Scheme I

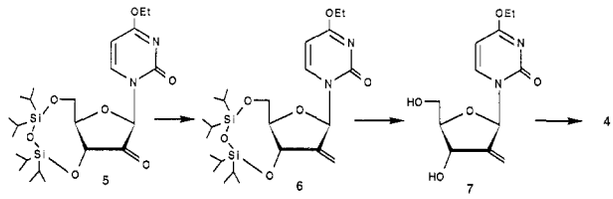

Table I. Inhibitory Effects of DMDC (4), ara-C, and 5-FU on the Growth of Various Mammalian Cell Lines in Vitro[a]

cell line	IC_{50},[b] μg/mL		
	DMDC (4)	ara-C	5-FU
L1210[c]	0.11	0.097	0.32
CCRFCEM[d]	0.047	0.065	40
MOLT 4[e]	0.025	0.056	3.8
K562[f]	1.2	3.2	38
PC10[g]	60.5	>100	>100
SW480[h]	3.8	>100	3.3
TE2[i]	2.9	>100	3.9
T24[j]	3.7	>100	6.1

[a] Drug sensitivity assays were performed according to the method of Carmichael et al.[13] Each tumor cell line (1×10^4/well) was incubated in the presence or absence of compounds for 72 h. Then, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide was added and $OD^{(550-660}$nm) was measured. Percent inhibition was determined as follows: % inhibition = [1 − ($OD^{(550-660}$nm) of sample well/$OD^{(550-660}$nm) of control well)] × 100. [b] IC_{50} (μg/mL) was given as the concentration at 50% inhibition of cell growth. [c] Mouse leukemia. [d] Human T-cell acute lymphoblastoid leukemia. [e] Human T-cell acute lymphoblastic leukemia. [f] Human chronic myelogenous leukemia. [g] Human lung squamous cell carcinoma. [h] Human colon adenocarcinoma. [i] Human esophagus adenocarcinoma. [j] Human bladder transitional-cell carcinoma.

prepared by reaction of potassium hydride and methyltriphenylphosphonium bromide in dimethyl sulfoxide), the desired 2′-methylidene nucleoside (6) was obtained in 41% yield as a foam [MS m/z 510 (M^+); ^{1}H NMR ($CDCl_3$) δ 5.39 (dd, 1 H, 2′-vinyl proton, J = 2.9 Hz, J = 1.5 Hz), 5.69 (dd, 1 H, 2′-vinyl proton, J = 2.9 Hz, J = 1.2 Hz), 6.64 (d, 1 H, 1′-H, J = 1.5 Hz)]. Deprotection of 6 with tetra-*n*-butylammonium fluoride in tetrahydrofuran afforded 7 in 91% yield. Compound 7 was then converted to the target nucleoside, 2′-deoxy-2′-methylidenecytidine (4, DMDC), by treatment with methanolic ammonia in a sealed tube at 100 °C for 2 days and isolated in 81% yield as an HCl salt [mp >300 °C; ^{1}H NMR (D_2O) δ 5.51 (br s, 1 H, 2′-methylidene proton), 5.68 (br s, 1 H, 2′-methylidene proton), 6.64 (br d, 1 H, 1′-H, J = 1.8 Hz). Anal. ($C_{10}H_{14}ClN_3O_4$) C, H, N.] (Scheme I).

Biological Activity. The nucleoside, DMDC (4), ara-C, and 5-fluorouracil (5-FU) were tested for their ability to inhibit the growth of various tumor cells including human tumor cells in vitro. The IC_{50} values for these compounds are summarized in Table I. Ara-C showed inhibitory activity against mouse leukemic, human T-cell acute leukemic, and chronic leukemic cells, but not against human carcinoma and adenocarcinoma cells. By contrast, 5-FU exhibited a broad spectrum of activity to this range of cells. Although DMDC is an analogue of 2′-deoxycytidine, its spectrum of activity against tumor cells is quite different from that of ara-C. DMDC was active at rather low concentrations against not only mouse leukemic and human leukemic cell lines but also human carcinoma cell lines. Furthermore, DMDC is more active than 5-FU in T24 human bladder transitional-cell carcinoma cells and comparably active to 5-FU in SW480 human colon adenocarcinoma and human esophagus adenocarcinoma cells.

