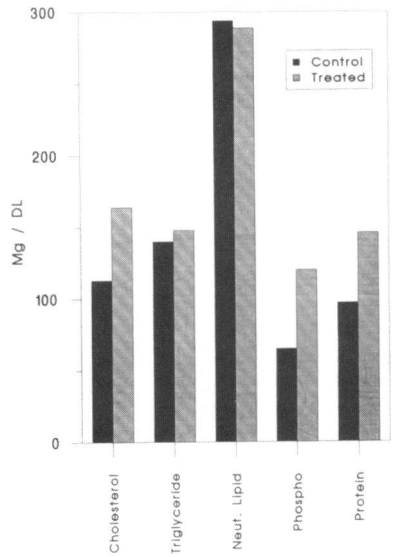


Figure 14. Bile excretion. Effects of compounds 5 and 7 at 8 mg/kg/day, orally after 14 days on Sprague-Dawley rats. Bile lipids.

Crossover in pharmacologic activity between antineoplastic, antibacterial, antiviral, immunomodulatory, and antiinflammatory activities is well documented for a number of chemically unrelated agents. Currently, evidence is accumulating to support the contention of a crossover between antineoplastic and hypolipidemic activity. For example, compactin, a hypocholesterolemic 3-hydroxy-3-methylglutaryl-Coenzyme A reductase (HMG-CoA reductase) inhibitor, inhibited DNA synthesis in L_{929} tumor cells (*18*). Similar types of crossover in activity between the 2,3-dihydrophthalazine-1,4-dione (*19*), sesquiterpene lactone (*20*), and benzohydroxamic acids (*21*) are known to occur. When the amine carboxyboranes were examined for hypolipidemic activity in CF_1 male mice, both serum cholesterol (18–48%) and triglyceride (19–77%) levels were reduced after 16 days at 20 mg/kg/day, ip (*22*) (Figure 8). When tested in Sprague-Dawley rats at 8 mg/kg/day orally for 14 days, both serum cholesterol (~35%) and triglyceride levels (32–58%) were reduced significantly (Figure 9). The reduction in lipids probably is the result of the amine carboxyboranes' inhibition of key enzyme activities in the *de novo* synthesis of cholesterol, i.e., HMG-CoA reductase, acetyl-CoA synthetase, fatty acids [i.e., citrate lyase, and triglycerides, such as *sn*-glycerol-3-phosphate acyltransferase and phosphatidylate phosphohydrolase (Figure 10)]. Rat tissue lipids, specifically cholesterol concentrations, were reduced in the liver

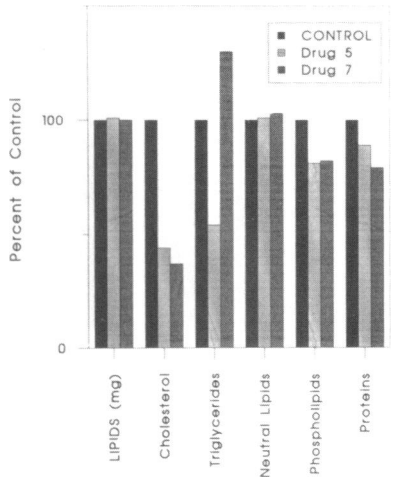


Figure 11. Rat liver lipids. Effects of compounds 5 and 7 at 8 mg/kg/day, orally after 14 days on Sprague-Dawley rats. Liver lipid concentrations.

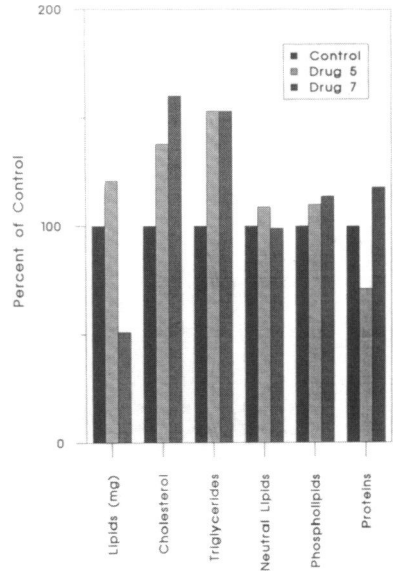


Figure 13. Rat feces lipids. Effects of compounds 5 and 7 at 8 mg/kg/day, orally after 14 days on Sprague-Dawley rats. Fecal lipid concentrations.

