measure the ability of various halogenated hydrocarbons to displace $[^{125}I]T_4$ from the high-affinity prealbumin binding site. The assay mixture contained 10 nM prealbumin, 0.4 nM $[^{125}I]T_4$ containing approximately 0.15 μCi, and varying concentrations of unlabeled compounds (10^{-5}–10^{-9} M) in 0.1 M imidazole acetate buffer containing 1 mM EDTA (pH 7.4) in a final volume of 0.5 mL. After incubation for 1 h at 25 °C, the mixture was then cooled to 0 °C, and protein bound $[^{125}I]T_4$ was isolated by gel filtration on Sephadex G-25 columns (bed volume of 2.0 mL) equilibrated with imidazole acetate buffer at 4 °C. A 0.4-mL aliquot of the incubation mixture was applied to the column. The protein fraction was eluted with an additional 1.2 mL of buffer. The following 1.6-mL fraction contained free, radioactive iodide. Free hormone binds tightly to the gel matrix[30] and does not elute in the volumes used. The remaining gel in the column was poured into a tube and the amount of ^{125}I was determined with a Packard Prias Auto Gamma Counter (60% counting efficiency).

The relative binding affinities of the various halogenated hydrocarbons for the prealbumin were obtained from the competitive binding assays shown in Figure 3.

The binding potency of each compound relative to that of T_4 (assigned a value of 100) was calculated as follows:

binding potency =

$$\frac{\text{concn of } T_4 \text{ at } 1/2 \text{ maximal of control}}{\text{concn of compd at } 1/2 \text{ maximal of control}} \times 100$$

The control value was obtained from incubation with $[^{125}I]T_4$ alone. The concentration of T_4 at half-maximal of control was 9.6×10^{-9} M at L-T_4 concentrations between 1.2×10^{-9} and 4.8×10^{-6} M. We determined the K_a of L-T_4 to be 5.8×10^8 M^{-1}, which is in agreement with the generally accepted range 10^7–10^8 M^{-1}. In this work, L-T_3 was found to have 5.3% the affinity of L-T_4, which compares favorably with the 9% affinity reported by others.[31]

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[³H]Batrachotoxinin A 20α-Benzoate Binding to Voltage-Sensitive Sodium Channels: A Rapid and Quantitative Assay for Local Anesthetic Activity in a Variety of Drugs

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[³H]Batrachotoxinin A benzoate ([³H]BTX-B) binds with high affinity to sites on voltage-dependent sodium channels in a vesicular preparation from guinea pig cerebral cortex. In this preparation, local anesthetics competitively antagonize the binding of [³H]BTX-B. The potencies of some 40 classical local anesthetics and a variety of catecholamine, histamine, serotonin, adenosine, GABA, glycine, acetylcholine, and calcium antagonists, tranquilizers, antidepressants, barbiturates, anticonvulsants, steroids, vasodilators, antiinflammatories, anticoagulants, analgesics, and other agents have been determined. An excellent correlation with the known local anesthetic activity of many of these agents indicate that antagonism of binding of [³H]BTX-B binding provides a rapid, quantitative, and facile method for the screening and investigation of local anesthetic activity.

The sodium channel of the plasma membrane of nerve or muscle cells functions as a voltage-sensitive gate for sodium ions.[1] Transitions between various resting, open, and inactivated states function to permit a specific increase in the permeability of the plasma membrane allowing influx of sodium ions followed by a return to a state which is relatively impermeable to sodium ions.[1] Local anesthetics modify the properties of the sodium channel resulting in a decrease in the flux of sodium ions. It has been proposed that local anesthetics bind to a site in the sodium channel complex, thereby promoting inactivation of the channel.[2] Local anesthetics appear to interact more rapidly with open conducting forms of the channel and to bind with higher affinity to inactivated forms of the channel.

Investigation of the nature of local anesthetic action has been aided by the discovery of neurotoxins which exhibit both specificity and high affinity for binding sites directly associated with the sodium channel. Radiolabeled derivatives of such toxins have provided molecular probes for at least three separate binding sites associated with the sodium channel. Two of these sites, the tetrodotoxin site and the scorpion toxin site, do not appear to be influenced by local anesthetics.[3,4] A third site, the batrachotoxin (BTX) binding site, is associated with the gating mechanism of the channel. The binding of BTX to the sodium channel prevents inactivation of the channel resulting in a massive influx of sodium ions and a persistent membrane depolarization.[5] Binding of a [³H]BTX analogue is inhibited by local anesthetics in a competitive or mutually exclusive manner.[6] Recent studies suggest that the binding of local anesthetics promotes an allosteric inhibition of binding of the [³H]BTX analogue, thus increasing the "off-rate" of BTX and shifting the sodium channel to an inactive form.[7] In the present report, [³H]batrachotoxinin A 20α-benzoate ([³H]BTX-B) has been utilized to

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compare the inhibition of BTX binding by local anesthetics and other compounds with local anesthetic activity. A preparation of resealed membrane vesicles from guinea pig cerebral cortex containing functional sodium channels[8] was used. Previous studies with such guinea pig vesicular preparations involved 14 local anesthetics and demonstrated a correlation between the ability of each compound to block the BTX-induced depolarization and to block the binding of [^{3}H]BTX-B.[6] Similar correlations between the potencies of 13 anesthetics as antagonists of [^{3}H]BTX-B binding in rat synaptosomes and electrophysiological potencies were recently reported by Postma and Catterall.[7] Some 40 classical local anesthetics and a variety of pharmacologically active compounds from other categories have now been assayed as inhibitors of [^{3}H]BTX-B binding. The inhibition constants are in general agreement with the local anesthetic properties of many of these drugs and the technique appears to provide a facile and quantitative screening assay for local anesthetic activity of new drugs and research agents.

Results

Classical Local Anesthetics. The effects of a large number of compounds known or presumed to have local anesthetic activity or "membrane effects" have been tested for their ability to displace [^{3}H]BTX-B from its binding site. Over half of some 40 classical local anesthetics exhibited at least 50% inhibition of [^{3}H]BTX-B binding at 10 μM. Nearly all of the anesthetics tested produced at least 50% inhibition of [^{3}H]BTX-B binding at 100 μM. The local anesthetics tested thus exhibited a wide range of activity with a 1230-fold difference between the most active compound euprocin (IC_{50} = 0.74 μM) and the least active compound, benzocaine (IC_{50} = 910 μM). Quinidine, quinine, quinacrine, and diphenhydramine were included in Table I under local anesthetics because of their well-known activity as "membrane stabilizers".[9] The 11 most potent of the classical local anesthetics were all compounds which have been used as topically applied anesthetics. The stereoselective local anesthetics RAC 109I and RAC 109II exhibited about an 8-fold difference in potencies vs. [^{3}H]BTX-B binding (Table I), consonant with their electrophysiological selectivity[10] and with recent data vs. [^{3}H]BTX-B binding in a rat synaptosome preparation.[7]

Adrenergic Antagonists. α_1-Adrenergic blockers, such as WB 4104 and prazocin, α_2-adrenergic blockers, such as yohimbine, corynanthine, and rauwolscine, and nonspecific α-blockers such as phentolamine and phenoxybenzamine were more potent than procaine in inhibiting binding of [^{3}H]BTX-B (Table I). A number of the β-adrenergic blockers including the β_2-adrenergic antagonist IPS 339, both *d*- and *l*-propranolol, and dichlorisoproterenol were potent blockers vs. binding of [^{3}H]BTX-B binding (Table I). Others such as sotalol and practolol were inactive.

