Potential CNS Antitumor Agents—Phenothiazines I: Nitrogen Mustard Derivatives

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Abstract □ Four phenothiazine derivatives containing the bis(β-chloroethyl)aminopropyl side chain were prepared and evaluated in the murine L-1210, P-388, and B-16 melanoma intraperitoneal tumor systems. Moderate P-388 activity was observed. An aminoethyl phenothiazine mustard was compared with the aminopropyl analogs and was superior in all test systems. None of the compounds tested against the murine ependymoblastoma brain tumor system was active.

Keyphrases □ Phenothiazines—derivatives containing bis(β-chloroethyl)aminopropyl side chain synthesized, CNS antitumor activity screened □ CNS antitumor activity—substituted phenothiazines synthesized, screened □ Antitumor activity, CNS—substituted phenothiazines synthesized, screened □ Structure–activity relationships—substituted phenothiazines synthesized, screened for CNS antitumor activity

Phenothiazine derivatives are well known for their central nervous system (CNS) activity (1–3). Several studies also showed that some common psychotropic agents within this family (*e.g.*, chlorpromazine) produce antitumor effects (4–7). Phenothiazines containing reactive alkylating groups also have been evaluated. Haloacyl derivatives (8, 9) were active in the sarcoma 180 system. Jackson and Shirley (10) prepared several phenothiazine derivatives containing the nitrogen mustard function. These compounds were reported to show no significant antitumor activity, but they were 10-aminoethyl derivatives rather than the more common 10-aminopropyl analogs.

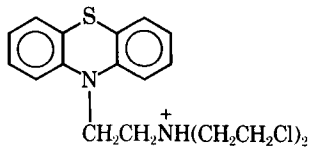
Three methylene groups between N-10 and the amino group in the side chain are known to be optimum for phenothiazine antipsychotic potency (1). Four aminopropyl nitrogen mustard analogs (X*a*–X*d*) were prepared and evaluated to determine whether the extra methylene group might enhance the antitumor activity and provide a potentially useful CNS antitumor agent. These compounds were compared with the aminoethyl analog (I) that had been reported and evaluated earlier (11, 12). This compound was among a group of similar materials that had activity in the Yoshida ascites system (11, 12).

Compound I was also prepared by Shirley *et al.* (13) for testing at the National Cancer Institute, but the results of the initial limited testing were not published. Therefore, I was used as a model 10-aminoethyl analog for comparison testing with the synthesized 10-aminopropyl compounds.

CHEMISTRY

The necessary intermediate compound, 2-ethylphenothiazine (VI), was prepared through the sequence (II–VI) described previously (12).

The bis(β-chloroethyl)aminopropyl derivatives of phenothiazine were synthesized as described in Scheme I (Table I). Intermediates VIII*a*–VIII*d* and IX*a*–IX*d* were oils which could be used in the next step without further purification.



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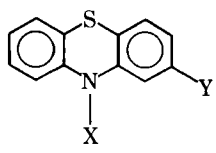
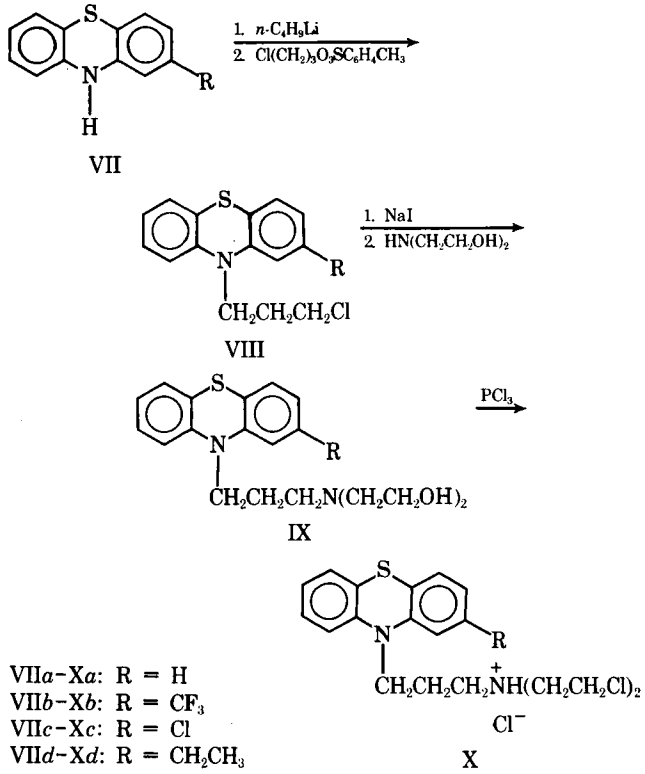