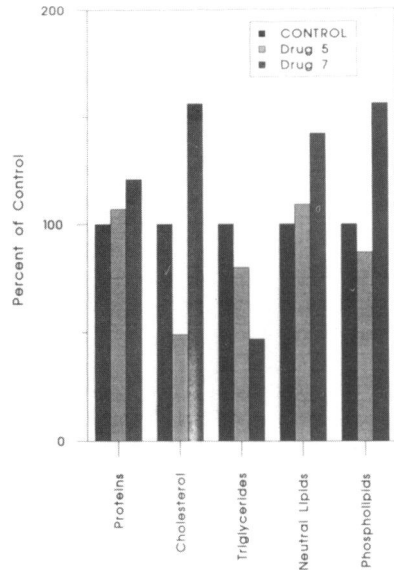


Figure 15. Chylomicron lipids. Effects of compounds 5 and 7 at 8 mg/kg/day, after 14 days orally on Sprague-Dawley rat. Chylomicron lipid concentrations.

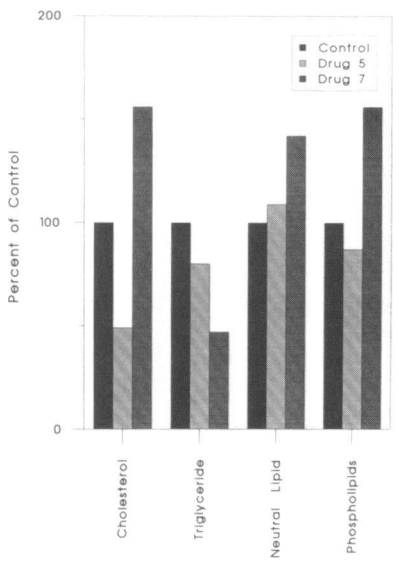


Figure 16. VLDL lipids. Effects of compounds 5 and 7 at mg/kg/day, after 14 days orally on Sprague-Dawley rat. VLDL concentrations.

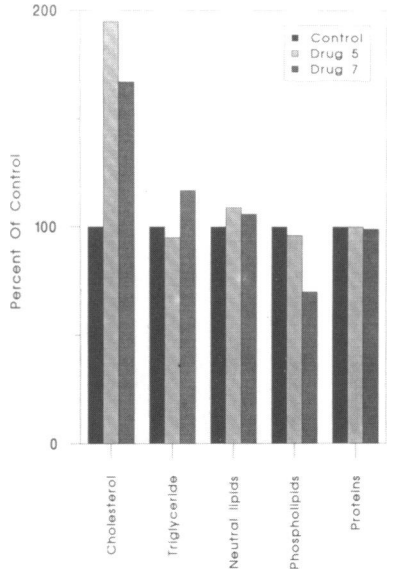


Figure 18. HDL lipids. Effects of compounds 5 and 7 at 8 mg/kg/day, after 14 days orally on Sprague-Dawley rat. HDL concentrations.

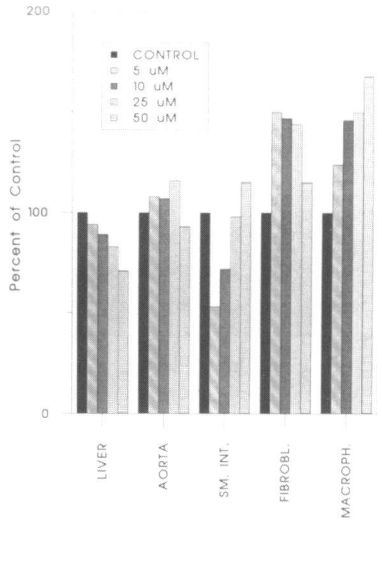


Figure 20. LDL degradation. Effects of compound 5 at 5, 10, 25, and 50 μM on activities in human liver and fibroblasts, rat aorta and small intestinal mucosa, and mouse macrophages after 5 hr. LDL degradation.

after 14 days administration (Figure 11) and in the aorta wall (Figure 12), but not in the small intestine (data not shown). On the other hand, lipids, particularly cholesterol and triglycerides, were increased in the feces (Figure 13) and bile (Figure 14). Further studies in rats showed that the serum lipoprotein lipid content was altered by drug treatment (Figures 15–18). Compound 5 was effective in lowering cholesterol content in the chylomicron, very low-density (VLDL) and low-density lipoprotein (LDL) fractions, while elevating the cholesterol content in the high-density lipoprotein (HDL) fraction. The magnitude of increase in HDL cholesterol was far superior to that of today's clinically used agents. Triglycerides were reduced in the chylomicron, VLDL, and LDL fractions.

The ideal clinical hypolipidemic agent should lower VLDL cholesterol content, since this is the means by which cholesterol is delivered to peripheral tissues, including the aorta plaques. In patients with atherosclerosis, the LDL cholesterol concentration is high and the HDL cholesterol oncentration is low. An effective hypolipidemic agent must reverse this ratio.