Histaminergic Antagonists. A variety of histaminergic antagonists were assayed vs. binding of [^{3}H]-BTX-B and most of the H_1 blockers were at least 10-fold more potent than procaine in antagonizing binding of [^{3}H]BTX-B. The most potent was cinnarizine (IC_{50} = 0.44 μM), an antihistamine entered in Table I as a calcium antagonist. Cyproheptadine gave an IC_{50} of 1 μM, making it more active than all but one of the local anesthetics. The H_2-specific blockers burimamide and cimetidine had low and very low activity, respectively.

Serotonin Antagonists. Cyproheptadine entered in Table I as a histamine antagonist is also a serotonin antagonist and was very potent vs. [^{3}H]BTX-B binding as was mianserin. The selective serotonin antagonist methysergide had relatively low activity.

Other Receptor Antagonists. Adenosine analogues and adenosine receptor antagonists such as theophylline, caffeine, and 8-phenyltheophylline had very low activity. The GABA antagonists bicuculline and picrotoxin were virtually inactive as was the $GABA_B$ receptor agonist baclofen. The glycine antagonist strychnine was relatively active vs. [^{3}H]BTX-B binding with an IC_{50} value of 21 μM. Cholinergic antagonists, such as dicyclomine, biperiden, and ditran, were relatively potent vs. [^{3}H]BTX-B binding with IC_{50} values less than 10 μM, while more classical nicotinic and muscarinic antagonists such as *d*-tubucurarine, scopolamine, and atropine were virtually inactive.

Calcium and Potassium Antagonists. The so-called "calcium antagonists" (see ref 11 for a review and references) included some of the most potent antagonists of [^{3}H]BTX-B binding. Prenylamine, fluperamide, and loperamide were indeed the most potent of the over 150 compounds which were tested with IC_{50} values of about 0.3 μM. Cinnarizine and flunarizine were almost as potent with IC_{50}'s of 0.44 and 0.6 μM, respectively. Verapamil, a widely used calcium antagonist, and its methoxy derivative D-600 showed similar potencies with IC_{50}'s of 3.3 and 3.6 μM. The two so-called "specific" calmodulin inhibitors W7 and W5[12] were relatively active versus [^{3}H]BTX-B binding with IC_{50}'s of 12.0 and 22.5 μM, respectively. Another potent calmodulin antagonist, calmidazolium,[13] was also relatively active vs. [^{3}H]BTX-B binding with an IC_{50} value of 7 μM. Diltiazem, structurally different from the above calcium antagonists, was less inactive. Nifedipine and a number of closely related dihydropyridines were much less potent and would have no effect on BTX-B binding at the concentrations where they block calcium channels. 2-*n*-Butyl-3-(dimethylamino)-5,6-(methylenedioxy)indene, a purported antagonist of internal calcium sites,[11] was quite potent vs. binding of [^{3}H]BTX-B with an IC_{50} value of 5.4 μM. Diazoxide and TMB-8, other purported blockers of internal sites of action or release of calcium,[11] had very low and virtually no activity, respectively. Ryanodine, a potent alkaloid purported to block release of internal calcium[14] had no effect on [^{3}H]BTX-B binding at 10 μM. The two potassium channel blockers 4-aminopyridine and tetraethylammonium chloride were inactive.

Tranquilizers. The various tranquilizers tested included many compounds which are dopamine antagonists, namely, the phenothiazines, thioxanthines, butyrophenones, and butaclamols: All of these dopamine antagonists were potent inhibitors of [^{3}H]BTX-B binding (Table I). The benzodiazepine class of tranquilizers, including diazepam and chlordiazepoxide, were relatively inactive in this assay system. Both diphenylmethane tranquilizers azacyclanol and hydroxyzine were relatively potent. Trazodone was relatively inactive. Reserpine was

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one of the most potent antagonists of BTX-B binding with an IC_{50} of 1.5 μM.

Antidepressants. The tricyclic antidepressants such as desipramine and imipramine were potent inhibitors of [^{3}H]BTX-B binding. Amitriptyline, protriptyline, and nortriptyline, which are also closely related in structure, gave inhibitions at 10 μM of about 70%. Norzimelidine and zimelidine were somewhat less active.

Analgesics. Morphine was inactive and codeine showed low activity at 10 μM. Levorphanol and dextrophan showed similar potencies with IC_{50} values of 25 and 18 μM, respectively.

Miscellaneous Compounds. Barbiturates had very low activity. Other anticonvulsants such as diazepam (vide supra), diphenylhydantoin, and carbamazepine also had low or no activity at 10 μM. Steroids exhibited a range of activity from virtually none for cholesterol to a 21% inhibition at 10 μM for testosterone. The synthetic estrogen diethylstilbestrol caused 30% inhibition at 10 μM. Two vasodilators, dilazep (IC_{50} = 0.6 μM) and hexobendine (IC_{50} = 1.5 μM), were extremely potent, while two other vasodilators, dipyridamole and papavarine, showed much lower activity. Two nonsteroidal antiinflammatories were tested: Flufenamic acid caused 27% inhibition at 10 μM, while indomethacin was virtually inactive at 10 μM. The anticoagulant anisindione was inactive.

Agents Affecting Cyclic AMP Generation. Forskolin, a general activator of adenylate cyclase,[15] was moderately active with an IC_{50} of 60 μM. This is however at least 10-fold higher than concentrations required for half-maximal activation of adenylate cyclase in intact cells. 2′,5′-Dideoxyadenosine, a general inhibitor of the enzyme,[16] was inactive. RMI 12330A, a purported selective inhibitor of adenylate cyclase,[17] was one of the most active compounds tested with an IC_{50} of 0.8 μM vs. [^{3}H]BTX-B binding. All of the various phosphodiesterase inhibitors[18-20] were relatively inactive in inhibiting the binding of [^{3}H]BTX-B.

