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Registry No. (1*S*,2*R*)-**2**, 109062-25-1; t(1*S*,2*R*)-**2**-N-amm, 108970-00-9; (1*S*,2*R*)-**3**, 108969-99-9; (1*S*,2*R*)-**3**-N-amm, 108969-95-5; (4a*R*,10b*S*)-**4**, 109062-23-9; (4a*R*,10b*S*)-4, 109119-95-1; (4a*S*,10b*R*)-**5**, 109062-21-7; (4a*S*,10b*R*)-**5**·HCl, 109119-94-0; *cis*-**6**·HCl, 82171-88-8; (4a*S*,10b*R*)-**6**, 109062-18-2; (4a*S*,10b*R*)-**6**·HCl, 109214-33-7; (4a*R*,10b*S*)-**6**, 109062-19-3; (4a*R*,10b*S*)-**6**·HCl, 109119-93-9; (4a*R*,10b*S*)-**6**-N-amm, 108969-96-6; (4a*R*,10b*S*)-**7**, 108969-98-8; (4a*R*,10b*S*)-**7**-N-amm, 108969-97-7; (4a*R*,10b*S*)-**8**, 109062-26-2; (4a*R*,10b*S*)-**8**-N-amm, 109062-24-0; (*R*)-(−)-*O*-methylmandelic acid, 3966-32-3; (4a*S*,10b*R*)-4-[(*R*)-2-methoxy-2-phenyl-1-oxoethyl]-10-methoxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline, 108969-93-3; (4a*R*,10b*S*)-4-[(*R*)-2-methoxy-2-phenyl-1-oxoethyl]-10-methoxy-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline, 109062-17-1; (4a*S*,10b*R*)-10-methoxy-4-(1-oxopropyl)-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline, 108969-94-4; 5,6,10b-octahydrobenzo[*f*]quinoline(4a*S*,10b*R*)-10-methoxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline, 109062-20-6; (4a*R*,10b*S*)-10-methoxy-4-(1-oxopropyl)-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline, 109062-27-3; (4a*R*,10b*S*)-10-methoxy-4-*n*-propyl-1,2,3,4,4a,5,6,10b-octahydrobenzo[*f*]quinoline, 109062-22-8.

Supplementary Material Available: X-ray data of (4a*R*,10b*S*)-**6**·HCl, including positional and thermal parameters, bond lengths, and bond angles (2 pages); tables of observed and calculated structure factors (19 pages). Ordering information is given on any current masthead page.

γ-Aminobutyric Acid Esters. 3. Synthesis, Brain Uptake, and Pharmacological Properties of C-18 Glyceryl Lipid Esters of GABA with Varying Degree of Unsaturation

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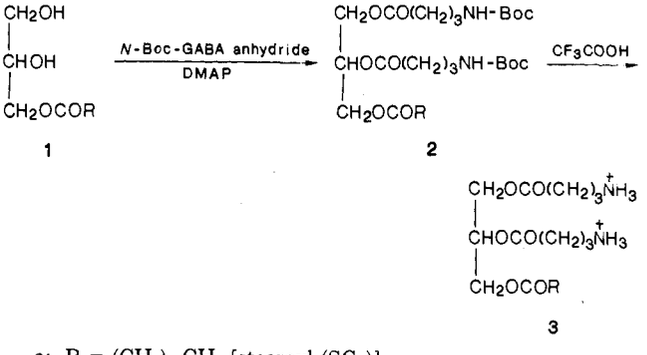
A series of ^{14}C-labeled and unlabeled di-γ-aminobutyric acid esters of glyceryl lipids having zero to three double bonds (stearoyl, oleoyl, linoleoyl, and linolenoyl) were synthesized. Measurements of the octanol/water partition coefficients of the compounds showed an increase with decreasing number of double bonds (i.e., from linolenoyl to stearoyl). The brain-uptake index went up from 31.5 (linolenoyl) to 45.1 (stearoyl) and similarly the brain-penetration index went up from 15 (linolenoyl) to 28 (stearoyl). Intraperitoneal injections of these di-GABA lipid esters produced a substantial inhibition of the general motor activity in mice at a dose of 30 mg/kg; the most active molecules were those containing two and three double bonds, i.e., the linoleoyl and linolenoyl derivatives. This is in reverse order to that predicted by brain-uptake and lipid-solubility properties, suggesting that the structure of the fatty acid side chain may be an additional factor in influencing biological activity.