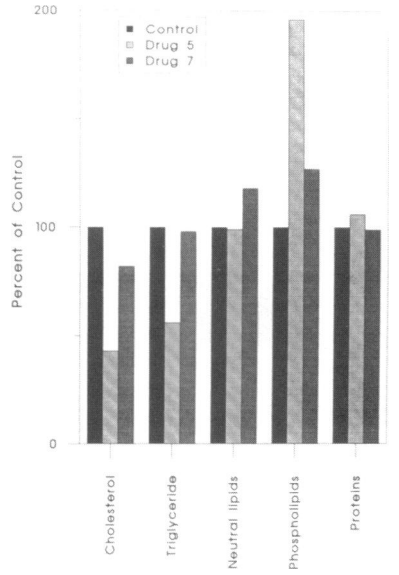
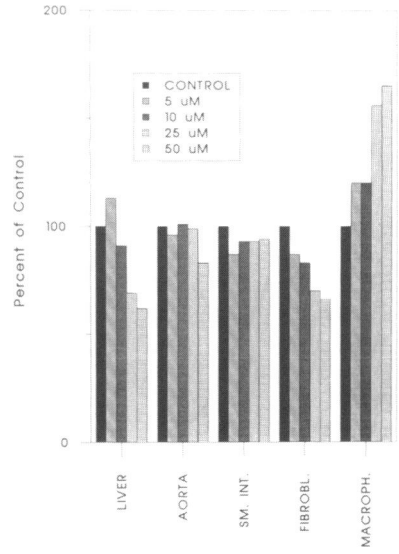


Figure 17. LDL lipids. Effects of compounds 5 and 7 at 8 mg/kg/day, after 14 days orally on Sprague-Dawley rat. LDL concentrations.



Figures 19. LDL receptor binding. Effects of compound 5 at 5, 10, 25, and 50 μM on activities in human liver and fibroblasts, rat aorta and small intestinal mucosa, and mouse macrophages after 5 hr. LDL receptor activity and internalization.

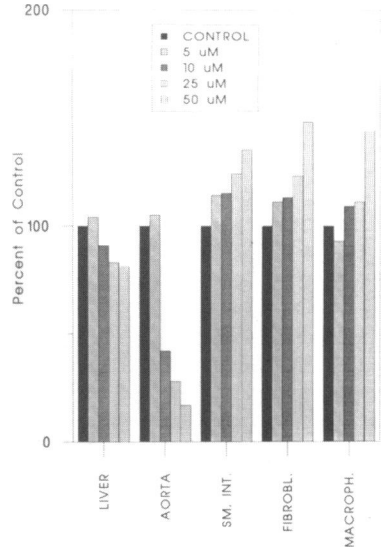


Figure 21. HMG-CoA reductase. Effects of compound 5 at 5, 10, 25, and 50 μM on activities in human liver and fibroblasts, rat aorta and small intestinal mucosa, and mouse macrophages after 5 hr. HMG-CoA activity.

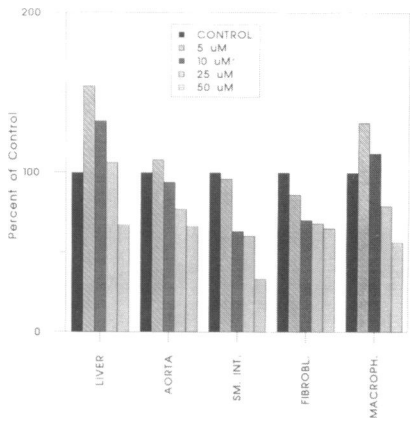


Figure 22. ACAT activity. Effects of compound 5 at 5, 10, 25, and 50 μM on activities in human liver and fibroblasts, rat aorta and small intestinal mucosa, and mouse macrophages after 5 hr. ACAT activity.

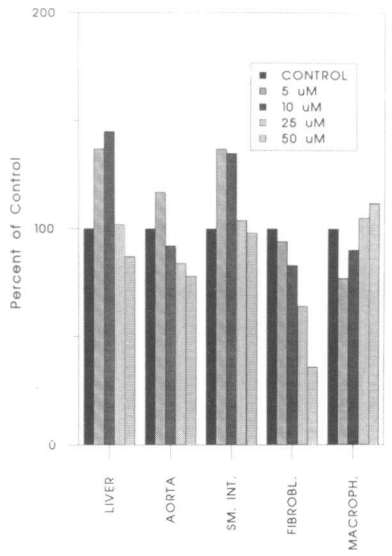


Figure 24. HDL degradation. Effects of compound 5 at 5, 10, 25, and 50 μM on activities in human liver and fibroblasts, rat aorta and small intestinal mucosa, and mouse macrophages after 5 hr. HDL degradation.

