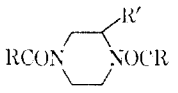
TABLE I[a]



No.	R	R′	Mp, °C[b]	Yield, %	Formula[d]	Analyses[c]	Act. appearing after 24 hr, wc[e,f]
I	$ClCH_2$	H	135–137				Inactive, toxic
II	$ClCH_2CH_2$	H	108–109				0.099, alkylating
III	$BrCH_2CH_2$	H	106–107				0.0066_1 alkylating
IV	CH_3CHCl	H	162–163				0.0066, sl inhib mitosis
V	CH_3CHBr	H	163–164				0.0066, sl inhib mitosis
VI	$CH_3CHClCH_2$	H	101–103				0.026, statmodieretic, sl alkylating
VII	$ClCH_2CHCH_3$	H	112–115				0.066, statmodieretic, sl alkylating (after 48 hr)
VIII	C_2H_5CHBr	H	128–130				0.0066, inhib mitosis, sl alkylating
IX	$(CH_3)_2CBr$	H	189–190				0.04, statmodieretic, sl alkylating
X	$(CH_3)_2CHCHBr$	H	185–187				Inactive, toxic
XII	$BrCH_2CH_2$	CH_3	82–83	27	$C_{11}H_{18}Br_2N_2O_2$	Br, N	0.0099, alkylating
XVI	$ClCH_2CHCH_3$	CH_3	119–120	77	$C_{13}H_{22}Cl_2N_2O_2$	Cl, N	0.033, statmodieretic, sl alkylating
XVII	C_2H_5CHBr	CH_3	136–137	23	$C_{13}H_{22}Br_2N_2O_2$	Br, N	0.0066, inhib mitosis, very sl alkylating
XVIII	$(CH_3)_2CBr$	CH_3	171–172	18	$C_{13}H_{22}Br_2N_2O_2$	Br, N	0.052, statmodieretic, sl alkylating
XIX	$(CH_3)_2CHCHBr$	CH_3	194–195	42	$C_{13}H_{26}Br_2N_2O_2$	Br, N	Inactive, toxic
XX	CH_3CHCH	H	188–189				Inactive

[a] For analytical data of other compounds see ref 1a and 3. [b] All melting points (capillary) are uncorrected. [c] Analytical values were within ±0.4% of theory. Halogen was determined mercurimetrically after alkaline hydrolysis, nitrogen by the Kjeldahl method. [d] The infrared spectra were taken as Nujol mulls using a UR-10 Zeiss spectrophotometer. 2-Methylpiperazine derivatives show characteristic maxima (cm^{-1}) in the ranges: ν amide I 1640–1650, I overtone 3250–3280; ν(asym) CH_2–X 3035–3050, C–Cl 750, C–Br 580–617. Complete spectral data are available on request. [e] Alkylating signifies a full chromatoclazic effect; slightly alkylating, that only an insignificant part of cells show chromatoclazic effect; statmodieretic, a strong formation of binuclear and plurinuclear cells.

Experimental Section

N,N′-Bis(haloacyl)-2-methylpiperazines.—All derivatives were obtained by the same method. Five grams (0.05 mole) of 2-methylpiperazine, 30 ml of $CHCl_3$, and 20 g of $NaHCO_3$ were treated with 0.12 mole of the appropriate haloacyl chloride or bromide in 30 ml of $CHCl_3$ (vigorous stirring and cooling at 5–8°). Then, 5 ml of H_2O and 10 g of $NaHCO_3$ were added, and stirring was continued for an additional 4 hr. After adding 20 g of anhydrous $MgSO_4$, the mixture was kept overnight. The flask content was filtered, and the precipitate was washed with 100 ml of $CHCl_3$. The $CHCl_3$ was distilled under reduced pressure, and the residue, usually an oil, was put aside for crystallization. The crude material was then purified by recrystallization from EtOAc–cyclohexane, then from EtOH.

Nitrogen Mustard Derivatives in the Phenothiazine and Benzophenothiazine Series

T. GERALD JACKSON AND DAVID A. SHIRLEY[1]

Department of Chemistry, The University of Tennessee, Knoxville, Tennessee 37916

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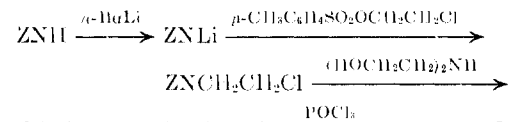
This paper reports the synthesis of selected nitrogen mustard derivatives in the phenothiazine and benzophenothiazine series for anticancer evaluation (Tables I–IV). The synthetic route chosen is that previously employed by Shirley, *et al.*,[2] and is shown in Scheme I.

