Table III. Potencies of Thienobenzothiazines to Increase HVA Levels in Rat Striatum

no.	ED_{300}[a]	95% CL
6	480	362-636
3	460	347-610
7	36.0	28.2-46.0
4	19.2	14.5-25.4
1	350	264-464
8	6.1	4.8-7.8
5	9.2	7.4-11.4
2	30.1	24.6-36.7
22	18.9	14.8-24.1
19	13.7	10.7-17.4
23	3.27	2.5-4.1
20	1.5	1.2-1.9
17	20.5	16.1-26.2
24	0.75	0.59-0.96
21	0.54	0.42-0.69
18	4.0	3.2-5.2

[a] Dose (μmol/kg) causing a HVA concentration threefold the control level.

washed with H_2O and dried over Na_2SO_4, and the ether was evaporated. The resulting oils were purified by column chromatography (10 g of Al_2O_3), vacuum distillation, or recrystallization from ethanol.

N-[3-[4-(2-Hydroxyethyl)-1-piperazinyl]propyl]thieno[1,4]benzothiazines 17-24. N-(3-Chloropropyl)thieno[1,4]benzothiazine (1.5 mmol) was dissolved in 15 mL of methyl ethyl ketone, and 130 mg of K_2CO_3, 65 mg of KI, and 1.5 mmol of 1-(2-hydroxyethyl)piperazine were added. The mixture was refluxed until a substantial amount of product was formed (TLC), which took sometimes 2 days. Even then unreacted starting material was still present. After water was added to the mixture, the bases were extracted with ether. Washing, drying over Na_2SO_4, and evaporation of the ether yielded yellow-brown sluggish oils, which were distilled in vacuo and converted into their HCl salts by the dropwise addition of a solution of HCl in ether. The precipitates were recrystallized from ethanol/ether.

Pharmacology. Materials and Methods. The compounds were dissolved in 0.9% NaCl immediately before use. The solutions were protected from light, and a small amount of ascorbic acid was added to prevent oxidation of the phenothiazines. Male albino Wistar rats, weighing 180-250 g (TNO, Zeist, The Netherlands), were injected intraperitoneally with 0.3-0.8 mL of the drug solution under investigation. Controls received an injection with saline-ascorbic acid solution. The rats were decapitated 2 h after the injection (or after various time intervals when time-effect curves were studied), and the corpus striatum was dissected.[14] HVA was assayed by a semiautomatic fluorometric method.[15] For each compound, four to six dose levels were studied and three to six rats were used for each dose. HVA in left and right striata was measured separately, as a control for the analytical procedure, and levels given are the mean of the bilateral parts. Doses, causing a threefold increase in HVA concentrations, were calculated from log dose-response curves.

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Synthesis and Anticancer Activity of Nitrosourea Derivatives of Phensuximide

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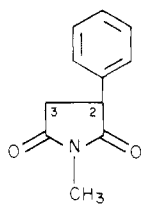
Nitrosourea derivatives **9-13** which utilize phensuximide (1) as the carrier were synthesized as potential central nervous system antitumor agents. The N-(2-chloroethyl)-N-nitrosourea **13** was active in the mouse ependymoblastoma brain-tumor system, as well as the intraperitoneal L1210 leukemia system.

The difficulty in the treatment of primary tumors of the central nervous system (CNS) and solid tumors metastasizing to the CNS from a variety of other primary sites, such as the breast and lungs, has been discussed.[1-4] However, some degree of success has been achieved with the development of new antitumor drugs with CNS activity. Several nitrosourea derivatives have been reported to exhibit activity against murine leukemia L1210 implanted intracerebrally.[5-7]

Driscoll and co-workers[2] have proposed that in the search for new antitumor drugs having CNS activity emphasis should be placed on structural types which penetrate the blood-brain barrier in significant concentrations and have antitumor activity. These workers have described the use of phenothiazines[3,4] and hydantoins[2] as carriers which penetrate the CNS to attach alkylating nitrogen mustard groups.