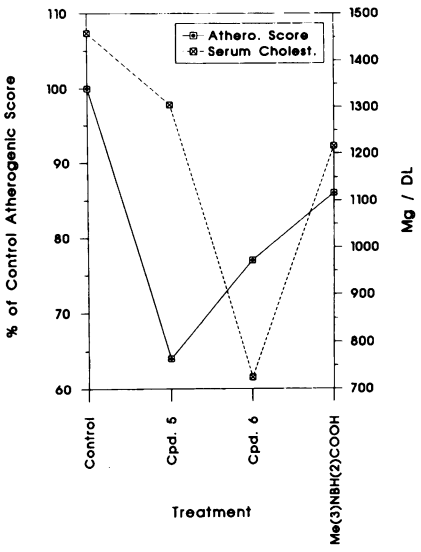


Figure 26. Effects of amine borane derivatives at 8 mg/kg/day, orally after 8 weeks on quail atherogenic scores and serum cholesterol concentrations.

Reversing the LDL/HDL cholesterol ratio has been shown to protect man from myocardial infarctions. The HDL fraction is responsible for the uptake of free cholesterol from peripheral cells (including aorta plaques) and conducting cholesterol to the liver for metabolism to cholic acid followed by excretion in the bile.

Because modulations of the serum lipoprotein fractions were favorable for treatment of atherosclerosis, we initiated studies to understand further the agents' effects on cellular lipid regulation (*23*). LDL receptors are located on peripheral cells which bind with high affinity to *apo*-B lipoproteins. LDL cholesterol complexes are taken up by the cell and merge with lysosomal vesicles from which hydrolytic enzymes are released. These enzymes digest the complex and release free cholesterol. These LDL receptors regulate the activities of HMG-CoA reductase, acyl-CoA cholesterol acyl transferase (ACAT), and cholesterol 7α hydrolase. We found the amine carboxyboranes caused a decrease in LDL receptor binding of ^{125}I-LDL and its internalization (Figure 19). Further, the degradation of the LDL-cholesterol complexes was reduced, whether the complex entered the cell through the high-affinity transport mechanism or a nonreceptor transport process (Figure 20). The net effect was that less free cholesterol was released in the cell. Macrophages, as scavenger mechanisms,

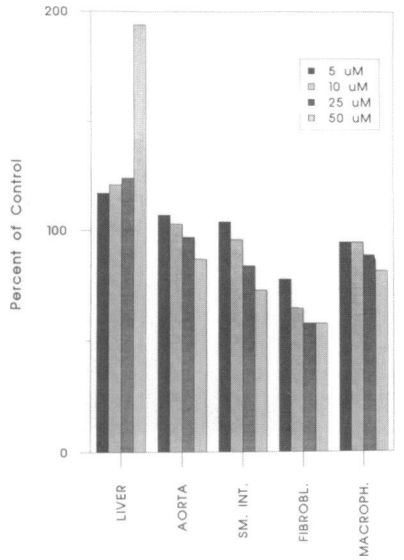


Figure 23. HDL receptor binding. Effects of compound 5 at 5, 10, 25, and 50 μM on activities in human liver and fibroblasts, rat aorta and small intestinal mucosa, and mouse macrophages after 5 hr. HDL receptor activity and internalization.

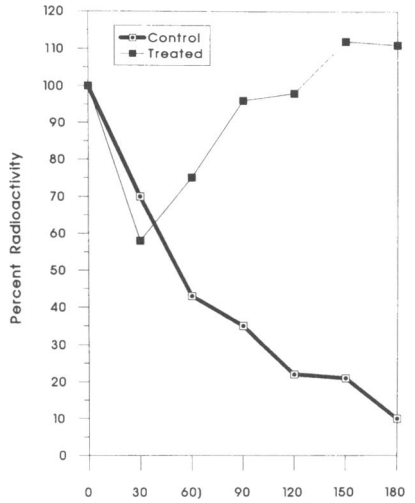


Figure 25. Effects of compound 5 on Sprague-Dawley rat *in situ* loop resorption of cholesterol.

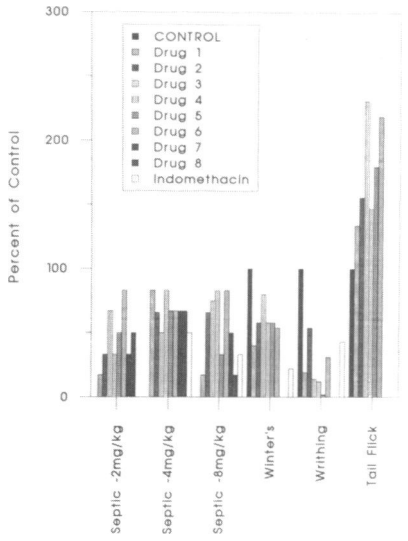


Figure 27. *In vivo* activity in mice. Effects of amine carboxyboranes at 8 mg/kg, ip on antiinflammatory tests. Septic shock, Winter's induced edema, writhing, and tail flick reflex.