The nitrogen mustard types were tested by CCNSC. Toxicity tests were performed by intraperitoneal daily injections in dose levels of 3.0–100 mg/kg using rats as the host. Three animals were used in each of four dose levels with injections being continued for 5 days. All test animals survived for 10 days in tests when the dose level did not exceed 33 mg/kg. In tests in which the dose level was 100 mg/kg the number of animals surviving varied with the compound under consideration. Tests were performed, using standard screening procedures, with the compounds against Walker carcinosarcoma 256, Dunning leukemia, Lewis lung carcinoma, lymphoid leukemia, Sarcoma 180, and human epidermoid carcinoma of the nasopharynx at dose levels through 200 mg/kg/day. None of the compounds showed significant activity.

SCHEME I



ZN = substituted phenothiazinyl and benzophenothiazinyl

Experimental Section

Elemental microanalyses were performed by Weiler and Strauss Microanalytical Laboratory, Oxford, England. Melting points were determined on a Mel-Temp melting point block. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

10-(2-Chloroethyl)-3-methoxyphenothiazine.[3]—*n*-BuLi (10 ml, 1.48 *M*) in hexane was added to a suspension of 3.0 g (13 mmoles) of 3-methoxyphenothiazine[4] in 75 ml of dry ether. The solution was stirred under reflux for 30 min and then cooled in an ice bath. A solution of 3.5 g (15 mmoles) of 2-chloroethyl *p*-toluenesulfonate in 25 ml of dry ether was added. The mixture was

(1) To whom inquiries concerning this paper should be sent.

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TABLE I

N-CHLOROETHYL DERIVATIVES

$ZNCH_2CH_2Cl$

No.	ZN	Mp, °C	Eluent	Yield, %	Formula[f]
1	2-Chlorophenothiazinyl[c,3]	118–120	*a*	47	$C_{14}H_{11}Cl_2NS$[g]
2	2-(Trifluoromethyl)phenothiazinyl[d]	80–81	*b*	50	$C_{15}H_{11}ClF_3NS$[h]
3	9-Methylbenzo[*a*]phenothiazinyl	88–90	*a*	35	$C_{19}H_{16}ClNS$
4	10-Methylbenzo[*a*]phenothiazinyl	87–88	*a*	45	$C_{19}H_{16}ClNS$
5	Benzo[*b*]phenothiazinyl[e]	163–164	...	37	$C_{18}H_{14}ClNS$

[a] 3:1 ligroin (bp 70–90°)–C_6H_6. [b] Ligroin (bp 60–90°). [c] Woelm neutral alumina, activity grade 1. [d] Kindly supplied by Dr. Harry L. Yale of the Squibb Institute for Medical Research, New Brunswick, N. J. [e] Recrystallized from $CHCl_3$. [f] All compounds showed proper analytical values for C, H, N unless otherwise noted. [g] Not analyzed. [h] C: calcd, 54.62; found, 55.11.

TABLE II

2-[BIS(2-HYDROXYETHYL)AMINO]ETHYL DERIVATIVES

$ZNCH_2CH_2N(CH_2CH_2OH)_2$

No.	ZN	Mp, °C	Eluent	Yield, %	Formula[d]
1	2-Chlorophenothiazinyl	Oil	*a*	82	$C_{18}H_{21}ClN_2O_2S$
2	2-(Trifluoromethyl)phenothiazinyl	Oil	*b*	79	$C_{19}H_{21}F_3N_2O_2S$
3	9-Methylbenzo[*a*]phenothiazinyl	Oil	*a*	97	$C_{23}H_{26}N_2O_2S$
4	10-Methylbenzo[*a*]phenothiazinyl	Oil	*a*	90	$C_{23}H_{26}N_2O_2S$
5	Benzo[*b*]phenothiazinyl	94–95	*c*	72	$C_{22}H_{24}N_2O_2S$

[a] 4:1 C_6H_6–Me_2CO. [b] 9:1 C_6H_6–Me_2CO. [c] 7:3 C_6H_6–Me_2CO. [d] Difficulty was experienced in securing proper elementary analytical values on the bis(2-hydroxyethyl)aminoethyl derivatives. However, the bis(2-chloroethyl)aminoethyl derivatives and the hydrochlorides gave proper analytical values.

TABLE III

2-[BIS(2-CHLOROETHYL)AMINO]ETHYL DERIVATIVES[a]

$ZNCH_2CH_2N(CH_2CH_2Cl)_2$

No.	ZN	Yield, %	Formula[b]
1	2-Chlorophenothiazinyl	43	$C_{18}H_{19}Cl_3N_2S$
2	2-(Trifluoromethyl)phenothiazinyl	60	$C_{19}H_{19}Cl_2F_3N_2S$
3	9-Methylbenzo[*a*]phenothiazinyl	33	$C_{23}H_{24}Cl_2N_2S$
4	10-Methylbenzo[*a*]phenothiazinyl	67	$C_{23}H_{24}Cl_2N_2S$
5	Benzo[*b*]phenothiazinyl	59	$C_{22}H_{22}Cl_2N_2S$

[a] All compounds in this table were oils. [b] C, H, N analyses.