Discussion

The results presented in this study demonstrate the utility of using [^{3}H]BTX-B as a direct molecular probe for the sodium channel to determine the local anesthetic potency of compounds. The relative simplicity and sensitivity of the method readily lends itself to the evaluation of structure–activity of compounds designed as local anesthetics. The method is equally useful as a screen for local anesthetic activity of compounds in which local anesthetic activity may be an undesirable ancillary property.

Local anesthetic activity by the present method is defined as the ability of compounds to inhibit the specific binding of [^{3}H]BTX-B under conditions in which its apparent affinity has been enhanced by the presence of scorpion venom.[21] In the absence of scorpion venom, specific binding of [^{3}H]BTX-B is much more difficult to measure.[22] An essential component of the method is the use of vesicular entities which maintain a sodium-channel-dependent transmembrane potential. The vesicular preparation used here maintains a transmembrane potential of approximately 70 mV, which is linearly related through the Nernst equation to the log of the external potassium ion concentration.[8] The depolarization induced by the sodium-channel-specific neurotoxins, BTX, veratridine, grayanotoxin I, and aconitine in this vesicular preparation[8] exhibits the same rank and potency as their ability to increase the initial rates of $^{22}Na^+$ uptake by neuroblastoma cells.[23] Thus this easily prepared vesicular preparation provides an adequate model for functional sodium channels in intact nerve. Synaptosomes from rat brain[21,24] and with a crude vesicular preparation from whole mouse brain[6,22] have also been used to investigate binding of [^{3}H]BTX-B. In the present procedure the membrane potential is maintained by replacing sodium ions with choline and including 1 μM tetrodotoxin in the external media. Tetrodotoxin effectively blocks the influx of sodium ions and thus BTX-induced depolarization but has virtually no effect on the binding of [^{3}H]BTX-B.

The binding of [^{3}H]BTX-B, in the guinea pig vesicular preparation, is consonant with a single, saturable, noninteracting binding site with a K_d of 35 ± 5 nM (SEM) with a maximum number of sites of 1.5 ± 0.1 pmol/mg of protein.[6] Scatchard analysis of the effect of the local anesthetics dibucaine, QX-572, bupivacaine, and tetracaine indicate that the inhibition of [^{3}H]BTX-B binding by local anesthetics is competitive in nature.[6,7] The anticonvulsant drugs diphenylhydantoin and carbamazepine also inhibit binding of [^{3}H]BTX-B in a competitive manner.[24] Studies of the effect of local anesthetics on the influx of $^{22}Na^+$ into neuroblastoma cells following sodium channel activation by BTX have shown that benzocaine, yohimbine, lidocaine, procaineamide, diphenylhydantoin, and carbamazepine inhibit BTX action competitively.[4,25-27] Khodorov and co-workers have also provided extensive data on the inhibition of batrachotoxin-activated sodium channels in frog node of Ranvier by local anesthetics (see ref 28 and references therein). In guinea pig brain vesicular preparations, dibucaine, tetracaine, QX-572, diphenhydramine, piperocaine, and cocaine all competitively inhibited BTX-induced depolarization in the same order of potency as their inhibition of [^{3}H]BTX-B binding.[6] For these six local anesthetics, there was an excellent correlation between blockade of binding and inhibition of BTX-elicited depolarization. Lidocaine ethiodide was an exception, being much more potent vs. binding than vs. BTX-elicited depolarization. The inhibition constants for 12 local anesthetics (tetracaine, etidocaine, propranolol, prilocaine, mepivacaine, procaine, tocainide, lidocaine, W36017, benzocaine) vs. [^{3}H]BTX-B binding to rat synaptosomes showed an excellent linear correlation[7] with electrophysical data on tonic blockade of nerve conduction.[29]

Recent studies suggest that local anesthetics increase the rate of dissociation of [^{3}H]BTX-B from its binding site by an allosteric competitive inhibition mechanism.[7] This mechanism is compatible with the observed competitive inhibition of [^{3}H]BTX-B binding by local anesthetics.

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Table I. Inhibition of Binding of [^{3}H]BTX-B to Brain Vesicular Preparations by a Variety of Local Anesthetics and Other Drugs