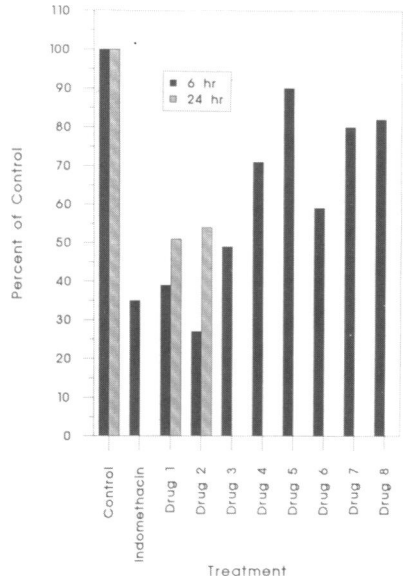


Figure 28. Mouse sponge evaluation: PMNs Flux (MPO). Effects of amine carboxyboranes at 8 mg/kg, ip, on antiinflammatory tests. PMN MPO activity flux into mouse sponges, SC at 6 and 24 hr.

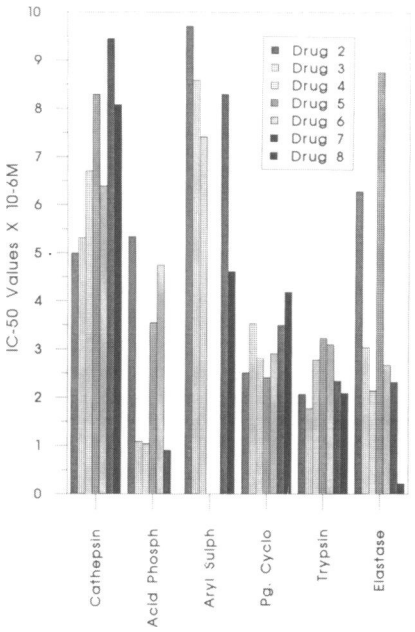


Figure 30. Macrophage enzyme activities. Fifty percent inhibition of enzyme activity (IC_{50}) values of amine carboxyboranes after 60 min. Effects on mouse macrophage cathepsin, acid phosphatase, aryl sulfatase, prostaglandin cyclooxygenase, typsin, and elastase activities.

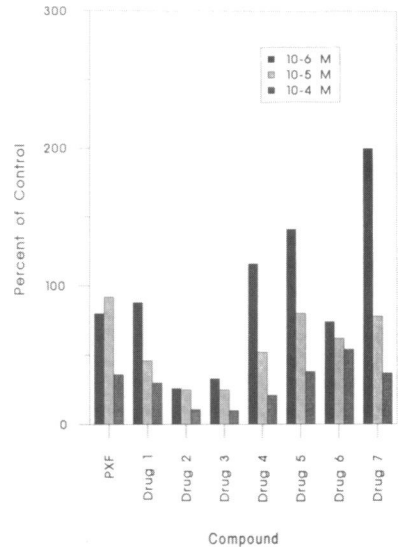


Figure 32. TNF-α release from LPS-induced macrophage s. Effects of amine carboxyboranes at several concentrations on cytokines. TNF-α levels from LPS-induced mouse IC-21 macrophages at 10^{-6}, 10^{-5}, and 10^{-4} M concentrations.

were responsible for the uptake of acetyl-LDL and β-VLDL to clear hyperlipidemic lipoproteins from the plasma and tissues. Tissue culture studies showed the agents accelerated the uptake and degradation of lipoproteins by the macrophages (Figure 19). Liver and aorta HMG-CoA reductase activity was inhibited by compound 5 in a concentration-dependent manner. However, HMG-CoA reductase activity in small intestinal mucosa, fibroblasts, and macrophages was not reduced (Figure 21). Inhibition of the activity of the ACAT enzyme (the enzyme that converts cholesterol to cholesterol esters, after treatment with amine-carboxyboranes [Figure 22]) should reduce plaque growth because deposition of cholesterol esters is related

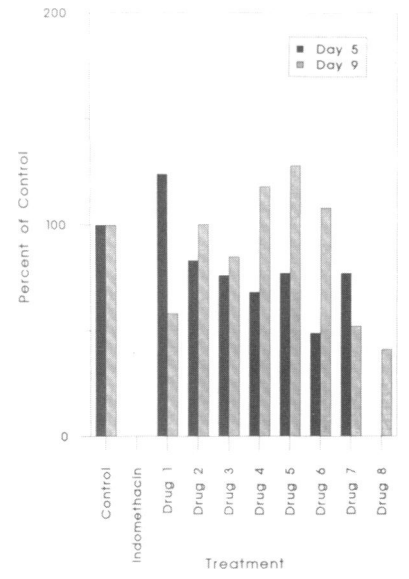


Figure 29. Mouse sponge evaluation: macrophage/monocyte flux (NAG). Effects of amine carboxyboranes at 8 mg/kg, ip, on antiinflammatory tests. Macrophage/monocytes (NAG) flux into mouse sponges, sc on days 5 and 9.