The effect of DMDC and ara-C on the synthesis of DNA, RNA, and proteins was also examined with L1210 cells. At 10 μg/mL, both DMDC and ara-C inhibited incorporation of [^{3}H]thymidine into DNA by 98%, while no inhibition of RNA synthesis (incorporation of [^{3}H]uridine) and protein synthesis (incorporation of [^{3}H]Leu) was observed. It is noteworthy that no significant deamination of DMDC was detected in 2 h by partially purified cytidine deaminase from mouse kidney.[11] Under similar conditions, cytidine and ara-C were deaminated (100% and 88%, respectively).

As this unique and broad spectrum of inhibitory activity of DMDC may be related to the allylic alcohol system in its structure, detailed studies on its mechanism of action in vitro as well as its activity in vivo[12] are being undertaken.

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Registry No. 4, 113648-25-2; 5, 113648-22-9; 6, 113648-23-0; 7, 113648-24-1; $H_2C{=}PPh_3$, 3487-44-3; uridine, 58-96-8.

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(±)-4-*tert*-Butyl-3-cyano-1-(4-ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octane: Synthesis of a Remarkably Potent $GABA_A$ Receptor Antagonist

Sir:

A great variety of potent convulsants including 1,4-disubstituted 2,6,7-trioxabicyclo[2.2.2]octanes, bicyclophosphorus esters, polychlorocycloalkanes, and picrotoxinin analogues act as noncompetitive $GABA_A$ receptor antagonists.[1-3] These toxicants and insecticides are considered to bind to a specific site(s) within the GABA receptor–ionophore complex and thereby to block the GABA-regulated chloride channel.[4-6] This specific site

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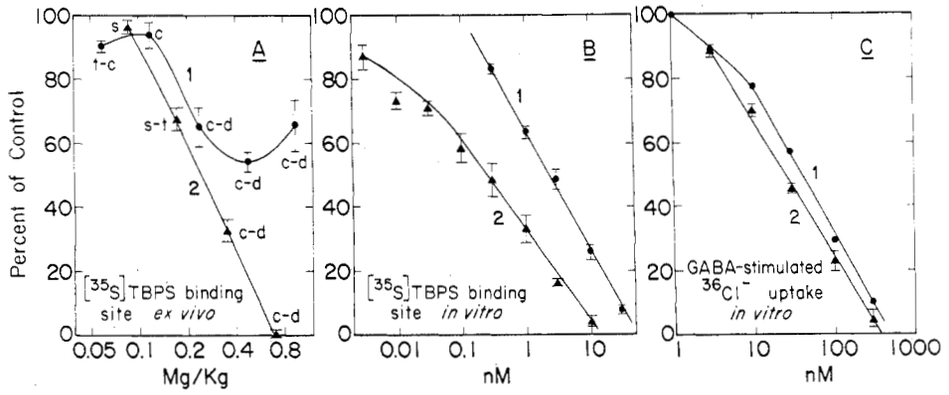
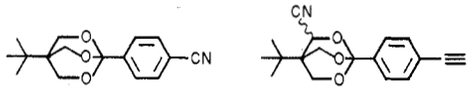


Figure 1. Relative potencies of compounds 1 and 2 as ex vivo (A) and in vitro (B) inhibitors of [^{35}S]TBPS binding to mouse brain membranes and as in vitro inhibitors of GABA-stimulated $^{36}Cl^-$ uptake by membrane vesicles from rat cerebral cortex (C). The poisoning symptoms[8] in A are designated as s (sedation), t (tremors), c (convulsions), and d (death) or dual designations (e.g., c–d) representing individual variations. Data in A and B are arithmetic means and in C are geometric means with SE ranges calculated from three to six replicates. The results for **1** in C are from Obata et al.[7]