compound[a]	IC_{50}, μM	% inhibition at 10 μM	% inhibition at 100 μM	source[b]
Local Anesthetics				
euprocin	0.74	95.7 ± 0.4		G′
diperodon	1.3	87.8 ± 1.1		M′
dibucaine	1.4	77.4 ± 1.9		J
phenacaine	1.7	81.1 ± 0.7		A
proparacaine	1.9	79.9 ± 0.3		K′
cyclomethycaine	2.0	81.9 ± 0.7		R
oxethazaine	2.3	83.9 ± 0.6		Q′
butacaine	2.7	75.3 ± 0.8		M′
benoxinate	2.9	73.4 ± 1.5		M′
quinidine	3.2	74.1 ± 1.3		Z
quinacrine	3.3	73.3 ± 0.1		I′
dimethisoquin	3.4	73.4 ± 0.2		J′
tetracaine	3.4	72.0 ± 1.2		I′
etidocaine	3.5	73.2 ± 0.4		C
QX 572[a]	3.9	71.6 ± 1.2		R′
proadifen	4.2	75.4 ± 0.2		J′
meproadifen iodide	4.2	72.0 ± 0.7		R′
quinine	4.4	69.3 ± 1.2		I′
bupivacaine methiodide	4.8	64.5 ± 2.1		S′
bupivacaine	5.4	63.6 ± 2.4		L′
diphenhydramine	6.0	59.8 ± 1.7		I′
hexylcaine	7.0	54.9 ± 1.4		V
meprylcaine	7.6	57.6 ± 0.9		M′
RAC 109I[a]	12.0	54.1 ± 1.5		C
piperocaine	13.0	43.6 ± 2.6		R
zolamine	19.2	35.9 ± 0.9	82.4 ± 0.5	G′
pramoxine	21.2	29.7 ± 1.9	86.3 ± 1.6	A
parethoxycaine	24.0	33.2 ± 1.1		K′
propoxycaine	24.0	30.0 ± 1.4		M′
cocaine	49.0	19.5 ± 3.1	63.2 ± 0.8	V
carticaine	51.0	17.1 ± 0.8	62.2 ± 0.8	L
prilocaine	54.0	14.2 ± 0.3	58.5 ± 1.4	C
butethamine	55.0	12.3 ± 0.6	63.2 ± 0.3	M′
diphenhydramine methiodide	64.0		69.0 ± 2.6	S′
procaine amide azide	65.0	13.4 ± 2.7	56.1 ± 0.7	R′
RAC 109II[a]	94.0	25.7 ± 2.0		C
lidocaine ethochloride (QX314)	97.0		52.4 ± 1.3	R′
procaine	110.0		47.3 ± 0.9	R′
butamben		7.0 ± 3.5	36.8 ± 0.4	A
lidocaine	240.0	9.8 ± 1.1	31.4 ± 4.7	R′
piperocaine methiodide	360.0	9.6 ± 3.0	31.4 ± 1.0	S′
mepivicaine		5.3 ± 0.8	29.3 ± 1.5	L′
procaine amide		7.8 ± 1.0	22.5 ± 2.0	K′
benzocaine	910.0	5.1 ± 3.7	0.0 ± 0.5	R′
Adrenergic Antagonists				
WB 4104[a]	2.4	67.7 ± 2.3		R′
phentolamine	8.4	54.6 ± 1.8		J
prazosin	10.1	58.6 ± 2.4		A′
corynanthine		33.7 ± 1.6		B
yohimbine	33.0	25.9 ± 4.6		I′
phenoxybenzamine	45.0	16.4 ± 1.5		J′
rauwolscine		13.1 ± 1.5		E′
piperoxan	61.0		58.8 ± 0.5	R′
β-Adrenergic Antagonists				
IPS 339[a]	3.7	72.5 ± 0.5		J
ICI 118551[a]	<10	64.2 ± 2.2		N
dichloroisoproterenol	<10	53.0 ± 6.0		B′
d-propranolol	10.0	50.0 ± 0.6		D
l-propranolol	13.0	42.6 ± 1.3		D
bunolol		27.3 ± 3.1		O′
butoxamine	27.0	26.8 ± 2.1		H
metoprolol	117.0	14.0 ± 1.7	47.3 ± 2.9	J
practolol		8.4 ± 1.7		D
timolol		8.0 ± 1.5		M′
d-(nitrophenyl)-2-(isopropylamino)ethanol		7.9 ± 1.1		H′
sotalol			4.6 ± 1.1	B′
Histaminergic Antagonists				
cyproheptadine	1.0	83.0 ± 0.3		I′
diphenidol	3.4	65.1 ± 2.7		J′
promethazine	6.9	61.0 ± 1.2		Q′
chlorpheniramine	10.0	49.7 ± 3.6		S
d-brompheniramine	11.0	46.0 ± 1.5		R′
pyrilamine		44.5 ± 1.2		O
l-brompheniramine	16.0	44.0 ± 2.6		R′
pheniramine	33.0	24.9 ± 4.3		S
burimamide		12.9 ± 2.6		J′
cimetidine		0.0 ± 0.3	4.1 ± 0.6	J′
Serotonergic Antagonists				
methysergide			17.8 ± 0.5	F′
mianserin	5.5	57.4 ± 2.0		R′
Adenosine Agonists and Antagonists				
8-phenyltheophylline		10.9 ± 1.6		I
caffeine	660	9.7 ± 2.8		I
theophylline		6.9 ± 1.4	7.0 ± 1.3	I
N^6-(*R*)-1-Phenyl-2-isopropyladenosine		2.2 ± 0.9		F
2-chloroadenosine		1.3 ± 0.6	0.9 ± 3.5	I
8-*p*-sulfophenyltheophylline		0.0 ± 1.6	2.9 ± 2.6	C′
GABA and Glycine Agonists and Antagonists				
strychnine	21.0	35.7 ± 2.4		I′
picrotoxin	700	22.7 ± 9.7		I′
d-baclofen		11.7 ± 1.2		R′
l-baclofen		6.2 ± 4.1		R′
bicuculline			0.0 ± 1.8	I′
Cholinergic Antagonists				
dicyclomine	2.8	75.0 ± 1.7		M′
biperiden	<10	62.9 ± 0.4		M′
ditran[a]	<10	53.0 ± 1.0		Q
scopolamine		3.1 ± 0.3	4.3 ± 1.4	I′
d-tubocurarine		0.8 ± 3.0		I
atropine		0.0 ± 4.5		I′
"Calcium Antagonists"				
loperamide	0.27	100 ± 0		R′
fluperamide	0.28	100 ± 0		R′
prenylamine	0.30	98.8 ± 0.4		L
cinnarazine	0.44	94.6 ± 0.5		R′
flunarazine	0.6	96.7 ± 0.8		R′
bepredil	0.84	96.2 ± 2.0		R′
verapamil	3.3	74.4 ± 1.3		P
methoxyverapamil (D600)	3.6	67.1 ± 1.8		P
calmidazolium	7.0	68.3 ± 2.7		F
W7[a]	12.0	46.8 ± 1.4		D′
2-*n*-butyl-3-(dimethylamino)-5,6-(methylenedioxy)indene	12.5	45.0 ± 2.6		T′
tiapamil	13.5			R′
W5[a]	22.0	33.3 ± 0.9		D′
nisoldipine	26.0	25.9 ± 1.4		X
nitrendipine	36.0	23.1 ± 2.0		X
nifedipine	37.0	26.0 ± 2.6		K
felodipine	37.0	20.1 ± 2.0		R′
diltiazem	45.0	36.2 ± 2.0		T
diazoxide		12.8 ± 3.7		R′
TMB-8[a]		3.4 ± 2.1	21.6 ± 7.8	R′
ryanodine		0		R′
Potassium Channel Blockers				
4-aminopyridine		0.5 ± 1.2	3.8 ± 1.9	B
tetraethylammonium chloride		0.0 ± 1.4		B
Tranquilizers				
phenothiazines				
fluphenazine	3.7	72.8 ± 1.0		K′
chlorpromazine	4.3	72.2 ± 1.0		R′
trifluoperazine	5.0	79.8 ± 0.6		J′
promazine	5.4	65.3 ± 2.3		J′
thioridazine	6.5	63.5 ± 2.0		J′
chlorpromazine sulfoxide		18.0 ± 2.4	69.1 ± 0.5	R′