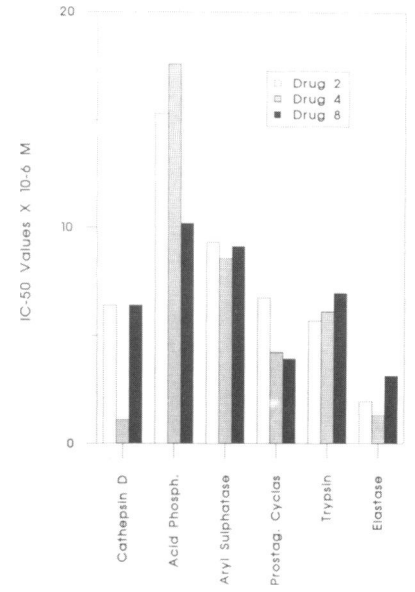


Figure 31. Be Sal osteoporotic cells enzyme activities. Fifty percent inhibition of enzyme activity (IC_{50}) values of amine carboxyboranes after 60 min. Be Sal human osteoporotic cells cathepsin, acid phosphatase, aryl sulphatase, prostaglandin cyclooxygenase, trypsin, and elastase activities.

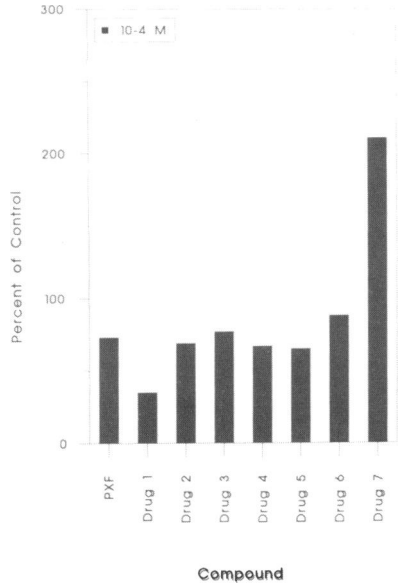


Figure 33. Il-1 Release from LPS-induced macrophages. Effects of amine carboxyboranes at several concentrations on cytokines. Il-1 release from LPS-induced IC-21 LPS-induced macrophages at 10^{-4} M.

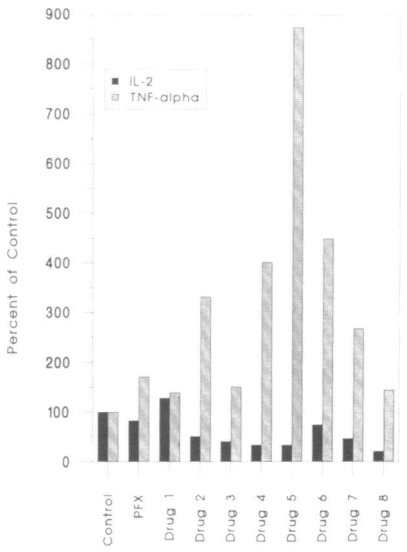


Figure 34. Il-2 and TNF-α in plasma from LPS-induced CF-1 mice. Effects of amine carboxyboranes at several concentrations on cytokines. Plasma tumor necrosis factor (TNF-α) and Il-2 levels 90 min after LPS-induction in CFI mice treated 2 hr prior to LPS with amine carboxyboranes at 8 mg/kg ip.

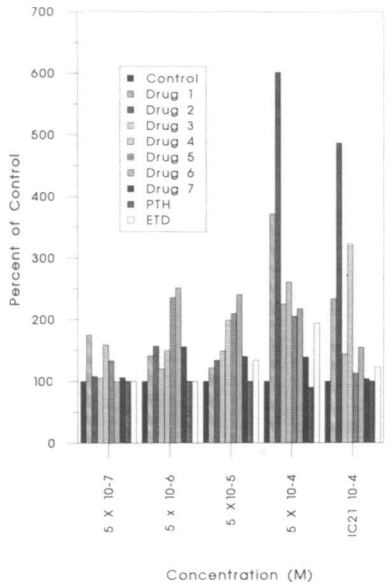


Figure 36. Calcium uptake by rat UMR-106 cells. ^{45}Calcium uptake of rat UMR-106 and IC-21 macrophage osteosarcoma cells from 5 x 10^{-7} to 5 x 10^{-4} M concentrations of amine carboxyboranes.