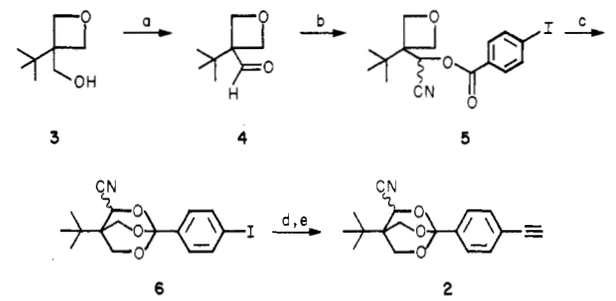
is conveniently assayed by binding studies with *t*-butyl-[^{35}S]bicyclophosphorothionate ([^{35}S]TBPS).[4] There is a significant correlation with various cage convulsants between their potency for inhibiting [^{35}S]TBPS binding to human and mouse brain membranes and their activity for blocking GABA-stimulated chloride-36 uptake in rat cerebral cortex.[7] The most potent reported inhibitor of TBPS binding and GABA-mediated chloride conductance is 4-*tert*-butyl-1-(4-cyanophenyl)-2,6,7-trioxabicyclo-[2.2.2]octane (**1**).[7,8] Here we report a new $GABA_A$ receptor antagonist, (±)-4-*tert*-butyl-3-cyano-1-(4-ethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octane (**2**), which is 10-fold more potent than **1** at the [^{35}S]TBPS binding site and is in fact the most potent known $GABA_A$ receptor antagonist.



1 2

Chemistry. Compound **1** was prepared as described earlier.[8] Our synthesis of **2** (Scheme I) was initiated by oxidation of 3-*tert*-butyl-3-(hydroxymethyl)oxetane (**3**)[8] with pyridinium chlorochromate (1.5 equiv) in dichloromethane under nitrogen to give 3-*tert*-butyl-3-formyloxetane (**4**) (mp 120 °C dec) recovered in 80% yield by chromatography on Florisil with ether. Treatment of freshly prepared **4** and iodobenzoyl chloride (1.4 equiv) in ether with sodium cyanide (3.6 equiv) in water with stirring overnight under nitrogen gave (±)-3-*tert*-butyl-3-[[(4-iodobenzoyl)oxy]cyanomethyl]oxetane (**5**) (via in situ formation of the oxetane cyanohydrin) recovered in 90% yield on extraction with ether. Lewis acid catalyzed rearrangement[8,9] of oxetane ester **5** to (±)-4-*tert*-butyl-3-cyano-1-(4-iodophenyl)-2,6,7-trioxabicyclo[2.2.2]octane (**6**) was carried out by treatment with boron trifluoride etherate (1.3 equiv) in dry dichloromethane at −70 °C under nitrogen. Recovery of **6** (58%) (mp 155–157 °C) involved neutralization with triethylamine, partitioning between dichloromethane and water, and chromatography of the organic phase on basic alumina with hexane/dichloromethane (4:1). Palladium-catalyzed coupling[10] of aryl iodide **6** with (trimethylsilyl)acetylene (1.5 equiv) in the presence of catalytic quantities of bis(trimethylphosphine)palladium(II) chloride and copper(I) iodide in dry diethylamine at 25 °C overnight afforded the trimethylsilyl-protected ethynylphenylbicyclooctane (99%) recovered by evaporation of the reaction mixture and partitioning between water and ether. The trimethylsilyl group was removed with K_2CO_3 in dry methanol[11] or with tetrabutylammonium fluoride[12] followed by purification on basic alumina with hexane/dichloromethane (4:1) to give **2** (57%) (pale yellow needles from hexane/dichloromethane, mp 123–125 °C). The products were identified by PMR spectroscopy (300 MHz, $CDCl_3$), mass spectrometry (MS), and elemental analysis.[13]