Several neurological and neuropsychiatric disorders such as epilepsy and Huntington's disease have been reported[1-3] to be associated with a decrease in γ-aminobutyric acid (GABA) levels in the central nervous system (CNS). This has led to the search for GABA agonists that might improve the poor uptake of GABA through the blood–brain diffusion barrier.[4] In previous studies we demonstrated that certain cholesteryl[5] and glyceryl[6] esters can easily penetrate the blood–brain diffusion barrier to function as prodrugs which release GABA into the CNS after hydrolysis by esterases. Thus 1-linolenoyl-2,3-bis(4-aminobutyryl)propane-1,2,3-triol (LG_2) and 1,2-dilinolenoyl-3-(4-aminobutyryl)propane-1,2,3-triol were found to have a 75- and 127-fold greater uptake than that of GABA and to have electrophysiological[7] and pharmacological activity.[6]

Scheme I[a]



a: R = $(CH_2)_{16}CH_3$ [stearoyl (SG_2)]
b: R = $(CH_2)_7(CH{=}CHCH_2)(CH_2)_6CH_3$ [oleoyl (OG_2)]
c: R = $(CH_2)_7(CH{=}CHCH_2)_2(CH_2)_3CH_3$ [linoleoyl (L^2G_2)]
d: R = $(CH_2)_7(CH{=}CHCH_2)_3CH_3$ [linolenoyl (LG_2)]
[a] DMAP, 4-(dimethylamino)pyridine.

In this paper we have extended this study to investigate the effect of varying the degree of unsaturation in the C-18 lipid moiety of the glyceride molecule on the neuropharmacological and brain-uptake properties of the molecule. Thus, we have synthesized linoleoyl, oleoyl, and stearoyl glyceryl lipids containing two GABA ester groups in each

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Table I. Brain Penetration Index Values for the GABA Lipids

compd	dose, mg/kg (mmol/kg)	[brain],[a] nmol/g	[liver],[a] nmol/g	BPI, %
GABA[b]	3–39 (0.03–0.38)	0.04–1.81	4.2–165	0.96 ± 0.09
SG_2·2OAc (**3a**)	44 (0.07)	1.89 ± 0.24	6.92 ± 0.96	28 ± 7
OG_2·2OAc (**3b**)	36 (0.06)	1.07 ± 0.42	6.32 ± 1.79	17 ± 4
L^2G_2·2OAc (**3c**)	54 (0.08)	1.32 ± 0.37	9.19 ± 1.58	14 ± 4
LG_2·2OAc (**3d**)	40 (0.06)	1.07 ± 0.25	7.86 ± 2.52	15 ± 8
L^2G_2 (**3c**)	41 (0.08)	7.74 ± 2.89	8.00 ± 0.99	97 ± 34
LG_2[b] (**3d**)	100 (0.19)	2.81 ± 0.36	3.79 ± 0.58	75 ± 13

[a] The amount of the drug was calculated from the total radioactivity found in brain and liver tissue. BPI = ([brain]/[liver]) × 100, at 5 min after sc administration of labeled test compound. The values represent mean ± SD for three measurements except LG_2·2OAc, which is an average of two measurements. [b] Reference 5.

compound. The ^{14}C-labeled GABA derivatives were also synthesized for measurements of the brain-uptake properties. The results suggest that all the compounds can readily penetrate the blood–brain barrier and that maximal pharmacological activity is associated with the compounds containing the highest degree of unsaturation.

Chemistry

Scheme I shows the general procedure used for the synthesis of the lipid esters of GABA.