Table I (Continued)

compound[a]	IC_{50}, μM	% inhibition at 10 μM	% inhibition at 100 μM	source[b]
thioxanthines				
chlorprothixene	<10	55.8 ± 3.8		M
cis-thiothixene		42.3 ± 1.6		A′
butyrophenones				
spiroperidol	0.6	98.7 ± 3.2		R′
droperidol	0.74	91.0 ± 4.3		U
haloperidol	1.2	85.3 ± 5.3		R′
benzodiazepines				
diazepam		15.2 ± 2.1	45.4 ± 5.5	M
chlordiazepoxide		4.4 ± 2.7	35.0 ± 1.7	M
Ro 15-1788[a] (antagonist)		6.9 ± 3.3	7.7 ± 1.3	M
diphenylmethanes				
hydroxyzine	<10	73.9 ± 0.7		M′
azacyclonol	14.0	43.2 ± 4.8		W
others				
reserpine	1.6	100 ± 0		B
l-butaclamol	2.0	87.1 ± 0.0		R′
d-butaclamol	3.6	77.2 ± 1.1		R′
trazodone		26.2 ± 1.1	79.6 ± 4.0	G
tetrabenazine	85	7.0 ± 3.0	55.0 ± 3.0	M
buspirone			0.0 ± 2.0	R′
Tricyclic Antidepressants				
desipramine	2.4	80.3 ± 2.2		N′
chlorimipramine	<10	76.6 ± 2.9		R′
amitriptyline	<10	73.8 ± 2.3		V
imipramine	3.6	72.7 ± 1.6		R′
protriptyline	<10	70.3 ± 1.0		V
nortriptyline	<10	69.4 ± 0.7		R
doxepin	<10	67.7 ± 0.4		A′
norzimelidine	10	48.2 ± 0.6		C
iprindol		42.4 ± 3.3		Q′
zimelidine		34.6 ± 0.4		C
Barbiturates				
phenobarbital		5.4 ± 1.2		P′
secobarbital		1.3 ± 4.4		R
pentobarbital		0.8 ± 1.2	7.6 ± 0.9	A
Anticonvulsants				
diphenylhydantoin		18.5 ± 1.9		Y
carbamazepine		1.6 ± 2.1		J
"Steroids"				
diethylstilbestrol		30.1 ± 4.0		I′
testosterone		21.7 ± 2.0		I′
β-estradiol		17.8 ± 1.6	53.3 ± 0.8	I′
cholesterol		3.3 ± 1.2		B
Vasodilators				
dilazep	0.6	99.3 ± 0.2		C
hexobendine	1.4	77.2 ± 2.6		W
dipyridamole		18.4 ± 1.9		E
papaverine		11.0 ± 0.6	56.0 ± 0.2	Z
Nonsteroidal Antiinflammatories				
flufenamic acid		26.7 ± 1.7		B
indomethacin		3.3 ± 1.0	11.2 ± 4.1	I′
Anticoagulants				
anisindione		1.3 ± 0.9	0.0 ± 3.0	M′
Analgesics				
levorphanol	25	33.0 ± 2.6		R′
dextrophan	18	38.2 ± 4.1		R′
codeine		5.7 ± 1.5		R′
morphine			5.3 ± 1.8	R′
Adenylate Cyclase Activators and Inhibitors				
forskolin	60		62.8 ± 2.1	I
RMI 12330A[a]	0.8		100 ± 1.0	W
2′,5′-dideoxyadenosine			0.0 ± 3.5	N
Phosphodiesterase Inhibitors				
HL 725[a]		17.4 ± 3.3		L
Ro 20-1724[a]		14.1 ± 2.2		M
etazolate (SQ 20009)		8.3 ± 3.3	20.1 ± 1.5	K′
rolipram (ZK 62771)		0.0 ± 3.1	8.5 ± 1.6	G′
3-isobutyl-1-methylxanthine		0.0 ± 1.8	0.0 ± 0.9	B

[a] Definitions of compounds designated by code numbers are as follows: QX 572, dimethylbis(phenylcarbamylmethyl)ammonium chloride; RAC 109I and 109II are the two enantiomers of *N*-[3-(diethylamino)propyl]-1,2,3,4-tetrahydronaphthalene-1-spirosuccinimide; WB 4101, (2,6-dimethoxyphenoxyethyl)-2-(aminomethyl)-1,4-benzodioxane, IPS 339, [(*tert*-butylamino)-3-hydroxy-2-propyloximino]-9-fluorene hydrochloride; ICI 118551, 2-(isopropylamino)-4-(4-indanoxy)-3-butanol; Ditran, *N*-ethyl-3-piperidyl phenylcyclopentylglycolate; W5, *N*-(6-aminohexyl)-1-naphthalenesulfonamide hydrochloride; W7, *N*-(6-aminohexyl)-5-chloro-1-naphthalenesulfonamide hydrochloride; TMB-8, (diethylamino)octyl 3,4,5-trimethylbenzoate; RMI 12330A, *N*-*cis*-2-phenylcyclopentylazacyclotridecan-2-imine hydrochloride; HL 725, 9,10-dimethoxy-2-(mesitylimino)-3-methyl-3,4,6,7-tetrahydro-2*H*-pyrimido[6,1-*a*]isoquinolin-4-one hydrochloride; RO 20-1724, 4-[3-(*n*-butyloxy)-4-methoxybenzyl]-2-imidazolidone. [b] Sources: A, Abbott Laboratories, N. Chicago, IL; B, Aldrich Chemical Co., Milwaukee, WI; C, Astra Pharmaceutical Products, Inc., Worcester, MA and Sodertalje, Sweden; D, Ayerst Labs, New York, NY; E, Boehringer Ingelheim Ltd., Ingelhein am Rhein, West Germany; F, Boehringer, Mannheim, Mannheim, West Germany; G, Bristol Myers Co., New York, NY; H, Burroughs Wellcome Co., Research Triangle Park, NC; I, Calbiochem Corp., San Diego, CA; J, Ciba-Geigy Corp., Ardsley, NY; K, Delbay Pharmaceuticals Inc., Bloomfield, NJ; L, Hoechst AG, Frankfurt am Main, West Germany and Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ; M, Hoffman-La Roche Inc., Nutley, NJ; N, Imperial Chemical Industries, Ltd., Macclesfield, Cheshire, Great Britain; O, K & K Labs, Plainview, NY; P, Knoll AB, Ludwigshafen am Rhein, West Germany; Q, Lakeside Laboratories, Milwaukee, WI; R, Lilly, Eli and Co., Indianapolis, IN; S, Mann Research Labs, Inc., New York, NY; T, Marion Labs Inc., Kansas City, MO; U, McNeil Labs Inc., Fort Washington, PA; V, Merck Sharp Dohme, West Point, PA; W, Merrell, Wm. S. Co., Cincinnatti, OH; X, Miles Labs, Elkhart, IN; Y, Park Davis Co., Detroit, MI; Z, S. B. Penick Co., New York, NY; A′, Pfizer Inc., Groton, CT; B′, Regis Chem. Co., Morton Grove, IL; C′, Research Biochemical Inc., Wayland, MA; D′, Rikaken Co., Ltd., Nagoya, Japan; E′, Roth, Carl GmBh Co., Karlsruhe, West Germany; F′, Sandoz Ltd., Basle, Switzerland; G′, Schering AG, Berlin, West Germany and Kenilworth, NJ; H′, Selvi and C. Milano, Milan, Italy; I′, Sigma Chemical Co., St. Louis, MO; J′, Smith Kline French Labs, Philadelphia, PA; K′, Squibb, E. R. and Sons Inc., New Brunswick, NJ; L′, Sterling-Winthrop Research Institute, Rensselaer, NY; M′, United States Pharmacopeia Convention, Inc., Rockville, MD; N′, USV Pharmaceutical Corp., Tuckahoe, NY; O′, Warner-Lambert Research Inst., Morris Plains, NJ; P′, Winthrop Laboratories, New York, NY; Q′, Wyeth Labs Inc., Philadelphia, PA; R′, cinnarazine, diazoxide, flunarazine dihydrochloride, and TMB-8 were supplied by Dr. D. J. Triggle, SUNY, Buffalo, NY; *d*- and *l*-butaclamol by Dr. J. Kebabian, NIH, Bethesda, MD; piperoxane hydrochloride by Dr. H. Thoa, NIMH, Bethesda, MD; meproadifen iodide by Dr. E. X. Albuquerque, University of Maryland, Baltimore, MD; imipramine hydrochloride, haloperidol, spiroperidol, and buspirone by Dr. S. Paul, NIMH, Bethesda, MD; chlorpromazine and chlorpromazine sulfoxide by Dr. A. A. Manian, NIMH, Bethesda, MD; chlorimipramine by Dr. A. Carlson, University of Goteborg, Sweden; procainamide azide by Dr. M. Schimerlik, Oregon State University, Corvallis, OR; felodopine, *d*- and *l*-brompheniramine, WB 4104, mianserin, bepredil, fluperamide, loperamide, and tiapamil by Dr. S. Snyder, Johns Hopkins University, Baltimore, MD; codeine hydrobromide, morphine hydrochloride, levorphanol and dextrophan by Dr. A. E. Jacobsen, NIADDK, Bethesda, MD; procaine hydrochlorde, lidocaine hydrochloride, benzocaine, lidocaine ethiodide, and QX-572 by Dr. L.-Y. M. Huang, NIH, Bethesda, MD; ryanodine by Dr. B. Witkop, NIADDK, Bethesda, MD; 2′,5′-dideoxyadneosine by Dr. C. Londos, NIADDK, Bethesda, MD; and *d*- and *l*-baclofen from Dr. W. J. Wojcik, NIMH, Washington, DC; S′, methiodides were prepared by standard methods (see ref 48); T, synthesized as described by Witiak et al.[49]