Our approach to the design of nitrosoureas capable of exhibiting CNS antitumor activity involved the use of phensuximide (1) as the carrier. The N-(2-chloroethyl)-



1

N-nitrosoureas have shown superior antitumor activity to other alkylnitrosoureas.[8] It was anticipated that the succinimide carrier group would penetrate the CNS. Therefore, less reactive alkylnitrosoureas could possibly

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(4) T. Hirata, G. Peng, and J. S. Driscoll, *J. Pharm. Sci.*, 67, 157 (1978).
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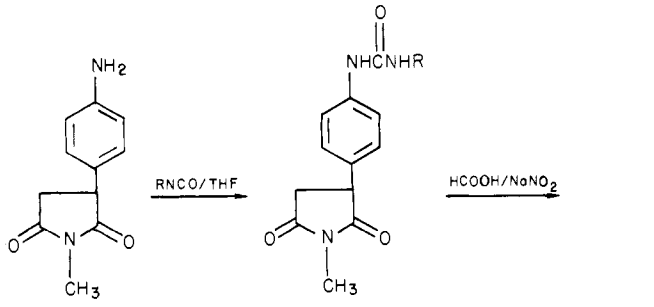
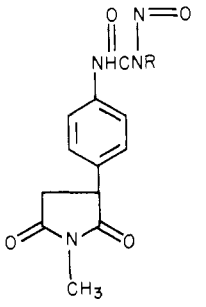
Scheme I



3

4, R = CH_3
5, R = C_2H_5
6, R = n-C_3H_7
7, R = n-C_4H_9
8, R = CH_2CH_2Cl



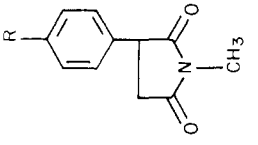
9, R = CH_3
10, R = C_2H_5
11, R = n-C_3H_7
12, R = n-C_4H_9
13, R = CH_2CH_2Cl

exhibit antitumor activity if present in adequate concentrations in the CNS. Nitrosourea groups were attached to the para position of the 2-phenyl group of **1**. This position was chosen since several para substituted derivatives of **1** have shown good anticonvulsant activity.[9] The relationship between the ability to penetrate the CNS and anticonvulsant activity has been discussed by Lien.[10] Therefore, we felt that these nitrosourea derivatives would have the ability to penetrate the CNS and exhibit antitumor activity.

Chemistry. Urea derivatives **4–8** (Table I) of phensuximide (**1**) were prepared by reaction of 2-(*p*-aminophenyl)-*N*-methylsuccinimide (**3**)[9] with an appropriate isocyanate in tetrahydrofuran (Scheme I). Nitrosation of the unsymmetrical ureas **4–8** can theoretically give mixtures of two isomeric nitrosoureas. However, the position of the nitrosation can be controlled to a large extent by utilization of a nitrosating medium of anhydrous formic acid.[8] Nitrosation of the ureas **4–8** with dry sodium nitrite powder and essentially anhydrous formic acid gave the nitrosoureas **9–13** (Scheme I) without difficulty. Isomeric purity is most clearly determined by NMR spectroscopy.[8] All of the nitrosoureas **9–13** (Table I) were homogenous on TLC and exhibited sharp melting points, and the NMR spectra were consistent with the assigned structures.

Biological Activity. Nitrosourea derivatives **9–13** were initially screened against the intraperitoneal L1210 mouse leukemia test system (Table II). Only the *N*-(2-chloroethyl)-*N*-nitrosourea derivative **13** exhibited significant

Table I. Physical and Chemical Data of Urea and Nitrosourea Derivatives of Phensuximide