In each case a monoacylglycerol (**1**) was esterified with 4-[(*tert*-butoxycarbonyl)amino]butyric anhydride (Boc-GABA anhydride) in the presence of 4-(dimethylamino)-pyridine to yield a triacyl glyceride (**2**). The glycerides were then treated with trifluoroacetic acid to remove the protecting group (Boc) from the amino terminus of the GABA moieties of the lipids (**3a–d**). These were then converted to the diacetate salts and stored at −17 °C to avoid the spontaneous decomposition which is promoted by the free amine. The compounds were also synthesized on a microscale as ^{14}C-labeled derivatives, in which all the radioactivity was localized on the GABA portion of the molecule, for use in brain-uptake studies.

Results and Discussion

Brain-Uptake Studies. We have used two methods to evaluate the capacity of these lipids to penetrate the blood–brain barrier of mice and rats.

(a) Brain Penetration Index (BPI) Method[5]. This method measures the quantity of a ^{14}C-labeled compound taken up into the brain as a percent of the amount of accumulating in liver per gram of tissue at 5 min following a subcutaneous (sc) injection.[5,6] The use of the sc route allows delivery of the compound via the bloodstream to the brain and the liver so that a direct comparison of uptake for the two organs can be made, as previously described.[5] It has the advantage of measuring the amount of the biologically available dose in the circulation which was taken up through the blood–brain barrier and avoids the problems that are generally encountered by sparingly soluble lipid esters which tend to remain largely at the site of injection. Table I gives the BPI values obtained for the GABA lipids. The BPI of GABA was found to be 1% irrespective of the mode of administration of the compound in the dose range[5] of 0.30–0.38 mmol/kg. This was increased by 75- and 97-fold for the linoleoyl- and linolenoylglyceryl lipids (**3c** and **3d**), respectively, injected as the free amines. The BPI values of these lipids became somewhat lower (14–17%) if they were administered as the diacetate salts. The highest BPI value (28%) was obtained for the diacetate salt of the stearoyl lipid (**3a**). This saturated lipid derivative also had the highest octanol/water partition coefficient (see Table II).

Table II. Brain-Uptake Index and Partition Coefficient Values for the GABA Lipids

compd[a]	no. of double bonds	octanol/water partition coefficient,[b] K	brain-uptake index (BUI)
GABA		0.004	2.0
SG_2 (**3a**)	0	8.6	45.1 ± 3.5
OG_2 (**3b**)	1	6.0	53.2 ± 1.1
L^2G_2 (**3c**)	2	4.2	25.9 ± 3.4
LG_2 (**3d**)	3	2.13	31.5 ± 3.6

[a] The GABA-lipid esters were used as the acetate salts. [b] The octanol/water partition coefficient was determined by using ^{14}C-labeled compounds. The lipids were used as the acetate salts except LG_2 (**3d**), which was used as the amine.

Table III. Effect of GABA Esters on the General Motor Activity of Mice

compd[a]	dose, mg/kg (mmol/kg)	percentage decrease in general motor activity with respect to vehicle control[b]
SG_2·2OAc (**3a**)	30 (0.046)	62.1 ± 12.8*[d]
OG_2·2OAc (**3b**)	30 (0.046)	74.0 ± 10.0*
L^2G_2·2OAc (**3c**)	30 (0.047)	89.3 ± 10.0*
LG_2·2OAc (**3d**)	30 (0.047)	82.6 ± 5.9*
stearoylglycerol[c]	16 (0.045)	+2.4 ± 12.5**[e]

[a] The drugs were injected as the diacetate salts in 0.14 M saline. [b] Cumulative activity scores over 60 min postinjection of the drug are expressed as the percentage decrease in general motor activity with respect to vehicle-injected control Balb-c mice (n = 9). [c] Stearoylglycerol was used as control (vehicle: 25% propylene glycol, water). [d] (*) Significant $p < 0.001$ (t test), analysis of variance shows group means are significantly different ($p < 0.02$). A t-test comparison of combined SG_2 and OG_2 groups vs. the combined L^2G_2 and LG_2 groups was also significant ($p < 0.005$). [e] (**) not significant.

The amounts of the drug entering the brain and liver were calculated from the total uptake of radioactivity in the tissue, and are expressed as nanomoles/gram of tissue. There is a large increase in the amounts of the drugs entering the brain as compared to GABA. The free amines of LG_2 and L^2G_2 had an even greater brain uptake when compared to their corresponding diacetate salts.