This interpretation is compatible with the electrophysiological model of Hille,[2] which suggests that local anesthetics bind more rapidly to the open or conducting from of the channel but with higher affinity to inactivated, nonconducting forms of the channel. Thus it appears that as a consequence of local anesthetic binding to a specific site(s) in the open form of the channel, the channel conformation is modified, the affinity of BTX binding is reduced, and

high-affinity binding of the local anesthetic to the modified inactive form of the channel results in reduction in sodium ion flux. The potency of local anesthetics thus reflect both (i) the affinity for allosteric binding site(s) which competitively influence the BTX-binding site and (ii) some parameter of the conversion of these sites to a high-affinity state associated with an inactivated state of the channel.

While the most current evidence suggests that the primary site of local anesthetic action is a protein component of the sodium channel, it is also clear that the amphipatic properties of classical local anesthetics are important to the potency of local anesthetics. Local anesthetics because of their amphipatic properties should tend to accumulate at the boundaries of nonpolar and polar regions of the channel–membrane complex.[2] Certainly, the presence of lipid-soluble groups in local anesthetics generally enhances anesthetic potency. Many previous studies have clearly established this relationship between lipid solubility and potency (see ref 30 for a review). These properties infer that the binding site for local anesthetics is adjacent to a large nonpolar region in the channel. Speculation of the topography of the nonpolar binding sites at which local anesthetics interact with voltage-dependent sodium channels is possible but may be of limited value since there are likely to be a relatively large number of such sites for compounds of widely differing structures. The present binding assay for interaction at such sites should facilitate rapid and quantitative investigation of the nature and topography of sites at which local anesthetics and other compounds affect the function of voltage-dependent sodium channels.

Local Anesthetics. The quantitative data on inhibition of binding of [^{3}H]BTX-B by the some 40 classical local anesthetics (Table I) is clearly similar to data on local anesthetic activity of these compounds in various biological systems (vide supra). There is an excellent correlation when inhibition of binding by tetracaine, etidocaine > bipivacaine > propranolol > prilocaine > procaine > lidocaine, mepivacaine > benzocaine is compared to these local anesthetics ability tonic blockade of nerve conduction.[2] Correlations of the binding data with local anesthetic activity derived from intact animal studies may not always be as satisfactory, due to pharmacokinetic effects. Intradermal administration of 12 compounds afforded the following rank order for local anesthetic activity: dimethisoquin > butamben, pramocaine > bupivicaine, etidocaine, parethoxycaine, proparacaine, carticaine, benzocaine, prilocaine > lidocaine > procaine.[31] All of these compounds are more potent than procaine in the binding assay except for butamben, benzocaine, and lidocaine. Butamben (a butyl ester) is 3–4-fold more potent than benzocaine (an ethyl ester) in both binding and intradermal infiltration assays. Of the other compounds, etidocaine, a close structural analogue of lidocaine is 30-fold more potent than procaine and 60-fold more potent than lidocaine in the binding assay, while being only 8-fold more potent than procaine and 2-fold more potent than lidocaine in the intradermal infiltration assay. In another series of compounds tested for local anesthetic activity, the following rank order pertained: dibucaine, tetracaine > propoxycaine > cocaine > lidocaine > quinidine, procaine.[32,33] This is the same rank order for potency which is found in the binding assay with the exception of lidocaine, which is 2-fold less potent than cocaine in the binding assay, and quinidine, which is over 30-fold more potent than procaine in the binding assay. In another study, the following order of potency was reported for anesthesia: bupivicaine > pramoxine > lidocaine > procaine.[34] This agrees with the rank order for the binding data. Procaine is often set as a reference compound for comparison purposes since it is a relatively weak local anesthetic and a very commonly used drug. In the binding assay 37 of 44 local anesthetics are more potent than procaine. For other classes of compounds only those which are more potent than procaine (IC_{50} = 110 μM) in inhibiting binding of [^{3}H]BTX-B have been considered relatively active.