Scheme I[a]



3 4 5 6 2

[a] (a) Pyridinium chlorochromate, CH_2Cl_2; (b) NaCN, 4-iodobenzoyl chloride, ether/H_2O; (c) $BF_3{\cdot}Et_2O$, CH_2Cl_2; (d) (trimethylsilyl)acetylene, Et_2NH, CuI, $(Ph_3P)_2PdCl_2$; (e) *n*-$Bu_4N^+F^-$, THF or K_2CO_3, CH_3OH.

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(13) Characterization data. **2**: Anal. ($C_{18}H_{19}NO_3$) C, H, N. [M + 1]$^+$ 298. ^{1}H NMR δ 1.05 (9 H, s, Me_3C), 3.10 (1 H, s, C≡CH), 4.00–4.55 (4 H, m, OCH_2CCH_2O), 4.95 (1 H, d, OCHCN), 7.50 (4 H, q, aromatic). Trimethylsilyl derivative of **2**: ^{1}H NMR δ 0.20 (9 H, s, Me_3Si), 1.05 (9 H, s, Me_3C), 4.00–4.50 (4 H, m, OCH_2CCH_2O), 4.95 (1 H, d, OCHCN), 7.45 (4 H, q, aromatic). **4**: ^{1}H NMR δ 1.05 (9 H, s, Me_3C), 4.50–4.70 (4 H, dd, CH_2OCH_2), 9.90 (1 H, s, CHO). **5**: ^{1}H NMR δ 1.10 (9 H, s, Me_3C), 4.60 (2 H, q, CH_2O), 4.75 (2 H, q, CH_2O), 5.80 (1 H, s, CHCN), 7.80 (4 H, q, aromatic). **6**: High-resolution MS (positive ion fast atom bombardment), calcd for $C_{16}H_{18}NO_3I$, (M + 1) 400.0410, found 400.0413. ^{1}H NMR δ 1.05 (9 H, s, Me_3C), 4.00–4.50 (4 H, m, OCH_2CCH_2O), 4.95 (1 H, d, OCHCN), 7.30 (2 H, d, aromatic), 7.70 (2 H, d, aromatic).

Pharmacology. The mouse intraperitoneal (ip) LD_{50} value for **2** at 24 h is 0.084 mg/kg, which is almost as toxic as **1** (LD_{50} = 0.060 mg/kg).[8] The poisoning symptoms[8] and toxicity of **2** are closely associated with its inhibition of the [^{35}S]TBPS binding site in the brains of mice 30 min after ip treatment (Figure 1A). Each assay involved a portion of the P_2 membrane fraction from the brains of two mice prepared in 0.32 M sucrose and washed three times with 1 mM EDTA by sedimentation, resuspension, and recentrifugation.[14] The final pellet was resuspended in 200 mM NaCl–50 mM sodium phosphate pH 7.4 assay buffer. Receptor assays involved various levels of the P_2 protein (50–300 μg, to ascertain the degree of linearity of binding) in 1 mL of assay buffer containing [^{35}S]TBPS (2 nM) alone or with unlabeled TBPS (2 μM) to correct for nonspecific binding (12% relative to the total binding). Following incubation for 30 min at 37 °C to achieve equilibrium between [^{35}S]TBPS and its binding site, the suspensions were subjected to rapid filtration on Whatman GF/C filters, three rinses with 2 mL of cold assay buffer, and liquid scintillation counting (LSC). The first symptoms of sedation and tremors by **2** appear at ~30% inhibition and convulsions are evident at ~70% inhibition of [^{35}S]TBPS binding. The behavior of **2** is the same as that of the polychlorocycloalkane insecticides in that the magnitude of inhibition at the [^{35}S]TBPS binding site correlates with the severity of the poisoning symptoms; however, **2** is at least 10-fold more potent as an ex vivo inhibitor than any of the polychlorocycloalkanes examined.[5,14] The slightly higher toxicity of **1** than **2** is not paralleled by their relative potencies in this ex vivo inhibition assay and the inhibition by **1** is not progressive with dose or consistently correlated with the poisoning symptoms. Perhaps the apparent anomalous behavior of **1** is due in part to partial dissociation from the binding site during membrane preparation and binding site assay.