(b) Brain Uptake Index (BUI) Method. The uptake of the compounds through the BBB was also measured by using the Oldendorf "brain uptake index" (BUI) technique.[8–10] In this method the amount of a ^{14}C-labeled compound entering rat brain during a single pass through the brain microcirulation in a 5-s period is determined as a percent of the amount of ^3H_2O (as a standard) injected with the sample. Table II gives the BUI data (uncorrected) for these GABA-lipid esters in comparison to GABA. The lipid esters of GABA had BUI values of 26–53%, a 15- to 25-fold increase above the results for GABA, with the unsaturated derivatives having the lower values.

Pharmacological Properties. The general motor activity of Balb-c mice was measured in a Stoelting electronic activity monitor (EAM) apparatus[11] for 60 min after an intraperitoneal (ip) injection of the diacetate salt of the lipid. All four lipids caused statistically significant ($p <$

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0.001) substantial decrease in the general motor activity in mice at a dose of 30 mg/kg (Table III). A control experiment using stearoylglycerol at an equivalent dose, by ip injection, indicated a small increase (2.4%, not significant) in activity in mice. Thus the stearoyl (**3a**) and oleoyl (**3b**) lipids resulted in a 62% and 75% decrease of the general motor activity, respectively, whereas the linoleoyl (**3c**) and linolenoyl (**3d**) lipids produced an 89% and 83% depression of the general motor activity, respectively. Analysis of variance showed the overall differences among the four lipid effects to be significant ($p < 0.02$). A specific comparison (t test) of the combined stearoyl and oleoyl lipid effects vs. the linoleoyl and linolenoyl lipid effects showed the difference to be highly significant ($p < 0.005$). These observations suggest that the fatty acid side chain of the di-GABA lipid might have an influence on the activity of the drug. It appears that an increase in the number of double bonds in the fatty acid side chain enhances the in vivo inhibitory effects of these lipids; this is, in fact, contrary to the expected result which is predicted by measurement of the octanol/water partition coefficients, BUI, and BPI measurements, which show a decrease with increased degree of unsaturation in the molecules. Thus the lipophilicity alone cannot be considered to be the only criterion for the pharmacological activity of these GABA lipids.

Conclusion

These newly synthesized C-18 glyceryl lipid esters of GABA represent a series of compounds that contain only one variable in their molecular structure, i.e., the number of double bonds present in their C-18 side chain. A study of their uptake into mouse brain showed that their BPI and BUI values were generally highest for the compounds which had the highest octanol/water partition coefficients and the least amount of unsaturation. Their neuropharmacological properties, however, as measured by their effects on the activity of mice, were found to increase approximately with the number of double bonds present, becoming a maximum for the linoleoyl and linolenoyl derivatives. Since the pharmacological activity of the compounds is obtained by the release of GABA by esterases present in the brain,[5,6] the role of the lipid could be a determinant in the rate of hydrolysis of a compound or a factor in the binding of the molecules at cell membranes in the vicinity of GABA receptor sites. We do not yet know which, if any, of these possibilities are important in influencing biological activity. An increase in unsaturation, however, represents a molecular factor that improves pharmacological activity.

Experimental Section

IR spectra were recorded on a Perkin-Elmer infracord spectrophotometer and are reported in reciprocal centimeters. NMR spectra were recorded on a CFT 20 spectrometer. The NMR and IR spectra were characteristic for the compounds. Elemental analyses were performed by the Midwest Microlab Ltd., Indianapolis, IN, and were in agreement (±0.4%) with the proposed structures. Thin-layer chromatography (TLC) was performed on 100-μm-thick precoated silica gel chromatogram sheets by Eastman. For column chromatography, silica gel (Merck Reagents, Kieselgel 60, 230–400 mesh) was used as the adsorbant. High-performance liquid chromatography (HPLC) was carried out on a Waters HPLC instrument with a refractive index detector.