Adrenergic Antagonists. Certain compounds with α- or β-adrenergic blocking activity have been extensively investigated for local anesthetic activity. Yohimbine and phentolamine are well-known to have local anesthetic activity[25,35] and are 3-fold and 10-fold, respectively, more potent than procaine in the binding assay. Prazocin is also 10-fold more potent than procaine, but this agent is very potent as a α_1-antagonist so its local anesthetic side effects[35] would be minimized. Another selective α_1-antagonist, WB 4101, was among the most potent inhibitors of [^{3}H]BTX-B binding, being about 40-fold more potent than procaine. It has local anesthetic activity, blocking sodium flux in neuroblastoma cells.[36] Phenoxybenzamine and piperoxan are both about twice as potent as procaine. The results with β-antagonists in the present binding assay are in good agreement with data on local anesthetic activity of β-antagonists in various systems.[37-41] Propranolol is well-known to have local anesthetic activity and both *d*- and *l*-enantiomers were potent blockers of [^{3}H]BTX-B binding. Sotalol is generally conceded to have no local anesthetic activity and was inactive in the binding assay. Practolol, *d*-(nitrophenyl)-2-(isopropylamino)ethanol, and timolol have no or very weak local anesthetic activity and were less potent than procaine in the binding assay. However, metoprolol which is generally classed as a β-antagonist with no local anesthetic activity was comparable in potency to procaine in inhibiting [^{3}H]BTX-B binding. Bunolol, another β-agonist reported to have little local anesthetic activity,[41] was about 4-fold more potent than procaine in the binding assay. Propranolol, dichlorisoproterenol, ICI 118551, and IPS 339 ranked with the most potent local anesthetics with respect to inhibition of binding of [^{3}H]BTX-B; all were at least 10-fold more potent than procaine.

Histaminergic Antagonists. Various antagonists of H_1-histamine receptors are generally recognized as having local anesthetic action.[9] In agreement with this, diphen-

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hydramine, cyproheptadine, diphenidol, promethazine, chlorpheniramine, and pyrilamine were comparable in potency to the more potent local anesthetics with respect to inhibition of [^{3}H]BTX-B binding: All were at least 10-fold more potent than procaine.

Other Receptor Antagonists. The cholinergic antagonists dicyclomine, biperidin and ditran were potent inhibitors of [^{3}H]BTX-B binding and structurally resemble compounds known to have potent local anesthetic activity. The glycine antagonists strychnine and *N*-methylstrychnine were potent inhibitors of binding and are known to have local anesthetic activity.[42]

Calcium Antagonists. Certain calcium antagonists are well-recognized to have potent local anesthetic activity[43-45] which often complicates their use for selective blockage of voltage-dependent calcium channels. Verapamil and its methoxy analogue D-600 were potent inhibitors of [^{3}H]BTX-B binding and are known to be potent local anesthetics, being 3–5-fold more potent than lidocaine.[43] Flunarazine and its desfluoro analogue, cinnarazine, were among the most potent inhibitors of [^{3}H]BTX-B binding. Flunarazine has been reported to be a relatively weak local anesthetic, being comparable in potency to lidocaine.[43] The dihydropyridine class of calcium antagonists (nisoldipine, nitrendipine, nifedipine, felodipine) were about 3-fold more potent than procaine as inhibitors of [^{3}H]-BTX-B binding. Local anesthetic activity of these compounds has not been reported, but they are usually used as calcium blockers at very low concentrations so that local anesthetic side effects will be minimal. Diltiazem is also generally considered a calcium antagonist without local anesthetic side effects.[45] It was, however, 2-fold more potent than procaine in inhibiting [^{3}H]BTX-B binding. The calmodulin antagonists W-7 and W-5 and calmidazolium showed marked inhibition of binding of [^{3}H]BTX-B binding at concentrations similar to those used to inhibit calmodulin-dependent processes (see ref 12, 13). The 3-(dimethylamino)-5,6-(methylenedioxy)indenes have been purported to be selective antagonists at internal calcium sites,[11] but the present data indicate that such compounds are 20-fold more potent than procaine in the present screen for local anesthetic activity. Diazoxide was inactive vs. [^{3}H]BTX-B binding and has been reported to have no local anesthetic activity.[46]

Tranquilizers. Among the various tranquilizers, the phenothiazines are well-known to have local anesthetic activity[46] and all of these compounds were very potent inhibitors of [^{3}H]BTX-B binding. Butyrophenone tranquilizers, such as spiroperidol, droperidol, and haloperidol, are extremely potent as neuroleptics and dopamine antagonists, and their potent local anesthetic activity is less well-known: Haloperidol was one of the most potent local anesthetics among a group of tranquilizers, being even more potent than chlorpromazine.[46] Haloperidol was also more potent than chlorpromazine in the present binding assay.

Diazepam was a relatively weak inhibitor of [^{3}H]BTX-B binding, being comparable in potency to procaine. Diazepam has been recently reported to block BTX-evoked sodium flux with a K_i of about 50 μM.[35] The two members of the diphenylmethane class of tranquilizers, namely, azacyclonal and hydroxyzine, were very potent inhibitors of [^{3}H]BTX-B binding as were *d*- and *l*-butaclamol and reserpine. Tetrabenamine was relatively inactive, being only slightly more potent than procaine.

Antidepressants. Tricyclic antidepressants are know to have local anesthetic activity.[46] All of the compounds of this class were very potent as inhibitors of [^{3}H]BTX-B binding.

Miscellaneous Compounds. The anticonvulsants diphenylhydantoin and carbamazepine have been previously reported to block BTX-evoked sodium fluxes[27] and to antagonize binding of [^{3}H]BTX-B.[24] The K_i values vs. sodium flux were 35–40 μM in neuroblastoma cells. The IC_{50} value for diphenylhydantoin vs. [^{3}H]BTX-B binding was about 40 μM, similar to that expected from the data of Table I. Remarkably, carbamazepine was less potent vs. [^{3}H]BTX-B binding (IC_{50} = 130 μM) than vs. BTX-evoked sodium flux.[24] Another class of anticonvulsants, the barbiturates, have very low local anesthetic activity[26] and were very weak inhibitors of [^{3}H]BTX-B binding (see ref 27 and Table I).

Certain of the vasodilators, namely, dilazep and hexobendine, are extremely potent inhibitors of [^{3}H]BTX-B binding and their structures are reminiscent of compounds known to have local anesthetic activity.

Conclusions. The results of the present screen of 44 local anesthetics and over 100 other pharmacologically active compounds vs. binding of [^{3}H]BTX-B to sites associated with the voltage-dependent sodium channel in nervous tissue document the utility of the method for probing potential local anesthetic activity. The few instances in which the results of the binding data do not conform with results from classical methods of determing local anesthetic activity are of interest, although such differences may in some instances only be the result of pharmacokinetic factors. There are, however, some compounds which are anomolously more active vs. binding of [^{3}H]BTX-B than they are vs. BTX-elicited depolarization (see ref 6). The converse is true in the case of carbamazepine.[24] A thorough investigation of effects of such compounds on binding and on ion flux in the same or similar preparations may provide insights into the function of voltage-dependent sodium channels.