compd	R	mp, °C	recrystn solvent	yield, %	IR cm^{-1} (KBr) NH	C=O[b]	CNH	N=O	formula	anal.[a]
4	NHCONHCH$_3$	201–203	CH$_3$CN	71	3390	1650	1575		$C_{13}H_{15}N_3O_3$	C, H, N
5	NHCONHCH$_2$CH$_3$	187.5–189	EtOAc	59	3390	1650	1560		$C_{14}H_{17}N_3O_3$	C, H, N
6	NHCONHCH$_2$CH$_2$CH$_3$	173–175	EtOAc	67	3390	1650	1590		$C_{15}H_{19}N_3O_3$	C, H, N
7	NHCONHCH$_2$CH$_2$CH$_2$CH$_3$	138–139.5	EtOAc	53	3450	1650	1575		$C_{16}H_{21}N_3O_3$	C, H, N
8	NHCONHCH$_2$CH$_2$Cl	141–142	EtOAc	66	3390	1655	1550		$C_{14}H_{16}ClN_3O_3$	C, H, N
9	NHCON(NO)CH$_3$	144.5–146	MeOH-CH$_3$CN	37	3330	1740	1600	1525	$C_{13}H_{14}N_4O_4$	C, H, N
10	NHCON(NO)CH$_2$CH$_3$	123–125	EtOH	42	3390	1755	1600	1525	$C_{14}H_{16}N_4O_4$	C, H, N
11	NHCON(NO)CH$_2$CH$_2$CH$_3$	115–117	EtOH	33	3335	1710	1600	1560	$C_{15}H_{18}N_4O_4$	C, H, N
12	NHCON(NO)CH$_2$CH$_2$CH$_2$CH$_3$	111.5–112.5	EtOH	57	3390	1710	1600	1540	$C_{16}H_{20}N_4O_4$	C, H, N
13	NHCON(NO)CH$_2$CH$_2$Cl	131.5 dec	EtOH	59	3330	1740	1600	1550	$C_{14}H_{15}ClN_4O_4$	C, H, N

[a] All compounds were analyzed for the elements shown and were within ±0.4% of the calculated value. [b] Urea or nitrosourea C=O.

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Table II. Activity of Succinimide Nitrosoureas against Mouse L 1210 Leukemia[a]

compd	dose range[b]	OD,[c] mg/kg	toxicity, day survivors[d]	animal wt diff, g, T − C	% T/C (cures)[e]
9	200-12.5	100	6/6	−1.2	127
10	200-12.5	200	6/6	−3.8	126
11	400-12.5	50	6/6	0.8	109
12	400-12.5	200	5/6	−2.8	120
13	50-6.25	25	6/6	−4.6	196 (2)
MeCCNU	50-6.25	25	6/6	−2.1	348 (6)

[a] Tests were carried out by A. D. Little under National Cancer Institute auspices. For a detailed description of the test protocol, see R. I. Geran, N. H. Greenberg, M. M. Macdonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, 3(2), 1 (1972). [b] Single intraperitoneal injection on day 1. [c] Optimum dose. [d] Recorded on the 5th day after injection of the compound. [e] A cure in this assay represents a 30-day survival.

Table III. Effect of Compound 13 on the Ependymoblastoma Tumor System[a]

compd	dose range[b]	OD,[c] mg/kg	toxicity, day survivors[d]	animal wt diff, g, T − C	% T/C (cures)[e]
13	12.0-0.75	12.0	9/10	−3.8	240 (2)
MeCCNU	16.0-1.0	16.0	10/10	−3.3	300 (5)
BCNU	16.0-2.0	16.0	10/10	−3.4	300 (9)

[a] See footnote *a* of Table II for test protocol. This test measures the ability of the compound to penetrate the CNS and exert antitumor activity. [b] Day 1-5 treatment schedule. [c] Optimum dose. [d] Recorded on the 5th day after the first injection of the compound. [e] A cure in this assay represents a 30-day survival.

reproducible activity, T/C of 196 and two cures in a group of six mice. However, some toxicity (weight loss greater than 4 g) was present. The fact that none of the *N*-alkyl-*N*-nitrosoureas **9–12** are active is in agreement with the results reported by Montgomery.[8]

The ependymoblastoma intracerebral test system was used to evaluate CNS antitumor activity.[11] Compound **13**, which was the only nitrosourea derivative to exhibit significant activity against mouse L1210, was tested in this tumor system. The nitrosourea **13** showed reproducible activity, a T/C of 240 with two cures in a group of 10 mice at a dose of 12.0 mg/kg (Table III). The activity of this aromatic nitrosourea **13** against the ependymoblastoma intracerebral tumor system is of interest, since previous workers[8] have stated that an aromatic ring may interfere with passage of the nitrosourea across the blood–brain barrier. The structural features of the phensuximide (**1**) carrier group, as well as an adequate log *p* value, allow the nitrosourea **13** to cross the blood–brain barrier and exhibit CNS antitumor activity. However, the compound does not appear to be as active as either MeCCNU or BCNU in this system.