Diazepam and phenobarbital administered ip at 10 and 100 mg/kg 15 and 5 min, respectively, before a 0.35 mg/kg dose of **2** greatly alleviate the poisoning symptoms at 30 min. However, these treatments do not significantly affect the ex vivo inhibition of the TBPS binding site 30 min after administering **2** with this regimen, i.e. (mean ± SD, n = 5–7) 68 ± 8% inhibition for **2** alone, 59 ± 15% inhibition for **2** with diazepam, and 61 ± 12% inhibition for **2** with phenobarbital.

The higher potency of **2** than **1** as an inhibitor of [^{35}S]TBPS binding evident ex vivo is also observed in vitro (Figure 1B). These assays were carried out as above but with EDTA/water-dialyzed brain P_2 membranes.[8] The inhibitors were introduced in 5 μL of dimethyl sulfoxide. Compound **2** with 50% inhibition at 0.25 nM is 10-fold more potent than **1** (2.5 nM) for in vitro inhibition of [^{35}S]TBPS binding.

The enhanced potency of **2** over **1** is also evident in inhibiting GABA-stimulated $^{36}Cl^-$ uptake by membrane vesicles from rat cerebral cortex.[7,15] The cerebral cortex homogenate in cold buffer (145 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 mM D-glucose, and 10 mM HEPES adjusted to pH 7.5 with Tris base) was centrifuged to obtain the 1000g pellet, which was washed by resuspension and recentrifugation. Various concentrations of **1** and **2** were preincubated with the brain vesicles (1.8 mg of protein) for 10 min at 30 °C and then GABA (100 μM final) and $^{36}Cl^-$ (0.1 μCi) were added and the uptake of $^{36}Cl^-$ was terminated 3 s later by addition of ice-cold buffer and rapid filtration, followed by LSC. GABA-stimulated $^{36}Cl^-$ uptake was calculated as the difference between basal uptake in the absence of GABA and total uptake in the presence of GABA. The enhanced potency of **2** over **1** in the [^{35}S]TBPS binding assay is also shown in the $^{36}Cl^-$ flux investigation, i.e., IC_{50} values of 25 and 40 nM, respectively (Figure 1C).

Because of its remarkable potency as a $GABA_A$ receptor antagonist, compound **2** is the best probe currently available for modeling the topography of the convulsant site of the chloride ionophore.

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Registry No. **2**, 114096-06-9; **3**, 99250-47-2; **4**, 107829-98-1; **5**, 114096-07-0; **6**, 114096-08-1; (±)-4-*tert*-butyl-3-cyano-1-(4-trimethylsilamethynylphenyl)-2,6,7-trioxabicyclo[2.2.2]octane, 114096-09-2; (trimethylsilyl)acetylene, 1066-54-2.

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Novel Antihypertensives Targeted at Dopamine β-Monooxygenase: Turnover-Dependent Cofactor Depletion by Phenyl Aminoethyl Selenide

Sir:

We and others have recognized the attractiveness of dopamine β-monooxygenase (DBM; EC 1.14.17.1) as a target point for modulation of adrenergic activity and thus the pharmacological potential of substrate analogues and inhibitors for this important enzyme of catecholamine metabolism.[1-7] While the biological role of DBM is the benzylic hydroxylation of dopamine (DA) to norepinephrine (NE), we have previously demonstrated several new kinetically facile monooxygenase activities for DBM—stereoselective sulfoxidation,[7] oxygenative keton-

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