Experimental Section

Compounds used for the binding assay included the following: tetrodotoxin, choline chloride, Hepes, and Tris from Calbiochem. Corp., La Jolla, CA; scorpion venom of *Leiurus quinquestriatus* from Sigma Chemical Co., St. Louis, MO; veratridine (no longer available at this source) from Aldrich Chemical Co., Milwaukee, WI. The sources of compounds tested vs. binding of [^{3}H]BTX-B are listed in the footnotes to Table I. [^{3}H]Batrachotoxinin A 20α-benzoate with a specific activity of 14 Ci/mmol was prepared as described.[32] It is now available from New England Nuclear.

Binding Assay with [^{3}H]Batrachotoxinin A 20α-Benzoate. The membrane vesicular preparation was from guinea pig cerebral cortical tissue.[47] The major components of this preparation have been characterized and appear to be resealed postsynaptic membranes with attached resealed presynaptic elements. Their preparation and assay are briefly as follows: Slices of cerebral cortical grey matter were prepared from the brain of one male Hartley guinea pig (200–250 g). The slices (approximately 1 g wet weight) were homogenized in 4 mL of buffer containing the following: 130 mM choline chloride, 50 mM Hepes buffer (ad-

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justed to pH 7.4 with tris(hydroxymethyl)aminomethane, approximately 23 mM Tris base), 5.5 mM glucose, 0.8 mM $MgSO_4$, and 5.4 mM KCl. The tissue was homogenized with 10–12 strokes of a glass-glass homogenizer. The final volume was adjusted to 10 mL and centrifuged at 1000*g* for 15 min. The pellet was washed once with 10 mL of buffer and resuspended in the same volume for binding studies. Incubations were carried out in a total volume of 250 μL containing 50 nM [^{3}H]BTX-B, 1 μM tetrodotoxin, 0.03 mg of scorpion venom, and about 400 μg of the particulate vesicular protein. Incubations for 30 min at 37 °C were terminated by dilution of the reaction mixture with 3 mL of wash buffer and filtration through a Whatman GF/C filter. Filters were washed three times with 3 mL of wash buffer. Filtration was accomplished with a Millipore filtration apparatus for single samples or with a Brandel Cell Harvester (Gaithersburg, MD) to filter sets of 24 samples. The results with both methods were identical. The wash buffer contained the following: 163 mM choline chloride, 5 mM Hepes (adjusted to pH 7.4 with Tris base), 1.8 mM $CaCl_2$, and 0.8 mM $MgSO_4$. Filters were counted in a Beckman scintillation counter using 10 mL of Hydroflour (National Diagnostics). The efficiency of tritium counting was 43%. Specific binding was determined by substracting the nonspecific binding, determined in the presence of 300 μM veratridine, from the total binding of [^{3}H]BTX-B. Specific binding was about 80% of total binding.

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Registry No. BTX-B, 78870-19-6; Na, 7440-23-5; Ca, 7440-70-2; K, 7440-09-7.

Bicyclic and Tricyclic Analogues of Anthramycin

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As analogues of pyrrolo[2,1-c][1,4]benzodiazepine antitumor antibiotics, such as anthramycin and tomaymycin, several benzo[1,4]diazepine imines and carbinolamine ethers were prepared and tested in vivo against P388 leukemia. Two different synthetic approaches, namely, a reduction of an aromatic nitro group with a concomitant cyclization and a reduction of a lactam, were employed to generate an imine or a carbinolamine moiety. Bicyclic analogues **6a′**, **6f**, and **6g** were found to be active, indicating that the pyrroline ring of anthramycin is not an absolute necessity for the antitumor activity. Compound **6g**, 3,4-dihydro-9-hydroxy-4-propargyl-5*H*-1,4-benzodiazepin-5-one, was at least as active as neothramycin although it was 5 times less potent. Among the tricyclic analogues, compounds **5**, **7a**, and **8b** were active against P388 leukemia, and they generally appear to be more potent than bicyclic analogues.

Pyrrolo[2,1-c][1,4]benzodiazepine antitumor antibiotics are a unique class of compounds represented by anthramycin (1) and tomaymycin (2).[1] Neothramycin,[2] one of the newest members of this class, and spadicomycin,[3] an anthramycin sodium hydrosulfite adduct, are currently in clinical trials in Japan. Previously, anthramycin and sibiromycin have been tried clinically with only limited success.[5]

On the molecular level, Hurley and co-workers proposed a possible mechanism of action of these agents.[5] According to this proposal, anthramycin (or other members of this class) fits in the minor groove of DNA, and it is bound by a labile aminal linkage between the N2 of guanine and the C11 of anthramycin. The secondary stabilizing force is provided by the hydrogen bonding of the C9 hydroxy group of anthramycin to the O2 of cytosine. The hydroxy group of tomaymycin, on the other hand, is presumed to be involved in bifurcated hydrogen bonding to the sugar and phosphate oxygens of DNA.[6] In 1979 Lown and Joshua prepared compounds **3**–**5** (Chart I) as models of pyrrolobenzodiazepine antibiotics.[7] They found that while compounds **3** and **5** readily added to nucleophiles (e.g., thiophenol), only **5** produced covalent attachment to DNA as shown by ethidium fluorescent assay.

Chart I

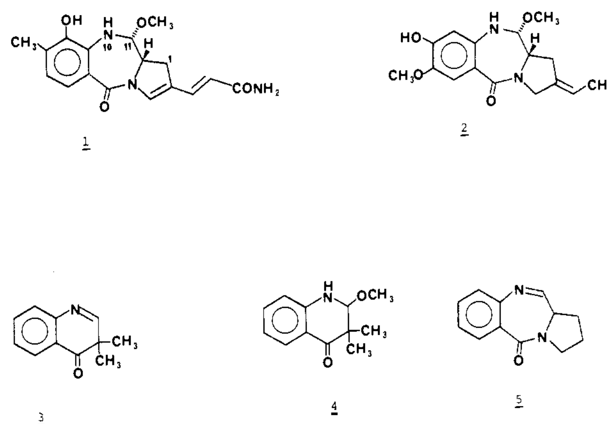
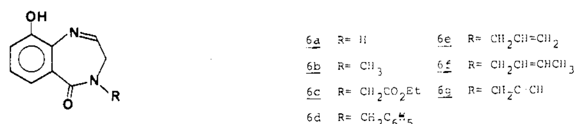
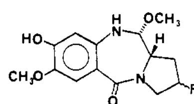
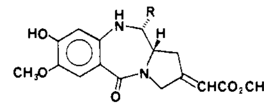


Chart II







These data and additional published data[8] suggested the possibility of rationally designing new analogues. We

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