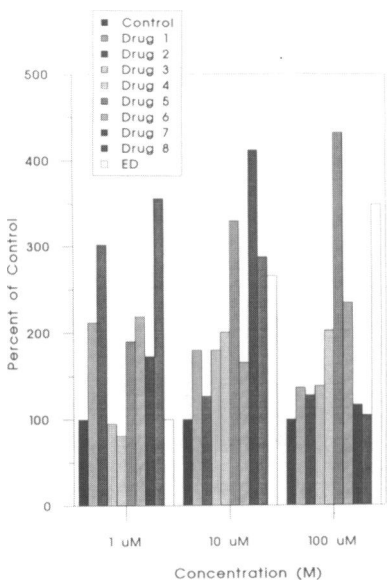


Figure 38. Proline incorporation by UMR-106 cells. ^{3}H-proline incorporation into collagen in presence of amine carboxyboranes at 1, 10, and 100 μM. Rat UMR-106 cells osteosarcoma.

directly to plaque size. This process increases with age of the individual and with hyperlipidemic disease states. Activation of neutral cholesterol ester hydrolase activity by the agents (data not shown), should increase the breakdown of cholesterol esters in fibroblasts and small intestinal mucosa cells. This would free cholesterol to attach itself to HDL for transport from the arterial plaque. Studies using human cultured liver cells demonstrated that the agents accelerated high-affinity binding of HDL via serum lipoprotein apoproteins Apo E and Apo AI (Figure 23), which would cause clearance of cholesterol from the serum compartment. However, HDL binding and degradation in other cells was decreased (Figure 24). Indeed, *in vivo* studies demonstrated fewer lipids in the rat

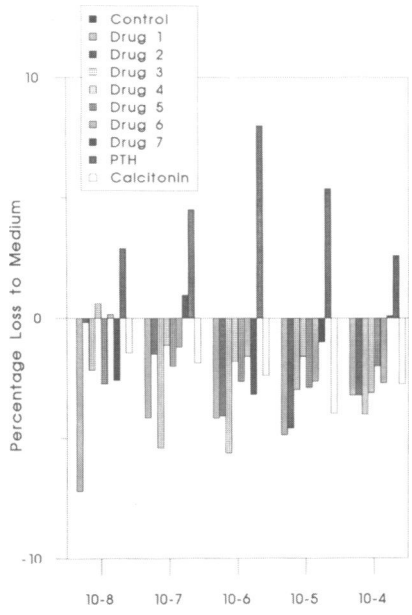


Figure 35. Bone calcium resorption in rat pup calvaria cultures. Bone ^{45}calcium resorption of mouse pup calvana bone at 10^{-8} to 10^{-4} M concentrations of amine carboxyboranes.

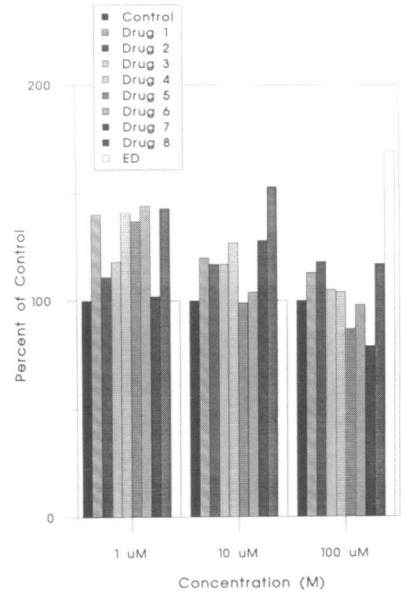


Figure 37. Proline incorporation: pup calvaria. ^{3}H-proline incorporation into collagen in presence of amine carboxyboranes at 1, 10, and 100 μM. Mouse CF1 pup calvaria bone.

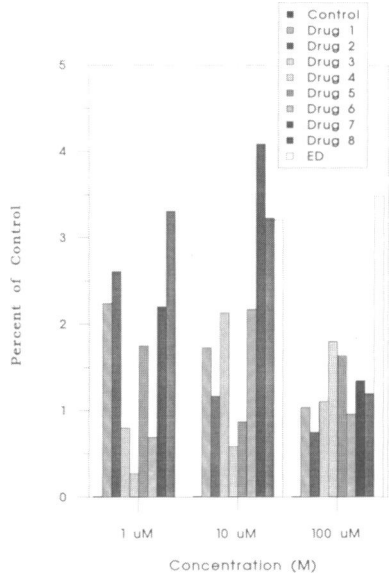


Figure 39. Percent increase in cellular collagen. ^{3}H-proline incorporation into collagen in presence of amine carboxyboranes at 1, 10, and 100 μM. Intracellular increase in ^{3}H-proline incorporation into collagen in rat UMR-106 cells.

aorta wall and more lipid excretion in the bile, suggesting that the drugs accelerated the HDL cholesterol reverse transport process.