II: X = Y = H
III: X = CH_3CO, Y = H
IV: X = Y = CH_3CO
V: X = H, Y = CH_3CO
VI: X = H, Y = C_2H_5

RESULTS AND DISCUSSION

Standard National Cancer Institute protocols (15) were followed. The results of *in vivo* studies in the intraperitoneal lymphoid leukemia L-1210, lymphocytic leukemia P-388, and B-16 melanocarcinoma models as well as in the intracerebral ependymoblastoma system are shown in Table II. Also included are *in vitro* KB cell culture assays. Compounds were considered active *in vivo* if they gave activity (T/C) values (15) equal to or greater than the following: L-1210 leukemia, 125%; P-388 leukemia, 125%; B-16 melanoma, 140%; and ependymoblastoma, 140%. Compounds were considered cytotoxic in the KB cell culture system if they possessed an LD_{50} of 4 μg/ml or less.

All compounds reported here, including intermediates, were evaluated in the L-1210 leukemia system. No intermediate was active. Only I and X*a* were marginally active against the L-1210 tumor. Significant activity was observed in the more sensitive P-388 system with I, X*a*, and X*d*. Marginal activity was observed with the trifluoromethyl (X*b*) and chloro (X*c*) analogs.



VII

VIII

IX

X

VII*a*–X*a*: R = H
VII*b*–X*b*: R = CF_3
VII*c*–X*c*: R = Cl
VII*d*–X*d*: R = CH_2CH_3

Scheme I

Table I—Physical and Chemical Data

Compound	Melting Point	Yield, %	Formula	Analysis, % Calc.	Found
X*a*	156–159°	42	$C_{19}H_{23}Cl_3N_2S \cdot \frac{1}{2}H_2O$	C 53.48 H 5.43 Cl 24.92 N 6.56 S 7.50	53.75 5.31 24.47 6.37 7.44
X*b*	181°	53	$C_{20}H_{22}Cl_3F_3N_2S$	C 49.44 H 4.56 Cl 21.89 N 5.77 S 6.60	49.01 4.72 21.77 5.73 6.59
X*c*	136–139°[a]	54	$C_{19}H_{22}Cl_4N_2S \cdot \frac{1}{2}H_2O$	C 49.47 H 5.03 Cl 30.75 N 6.07 S 6.95	49.85 4.90 30.93 5.78 6.94
X*d*	144°	70	$C_{21}H_{27}Cl_3N_2S$	C 56.56 H 6.10 Cl 23.86 N 6.28 S 7.19	56.25 6.09 23.56 5.95 7.50

[a] Lit. (14) mp 145°.

Earlier studies (5) indicated that chlorpromazine had a marked affinity for melanin-containing tissues and affected tumor growth in the B-16 melanoma system. It was suspected that this property might be a characteristic of the phenothiazine structure. However, of the four compounds tested, only I was active in that system.

The initial objective of this investigation was the preparation of potential CNS antitumor agents. Since intracerebral antitumor activity is normally significantly less than intraperitoneal activity in any given tumor system (16), the compounds in this study were considered to have insufficient L-1210, P-388, or B-16 activity intraperitoneally to be tested in the corresponding intracerebral tumor system. Three compounds were tested in the intracerebral ependymoblastoma murine brain tumor model (16, 17), but no activity was observed. This finding is in contrast to the high activity observed when hydantoins rather than phenothiazine were used as the carrier for the bis(β-chloroethyl)amino group (18). The poor activity might be related to the very high ($\log P > 7$) partition coefficients (19) calculated for the free base of the phenothiazine alkylating agents.