The initial results of this study indicate that the *N*-(2-chloroethyl)-*N*-nitrosourea, **13**, derivative is able to penetrate the CNS and exhibit antitumor activity. Further work is in progress in an attempt to increase the potency of succinimide carriers of alkylating *N*-(2-chloroethyl)-*N*-nitrosoureas.

Experimental Section

Spectra and Analyses. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. IR spectra were recorded as KBr pellets with a Perkin-Elmer 137 spectrophotometer. NMR spectra were recorded on a Varian EM 360A spectrometer. Chemical shifts are reported in parts per million (δ) relative to tetramethylsilane (1%) as the internal standard. Analytical data were obtained from Micro-Analysis, Inc., Wilmington, Del. TLC was performed on precoated silica gel plastic sheets (Macherey-Nagel). Column chromatography was carried out using MN-Kieselgel (70–325 mesh) as the adsorbent.

General Procedure for the Preparation of Ureas of Phensuximide, 4–8. The synthesis of 2-[*p*-[3-(2-chloroethyl)ureido]phenyl]-*N*-methylsuccinimide (8) is representative of the general method. A mixture of **3** (2.00 g, 9.80 mmol) and 2-chloroethyl isocyanate (Eastman Kodak) in SpectrAR grade $CHCl_3$ (75 mL) was stirred at room temperature for 2 h and evaporated under reduced pressure. The solid residue was dissolved in a $CHCl_3$–EtOAc mixture (150 mL) and washed with 10% HCl solution (50 mL) followed by H_2O (50 mL). The organic phase was dried (Na_2SO_4), filtered, and evaporated to afford a white solid. Recrystallization of the solid from EtOAc gave, in two crops, 2.01 g (66%) of **8**: NMR (Me_2SO-d_6) δ 2.73–4.77 (m, including s at 2.93 NCH_3, 10 H), 6.50 (t, 1 H, $NHCOCH_2$), 7.25 (d, J = 9 Hz, 2 H, Ar), 7.53 (d, J = 9 Hz, 2 H, Ar), 8.83 (s, 1 H, C_6H_5NHCO).

General Procedure for the Preparation of Nitrosoureas of Phensuximide, 9–13. The synthesis of 2-[*p*-[3-(2-chloroethyl)-3-nitrosoureido]phenyl]-*N*-methylsuccinimide (13) is representative of the general method. The urea **8** (1.50 g, 4.80 mmol) was dissolved in 99% HCOOH (10 mL), cooled to 0–5 °C, and treated with dry $NaNO_2$ (1.00 g, 14.6 mmol) in small portions over a period of 0.5 h. After stirring for 0.5 h, the reaction mixture was diluted with H_2O (10 mL), stirred for an additional 0.5 h at 0–5 °C, and extracted with EtOAc (3 × 50 mL). The combined EtOAc extracts were dried (Na_2SO_4), filtered, and evaporated to yield a yellow solid. The solid was dissolved in $CHCl_3$ and chromatographed on a silica gel column using $CHCl_3$–MeOH (9:1) as the solvent system. Removal of the solvents afforded 0.97 g (59%) of **13**. An analytical sample was obtained by recrystallization of the solid from EtOH to give pure **13**: NMR (Me_2SO-d_6) δ 2.75–4.43 (m, including s at 2.93, NCH_3, t at 3.73, J = 6 Hz; upfield half of A_2B_2 system due to $NNOCH_2CH_2Cl$ and t at 4.25, J = 6 Hz; downfield half of A_2B_2 system due to $NNOCH_2CH_2Cl$, 10 H), 7.43 (d, J = 9 Hz, 2 H, Ar), 7.83 (d, J = 9 Hz, 2 H, Ar).

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