The trimethylamine-carboxyborane derivative 5 accelerated cholesterol, triglyceride and neutral lipid excretion into the bile (Figure 14). Furthermore, the agent increased the bile flow by 48%. Individual bile acids demonstrated different concentrations after drug treatment; however, there was no evidence that the agent induced lithogenic effects like many hypolipidemic agents. *In situ* rat intestinal loop studies showed that agent 5 interfered with cholesterol absorption / reabsorption from the gut (Figure 25); however, it had no effect on cholic acid reabsorption (data not shown). This property of the agent would also account for the observed reduction of serum cholesterol over time.

In quail, treatment with amine-carboxyborane derivatives for 8 weeks reduced serum cholesterol concentrations and numbers of atherosclerotic lesions in the aorta (Figure 26). Some of the boron derivatives of this chemical class should be effective in reducing tissue lipids. These agents were effective in the treatment of genetic hyperlipidemic, normalogenic and hypolipidemic diseased mice, lowering lipid concentrations in an analogous manner, as indicated in the CF_1 mouse and rat studies. Further studies have indicated that the activities of the same lipid regulatory enzymes in miniature pig liver was inhibited by compound 5, which suggests that the compounds may be useful in agriculture in improving the quality of the meat products from domestic animals (*24*).

Another area where these compounds have demonstrated good pharmacologic activity is as antiinflammatory agents. These agents were particularly useful in the inhibition of induced edema, reduction of local pain associated with inflammation, and inhibition of centrally induced pain (*25*) (Figure 27). But more important, the agents protected against septic shock from lipopolysaccharides (LPS) better than any tested commercial agent. Selected agents were also demonstrated to be effective against chronic induced arthritis in rats at 2.5 mg/kg/day and active against pleurisy in rats. Subcutaneous implantation of sponges containing LPS (*26*) in mice showed that agents blocked PMN myeloperoxidase (MPO) activity's (Figure 28) and macrophage/monocyte N-acetylglucosaminidase (NAG) activity's (Figure 29) migration to the inflammation sites. These derivatives inhibited the activities of lysosomal enzymes from a number of tissues [e.g., PMNs, hepatocytes, and leukocytes, with the concentration necessary for 50% inhibition of enzyme activity (IC_{50} value) in the range of 10^{-6}M (Figures 30, 31)]. Although another characteristic of these agents is their potent inhibition of trypsin, elastase, and neutral cathepsin activities, in our hands they were less potent inhibitors of collagenase type I and II activities with IC_{50} values from 10^{-4} to 10^{-5}M (data not shown). The agents proved to be dual inhibitors of prostaglandin cyclo-oxygenase and 5′-lipoxygenase (*27,28*), with IC_{50} values in the range of 10^{-6}M. IC-21 macrophages incubated with the agents secreted less interleukin-1 (Il-1) and tumor necrosis factor-α (TNFα) cytokines (Figures 32,33). However, after treatment with the agents at 8 mg/kg in mice *in vivo* Il-2 concentrations were low in the blood and TN-α was high (Figure 34). This relationship between high amounts of TNF-α in bacterial or malarial infections and septic shock is not unique. The deleterious effects normally attributed to TNF-α (e.g., cachexia and endotoxic shock) may be incorrect. Rather, deleterious effects may be the result of other concurrently released cytokines, like Il-6 or Il-8, and not TNF-α.

Examination of the derivatives for their ability to block calcium resorption as antiosteoporotic agents was our next priority. We first demonstrated that inorganic calcium, phosphorus, and hydroxyproline were low in the urine but high in the blood after 21-day treatment at 8 mg/kg/day in mice. Four-day-old rat pup calvaria bone exchanged less calcium to the medium in the presence of drug from 10^{-8} to 10^{-4}M for 48 hr (*29*) (Figure 35). In rat UMR-106 cultured osteosarcoma cells, calcium resorption was blocked by the agent (*30*). These derivatives were more active than calcitonin and the bis-phosphate standard in blocking calcium resorption. Concurrent incorporation of calcium into the cell collagen was increased in rat UMR-106 cells, IC-21 macrophages, and Be Sal human osteoporosis cells in the presence of the agents (Figure 36). In addition, an increase in labeled collagen incorporation into cellular collagen was also observed in these cells as well as in the pup calvaria cultures (Figure 37) and UMR-106 cells (Figure 38). The exchange of proline to the medium over the next 48 hr was reduced significantly in the presence of drugs (Figure 39). In a lactating rat model in which rats were dosed orally for 14 days at 8 mg/kg/day, the amine boranes increased bone volume, weight, density, and ash weight, while elevating bone and serum calcium levels. In conclusion, the amine-carboxyborane derivatives demonstrated promise as therapeutic agents for a number of disease states.

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