Monoacylglycerols. The monoacylglycerols (**1a–c**; R = stearoyl, oleoyl, and linoleoyl) were purchased from Serdary Research Laboratories, London, Ontario, Canada. Monolinolenoylglycerol (**1d**) was synthesized as previously described.[6]

1-Stearoyl-2,3-bis[4-[(*tert*-butoxycarbonyl)amino]-butyryl]propane-1,2,3-triol (2a). A solution of monostearoylglycerol (**1a**) (250 mg, 0.698 mmol), Boc-GABA anhydride (580 mg), and 4-(dimethylamino)pyridine (180 mg) in 35 mL of benzene was stirred at room temperature under nitrogen atmosphere overnight. This was then washed sequentially with an ice-cold solution of 0.1 N HCl, 5% (w/v) solution of sodium bicarbonate, and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated to yield a viscous product, 504 mg (99%). TLC: R_f 0.54 [ethyl acetate/petroleum ether (1:1)] 0.77 [$CHCl_3/CH_3OH$ (9:1)]. IR (neat): 3430, 1766, 1697 cm^{-1}. Anal.[1] ($C_{32}H_{72}N_2O_{10}$) C (calcd 64.29, found 64.75) H, N. DCI MS: m/e (relative intensity) 730 ($M + H^+$), 747 ($M + NH_4^+$), 675 (15), 629 (55), 573 (42), 529 (33), 505 (53), 444 (85).

1-Stearoyl-2,3-bis(4-aminobutyryl)propane-1,2,3-triol (3a). A solution of 116 mg (0.150 mmol) of **2a** and 2 mL of trifluoroacetic acid was taken in 25 mL of methylene chloride and was stirred under nitrogen atmosphere at 4 °C overnight. This was concentrated and the residue was taken in 15 mL of chloroform. The chloroform solution was washed with dilute sodium bicarbonate mixed with brine, dried over anhydrous Na_2SO_4, and concentrated after addition of 35 μL of acetic acid to yield the diacetate of **3a** (100 mg, 97%). TLC: R_f 0.33 [(chloroform/methanol/acetic acid (9:3:0.5)]. IR (neat): 3250, 1730, 1670 cm^{-1}. Anal. of trifluoroacetate ($C_{33}H_{58}F_6N_2O_{10}\cdot 2H_2O$) C, H, N.

The ^{14}C-labeled stearoyl di-GABA glyceride (**3a**) was synthesized by the same procedure, starting from *N*-Boc[U-^{14}C]GABA anhydride, and had a specific activity of 0.427 mCi/mmol. TLC: Boc derivative of R_f 0.54 [EtOAc/petroleum ether (1:1)], 0.77 [$CHCl_3/CH_3OH$ (9:1)], diacetate R_f 0.33 [$CHCl_3/CH_3OH/CH_3COOH$ (9:3:0.5)].

1-Oleoyl-2,3-bis[4-[(*tert*-butoxycarbonyl)amino]-butyryl]propane-1,2,3-triol (2b). A mixture of monooleoylglycerol (264 mg, 0.745 mmol), Boc-GABA anhydride (620 mg), and 4-(dimethylamino)pyridine (181 mg) in 30 mL of benzene was stirred at room temperature overnight under nitrogen atmosphere. The reaction upon workup yielded 519 mg of the product (95%) as a viscous semisolid. TLC: R_f 0.54 [ethyl acetate/petroleum ether (1:1)], 0.77 [$CHCl_3/CH_3OH$ (9:1)]. IR (neat): 3550, 1690, 1750 cm^{-1}. Anal. ($C_{39}H_{70}N_2O_{10}$) C, H, N.

1-Oleoyl-2,3-bis(4-aminobutyryl)propane-1,2,3-triol (3b). A solution of oleoyl-di-Boc-GABA-glycerol (159 mg, 0.219 mmol) and trifluoroacetic acid (2-mL) in 20 mL of methylene chloride was stirred at 4 °C under nitrogen atmosphere overnight. By a procedure similar to that for **3a**, the reaction upon workup yielded 106 mg of the viscous product (92%). TLC: R_f 0.34 [(chloroform/methanol/acetic acid (9:3:0.5)]. IR (neat): 3260, 1740, 1680 cm^{-1}. Anal. acetate ($C_{33}H_{62}N_2O_{10}\cdot 1.5H_2O$) C, H, N.