Compounds X*a* and X*c* were reported to affect cell respiration and to increase murine survival time when injected with Ehrlich sarcoma cells (20). Borderline KB activity was observed only with the 2-ethyl derivative, X*d*, in our *in vitro* test. *In vitro* activity did not correlate with activity in any *in vivo* test system.

The antitumor results show the aminoethyl compound (I) to be clearly superior to the aminopropyl derivatives (X*a*–X*d*). This relationship between the number of methylene groups and antitumor activity is more similar to that observed for phenothiazine antihistaminergic activity (21) than that for antipsychotic activity. The antihistaminergic parallel is also consistent with the general trend among X*a*–X*d*, which indicates a reduction of antitumor activity with electronegative substitution in the 2-position of the phenothiazine ring.

Based on available data, further synthetic studies with phenothiazines as carriers for nitrogen mustard groups are not planned.

EXPERIMENTAL[1]

10-Bis(2-chloroethyl)aminoethylphenothiazine (I)—This compound was prepared and supplied by Shirley *et al.* (13).

10-Acetylphenothiazine (III)—Phenothiazine (50.0 g, 0.25 mole) was refluxed in a mixture of acetic anhydride (50 ml) and xylene (90 ml) for 6 hr. Upon cooling, the resulting crystals were filtered and washed with petroleum ether to give 55 g (92%) of a light-gray-green powder, mp 197° [lit. (22) mp 197–198°].

2,10-Diacetylphenothiazine (IV)—This compound was prepared on a 40-g scale in 74% yield by the method of Takada and Nishimura (23). The crude solid was used without purification for the next step in the sequence.

2-Acetylphenothiazine (V)—Crude IV was hydolyzed with 10% HCl in acetic acid at reflux for 15 min. The resulting product was recrystallized from benzene to give yellow crystals, mp 188–190° [lit. (23) mp 189–195°].

2-Ethylphenothiazine (VI)—This compound was prepared on a 6-g scale. Recrystallization from hexane and benzene gave crystals, mp 132–133° [lit. (23) mp 135°].

2-(Trifluoromethyl)-10-[3-[bis(2-hydroxyethyl)amino]propyl]phenothiazine (IX*b*) (General Procedure for IX*a*–IX*d*)—To a solution of 5.21 g (19.5 mmoles) of 2-trifluoromethylphenothiazine in 120 ml of dry ether was added 15 ml of 1.6 *M* butyllithium (24 mmoles) in hexane under dry, deoxygenated, nitrogen gas (passed through alkaline pyrogallol solution, calcium chloride, and potassium hydroxide tubes in that order). The solution was stirred under reflux for 30 min and cooled in an ice bath. A solution of 5.25 g (21 mmoles) of γ-chloropropyl *p*-toluenesulfonate in 30 ml of dry ether was added. The mixture was stirred (ice bath) for 1 hr and then at room temperature for 18 hr. A brown precipitate was formed.

Benzene and water were added to the reaction mixture; the benzene layer was then separated, washed with saturated sodium chloride, and dried over anhydrous sodium sulfate. Evaporation of benzene *in vacuo* gave a dark oil, which was mixed with 3.05 g (20 mmoles) of sodium iodide and 50 ml (520 mmoles) of diethanolamine. The stirred mixture was heated at 150–165° for 18 hr. After cooling, 100 ml of water was added to the reaction mixture and the resulting emulsion was extracted with ether. The ether extracts were washed with water and extracted with cold 5 *N* HCl. 2-(Trifluoromethyl)phenothiazine (850 mg) was recovered from the ether layer. Dark oil drops were present in the aqueous layer. The aqueous layer was made alkaline with concentrated potassium hydroxide and extracted with ether.