TABLE IV

2-[BIS(2-CHLOROETHYL)AMINO]ETHYL HYDROCHLORIDES

$ZNCH_2CH_2N(CH_2CH_2Cl)_2 \cdot HCl$

No.	ZN	Mp, °C	Yield, %	Formula[a]
1	2-Chlorophenothiazinyl	140–142	83	$C_{18}H_{20}Cl_4N_2S$
2	2-(Trifluoromethyl)phenothiazinyl	140–141	97	$C_{19}H_{20}Cl_3F_3N_2S$
3	9-Methylbenzo[*a*]phenothiazinyl	176–177	94	$C_{23}H_{25}Cl_3N_2S$
4	10-Methylbenzo[*a*]phenothiazinyl	160–161	96	$C_{23}H_{25}Cl_3N_2S$
5	Benzo[*b*]phenothiazinyl	151–153	88	$C_{22}H_{23}Cl_3N_2S$

[a] C, H, N analyses.

stirred at ice-bath temperature for 1 hr and at room temperature for 18 hr. An almost colorless precipitate formed in the course of the reaction. C_6H_6 and H_2O were added to the reaction mixture. The C_6H_6–Et_2O layer was separated and concentrated by evaporation. Ligroin (bp 70–90°) was added to produce a 3:1 mixture by volume of ligroin and benzene. Chromatographic separation of the solution over Alcoa grade F-20 alumina, using a 3:1 mixture of ligroin (bp 70–90°) and benzene as eluent, yielded 2.5 g (67%) of white solid, mp 82–83°.

10-{2-[Bis(2-hydroxyethyl)amino]ethyl}-3-methoxyphenothiazine.—A solution of 2.0 g (6.9 mmoles) of 10-(2-chloroethyl)-3-methoxyphenothiazine in 30 ml of diethanolamine was stirred at 140–150° for 30 hr and cooled to room temperature, and 50 ml of cold H_2O was added. The suspension was extracted ($CHCl_3$) and the extract was washed (H_2O). The $CHCl_3$ was evaporated and the resulting oil was dissolved in C_6H_6. The solution was chromatographed over 60–100 mesh Florisil. Elution with C_6H_6 yielded a small amount of 10-(2-chloroethyl)-3-methoxyphenothiazine. The product, an oil, was eluted with a 1:4 mixture of acetone and benzene. The yield was 1.8 g (73%). *Anal.* ($C_{19}H_{24}N_2O_3S$) H, N; C: calcd, 63.30; found, 63.77, 63.80.

10-{2-[Bis(2-chloroethyl)amino]ethyl}-3-methoxyphenothiazine.—$POCl_3$ (10 ml) was added slowly to 2.0 g (6.8 mmoles) of the corresponding hydroxyethyl compound at ice temperature. The mixture was allowed to warm slowly to room temperature and then heated on a steam bath for 1 hr. Excess $POCl_3$ was removed under reduced pressure and the residual oil was dissolved in acetone. The solution was poured over crushed ice and neutralized (Na_2CO_3). The resulting solution was extracted several times ($CHCl_3$). The combined extracts were washed (H_2O), concentrated, and placed on a chromatographic column of Florisil. The product was eluted with C_6H_6 to yield 0.5 g (19%) of oil. Conversion to the **hydrochloride** gave, after crystallization from $CHCl_3$–Et_2O, 94% yield of colorless crystals, mp 108–110°. *Anal.* ($C_{19}H_{23}Cl_3N_2OS$) C, H, N.

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Cysteine Analogs as Potential Amino Acid Antagonists in Bacteria

WALTER A. ZYGMUNT AND TELLIS A. MARTIN

Mead Johnson Research Center, Evansville, Indiana 47721

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Relatively few compounds have been reported to be cysteine antagonists in microorganisms. Allylglycine[1] inhibits in part utilization of cysteine in bacteria and yeast. Klubes and Schultze[2] found that S-(1,2-dichlorovinyl)cysteine inhibited growth of *Escherichia coli* and showed that the L enantiomer was the more active isomer. Cysteine thioethers[3] from chloroethylenes have also been reported to inhibit growth of fungi and algae.

In the present studies, 21 cysteine or cystine analogs were tested as inhibitors of cysteine or cystine utiliza-

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