By the same route the ^{14}C-labeled oleoyl di-GABA lipid was synthesized starting from labeled *N*-Boc-[U-^{14}C]GABA anhydride. Overall yield, 77%; specific activity, 0.435 mCi/mmol. TLC: Boc derivative R_f 0.54 [EtOAc/petroleum ether (1:1)], 0.77 [$CHCl_3/CH_3OH$ (9:1), diacetate R_f ($CHCl_3/CH_3OH/CH_3COOH$ (9:3:0.5)].

1-Linoleoyl-2,3-bis[4-[(*tert*-butoxycarbonyl)amino]-butyryl]propane-1,2,3-triol (2c). A solution of monolinoleoylglycerol, 295 mg (0.83 mmol), Boc-GABA anhydride (650 mg), and 4-(dimethylamino)pyridine (205 mg) in 35 mL of benzene was stirred at room temperature under nitrogen atmosphere overnight. By a procedure similar to that for **2a**, the product (**2c**) was obtained as a viscous material, 500 mg (0.69 mmol) (86%). TLC: R_f 0.54 [ethyl acetate/petroleum ether (1:1)], 0.77 [$CHCl_3/CH_3OH$ (9:1)]. IR (neat): 3450, 1725, 1680 cm^{-1}. Anal. ($C_{39}H_{68}N_2O_{10}$) C, H, N.

1-Linoleoyl-2,3-bis(4-aminobutyryl)propane-1,2,3-triol (3c). A solution containing 1-linoleoyl-2,3-bis[4-[(*tert*-butoxycarbonyl)amino]butyryl]propane-1,2,3-triol, 150 mg (0.21 mmol) and 1.5 mL of trifluoroacetic acid in 15 mL of methylene chloride was stirred at 4 °C under nitrogen atmosphere overnight. This was concentrated and the residue was taken up in 15 mL of chloroform. The chloroform solution was washed with dilute sodium bicarbonate solution, dried (Na_2SO_4), and concentrated after addition of 40 μL of acetic acid to yield 138 mg of product (99%) as viscous material. TLC: R_f 0.34 [chloroform/methanol/acetic acid (9:3:0.5)]. IR (neat): 3250, 1720, 1655 cm^{-1}. Anal. of trifluoroacetate ($C_{33}H_{54}F_6N_2O_{10}\cdot 1.5H_2O$) C, H, N.

This compound was also prepared as ^{14}C-labeled (specific activity, 0.540 mCi/mmol), by using the above procedure using *N*-Boc[U-^{14}C]GABA anhydride. TLC: Boc derivative R_f 0.54

[EtOAc/petroleum ether (1:1)], 0.77 [$CHCl_3/CH_3OH$ (9:1)], diacetate R_f 0.34 [$CHCl_3/CH_3OH$/CH COOH (9:3:0.5)].

Brain-Uptake Studies. Brain Penetration Index Measurements. Each of the ^{14}C-labeled lipids was dissolved in 15% propyleneglycol in water (0.3–0.4 mL) and then injected subcutaneously (sc) into Balb-c mice (male) (20 ± 2 g). After 5 min the animals were sacrificed by cervical fracture, the brain and liver were removed, weighed, and homogenized in 8 and 10 mL, respectively, of brain protein solvent[12] (phosphate buffer containing sodium dodecyl sulfate and EDTA at pH 7.6), and aliquots were counted for ^{14}C in 10 mL of liquiscent (National Diagnostics) with a Beckman liquid scintillation counter. The ^{14}C counts were used to calculate the total quantity of the compound present in brain and liver per gram of tissue. The ratio of the amount in bain as a percent of that present in liver at 5 min was taken as the brain penetration index (BPI). BPI = ([brain]/[liver]) × 100.