The ether solution was dried (sodium sulfate) and evaporated to give 4.0 g of an oil, which was then chromatographed on silica gel (60–200 mesh, 100 g). Unreacted 2-(trifluoromethyl)phenothiazine was first eluted with benzene, and then a homogeneous oily product (3.5 g, 44%) was eluted with benzene–acetone (1:1); NMR ($CDCl_3$): δ 1.90 (2H, t, $CH_2CH_2CH_2$), 2.53 (6H, distorted triplet, CH_2 adjacent to ammonium nitrogen), 2.71 (2H, s, OH), 3.50 (4H, t, CH_2OH), 3.95 (2H, t, CH_2 adjacent to N-10), and 6.8–~ 7.2 (7H, m, aromatic).

All derivatives in this series were oils. The following yields were obtained: IX*a*, 40%; IX*b*, 44%; IX*c*, 55%; and IX*d*, 52%. Compounds obtained in this manner were used without further purification to obtain the final products.

General Procedure for 2-Substituted 10-[3-[Bis(2-chlo-

[1] Melting points were recorded on a Thomas-Hoover melting-point apparatus and are uncorrected. Elemental analyses were performed by the National Institute of Arthritis, Metabolism, and Digestive Diseases, National Institutes of Health, Bethesda, Md. Phenothiazine and its 2-chloro and 2-trifluoromethyl analogs were obtained commercially. NMR spectra were obtained on a Varian HA-100D spectrometer, with tetramethylsilane as an internal standard.

Table II—Biological Activity[a]

Compound	Antitumor Activity								KB Cell Culture Activity, μg/ml[e]
	L-1210 Lymphoid Leukemia[b]		P-388 Lymphocytic Leukemia[b]		B-16 Melanocarcinoma[c]		Ependymoblastoma[d]		
	OD[f]	T/C[g]	OD	T/C	OD	T/C	OD	T/C	
I	300[h]	141	35	210	12.5	147	30	126	100
Xa	25	125	12.5	169	12.5	128	25	110	100
Xb	25	113	12.5	128					59
Xc	50	122	6.25	125	6.25	138			19
Xd	12.5	111	12.5	163	12.5	115	25	108	5

[a] Protocols and test systems were described in Ref. 15. [b] Day 1, 5, and 9 treatment schedule, unless noted otherwise. [c] Day 1–9 treatment schedule of intraperitoneally implanted tumor. [d] Day 1–5 treatment schedule: intraperitoneal treatment of intracerebrally implanted tumor. [e] Concentration required to inhibit cell growth to 50% that of the control. Cells were derived from a human epidermoid carcinoma of the mouth. [f] Optimum dose (milligrams per kilogram per injection). [g] T/C = (treated survival ÷ control survival) × 100%. [h] Day 1 only treatment schedule.

roethyl)amino]propyl]phenothiazine Hydrochlorides (Xa–Xd)—A solution of 4 mmoles of phosphorus trichloride in 10 ml of dry benzene was added dropwise to a solution of 2 mmoles of 2-substituted 10-[3-[bis(2-hydroxyethyl)amino]propyl]phenothiazine in 20 ml of dry benzene. The solution was heated gently during the 30-min addition period. The reaction solution was then refluxed for 3.5–5 hr, cooled, and treated with 20 ml of water.

Sodium carbonate was added to the cold mixture with vigorous shaking until the aqueous layer turned to a thick paste. The mixture was extracted repeatedly with benzene. The benzene extracts were dried (sodium carbonate) and evaporated to give an oil, which was dissolved in 30 ml of dry ether and treated with hydrogen chloride gas. The resulting crude hydrochloride was recrystallized from chloroform–ether; NMR ($CDCl_3$) for Xc: δ 2.35 (2H, broad, $CH_2CH_2CH_2$), 3.33 (6H, t, CH_2 adjacent to ammonium nitrogen), 3.85 (4H, t, CH_2Cl), 4.06 (2H, t, CH_2 adjacent to N-10), and 6.8 –~ 7.2 (7H, m, aromatic).

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