Brain Uptake Index Measurements. Male Sprague–Dawley rats (200–300 g) were deeply anesthetized with Nembutal (Abbott Laboratories, Chicago), and the right carotid artery was dissected free. A 0.15-mL bolus of ^3H-labeled water and a ^{14}C-labeled test compound dissolved in 0.01 M HEPES (pH 7.4) was rapidly injected into the artery, and the animal was decapitated 5 s later.[13] The brain was quickly removed and the ipsilateral forebrain dissected out, rinsed, and homogenized in 0.5 mL of buffer consisting of 0.1% SDS, 6 M urea, 20 mM EDTA, and 10 mM sodium phosphate (pH 7.4). Duplicate aliquots of the homogenate were added to 10 mL of Liquiscint (National Diagnostics), and the radioactivity was determined in a Beckman liquid scintillation counter. The results were corrected for quenching and spillover. A brain uptake index (BUI)[9] was calculated for each compound by the formula:

$$BUI = [^{14}C \text{ cpm}/^{3}H \text{ cpm}]\text{brain}/[^{14}C \text{ cpm}/^{3}H \text{ cpm}]\text{injectate}$$

Pharmacology. The lipid conjugate of GABA was used as the diacetate salt dissolved in 0.14 M saline. An injection volume of 0.1–0.2 cc for 20 ± 2 g Balb-c mice was used. Suppression of general motor activity following an intraperitoneal (ip) injection of each test compound in comparison to vehicle control was carried out on a Stoelting electronic activity monitor (EAM) apparatus.[11] Each data point represents the mean of the general motor activity of nine test mice, as compared to the results for nine control mice injected with the vehicle (0.14 M saline).

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Registry No. **1a**, 123-94-4; **1b**, 111-03-5; **1c**, 2277-28-3; **2a**, 108920-48-5; **2b**, 108920-49-6; **2c**, 108920-50-9; **3a**, 108920-51-0; **3a**·2HOAc, 108920-52-1; **3a**·2TFA, 108920-58-7; [^{14}C]-**3a**·2HOAc, 108920-61-2; **3b**, 108920-53-2; **3b**·2HOAc, 108920-54-3; [^{14}C]-**3b**·2HOAc, 108920-63-4; **3c**, 108920-55-4; **3c**·2HOAc, 108920-56-5; **3c**·2TFA, 108920-59-8; [^{14}C]-**3c**·2HOAc, 108920-65-6; **3d**, 93383-17-6; **3d**·2HOAc, 108920-57-6; Box-GABA anhydride, 89231-63-0; [^{14}C]-Boc-GABA anhydride, 89231-64-1.

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Potential Antitumor Agents. 52. Carbamate Analogues of Amsacrine with in Vivo Activity against Multidrug-Resistant P388 Leukemia

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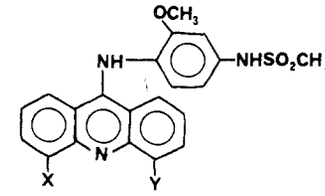
Study of a series of aniline-substituted 9-anilinoacridines related to the antileukemic drug amsacrine showed that a 1′-carbamate group provided increased activity against the multidrug-resistant P388/ADR leukemia subline in vivo. Since activity against such resistant tumors is of great clinical significance, a series of acridine-substituted carbamate derivatives were evaluated against both wild-type and ADR/resistant P388 leukemia and the Lewis lung solid tumor in vivo. Structure–activity relationships for all three tumor lines were similar, with 3-halo-5-methyl and 3-halo-5-methoxy compounds proving the most active. This substitution pattern also provided the highest DNA binding. Such compounds (particularly the 3-chloro-5-methyl and 3-chloro-5-methoxy) have in vivo activity against wild-type P388 and Lewis lung comparable to that of the best amsacrine analogues previously developed (>50% cures), as well as P388/ADR activity. This work essentially completes the development of the amsacrine series of antitumor agents.

The acridine derivative amsacrine (**1**) is a useful clinical antitumor drug, albeit with a limited spectrum of action.[1,2] Work in our laboratory on analogues of amsacrine has concentrated on structural variants with a broader spectrum of action against experimental tumors, using particularly the mouse Lewis lung carcinoma.[3] This work has led to the 4-methyl-5-(methylcarbamoyl) analogue (**2**; CI-921, NSC 343 499), which is currently in clinical trial,[4] and other derivatives now under advanced evaluation.[5-8]



(1) X = Y = H

(2) X = CH_3; Y = CONHCH$_3$

One significant aspect of the clinical profile of amsacrine that has received little attention is the reports[9,10] of its

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