TABLE I
THE EFFECT OF CROSEMPERINE ON EHRLICH ASCITES TUMOR-BEARING MICE

Dose, mg/kg	No. of daily doses	Survivors	Mean PCV[a] (Test/Control)	T/C
400	1	0/4	...	...
200	1	4/6	2.45 ± 0.4/3.4 ± 0.6	0.72
100	1	6/6	2.46 ± 0.5/3.4 ± 0.6	0.72
50	5	0/5	...	...
25	5	4/5	1.0 ± 0.3/2.2 ± 0.4	0.45

[a] Packed cell volume in milliliters ± standard deviation.

et al.[5] The latter compound was not lethal at 200 mg/kg administered daily for 11 days. If biological activity depends upon opening of the cyclic diester ring structure, these data indicate that crosemperine may be more susceptible to enzymic attack than monocrotaline. Alternatively, the methylated nitrogen of the present compound may alter its distribution properties, resulting in increased toxic effects to the host but reduced toxic effects to the tumor.

Crosemperine was tested up to 1600 μg/ml concentration against *Staphylococcus aureus, Escherichia coli, Bacillus cereus, Saccharomyces cerevisiae, Candida albicans,* and *Aspergillus niger*, using a serial dilution method[7] and was found inactive.

(7) R. F. Smith, D. E. Shay, and N. J. Doorenbos, *J. Bacteriol.*, **85**, 1295 (1963).

Cytostatic Bis(haloacyl) Derivatives of Piperazine and 2-Methylpiperazine

STEFAN GROSZKOWSKI, JULIUSZ SIENKIEWICZ, LILIANA NAJMAN, RODICA OTELEANU, AND MARIA RETEZEANU

Department of Pharmaceutical Chemistry, Medical Academy, Łódź, Poland, and Institute of Pharmaceutical Research, Bucharest, Rumania

Received November 18, 1967

Disubstituted haloacylpiperazines were of interest to us as intermediates which can be used to obtain amine derivatives.[1] Carbon and co-workers[2a] discovered antitumor activity for N,N′-bis(3-bromopropionyl)piperazine, introduced recently under the name pipobroman,[2b] and N,N′-bis(3-chloropropionyl)piperazine was obtained some years ago by one of us.[1a] We decided to synthesize a series of derivatives[3] and to examine their cytostatic activity. Also the mechanism of action of these compounds has not yet been elucidated.[4] In order to explain the relation between chemical structure and cytostatic activity of the compounds, we varied both the acyl substituent, introducing alkyl groups and a halogen atom in positions 2 or 3, and the amine part, replacing piperazine by 2-methylpiperazine.

(1) (a) S. Groszkowski, *Roczniki Chem.*, **34**, 707 (1960); **38**, 229 (1964); (b) S. Groszkowski and J. Sienkiewicz, *Ann. Pharm. Franc.*, in press.

(2) (a) R. J. Stein, J. A. Carbon, J. Langdon, and R. K. Richards, *J. Lab. Clin. Med.*, **56**, 949 (1960); (b) Vercyte®.

(3) (a) S. Groszkowski, J. Sienkiewicz, and L. Najman, *Farmacia* (Bucharest), **15**, 263 (1967); (b) S. Groszkowski, L. Korzycka, and A. Wesolowski, *Roczniki Chem.*, in press.

(4) (a) J. Louis, R. J. Rohn, and R. W. Monto, *Proc. Am. Assoc. Cancer Res.*, **3**, 246 (1961); (b) T. J. McNair, E. A. Wibin, E. T. Hoppe, J. L. Schmidt, and F. A. de Peyster, *J. Surg. Res.*, **3**, 130 (1963); (c) C. E. Nasjleti, J. M. Walden, and H. H. Spencer, *Cancer. Res.*, **25**, 275 (1965).

The compounds were obtained by condensation of the appropriate haloacyl chlorides or bromides with piperazine hexahydrate or 2-methylpiperazine in chloroform in which almost all derivatives of haloacylpiperazines are readily soluble. The acid chlorides give higher yields than bromides. The piperazine derivatives were obtained in good yield as solids which can be easily purified by recrystallization.[1,3] The new derivatives of 2-methylpiperazine described in this paper, excluding XVI and XIX obtained in the solid state, are (as crude products) oils or resins, crystallizing slowly and decomposing during distillation under reduced pressure. Among them we obtained XII and XVI–XIX in a pure crystalline form. We could not obtain in a pure state the products of condensation of 2-chloropropionyl, 2-bromopropionyl, 3-chloropropionyl, and 3-chlorobutylryl chlorides with 2-methylpiperazine. Therefore, these compounds were not submitted to biological investigations.

The derivatives of 2-methylpiperazine and the derivatives of piperazine previously described[1a,3] were examined for cytostatic activity by the test of Constantinescu and co-workers.[5] The method is based upon the determination of the smallest amount of compound which, dissolved in a 1.0 m*M* caffein solution (0.0212 wt %), will, after 24 hr at 25 ± 1°, induce the characteristic alteration of all the mitotic figures of cariokinesis in the wheat radicular meristemas introduced into the solution. The alterations are identical with those produced by the same agent in healthy and cancerous animal tissues with an intense proliferative activity. These "chromatoclazic"[6] changes consist mainly of mitosis protraction, chromosome fragmentation, the formation of ana- and telophasic bridges, the appearance of micronuclei, and the grouping of chromatin in clusters of various sizes. Under the same conditions, but without the addition of caffein, only a slight inhibition of mitosis with no "chromatoclazic" effects can be observed. On the other hand, the caffeine solution alone, depending on concentration, induces a weak "statmodieretic"[5,7] activity, which consists of the formation of polynuclear cells. It should also be emphasized that cytostatic substances, which show a mechanism of action different from alkylation, do not induce any of the previously described changes in the cariokinesis pictures. They produce, at most, an inhibition of mitosis.

Among 16 substances examined, pipobroman (III) showed (Table I) highest alkylating activity, followed by XII and II. Substances I, X, XIX, and XX did not show cytostatic activity. The remainder increased the statmodieretic activity of caffein and had weak antimitotic or alkylating properties.

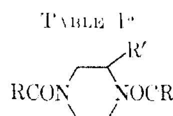
On the basis of experimental data, it can be concluded that the bromine derivatives show a higher activity than chloro analogs. Position 3 is much more favorable than 2. The introduction of a methyl radical or branched acyl group into the ring reduces the alkylating activity. The absence of the halogen atom or too small or too large acyl radicals abolish activity.

(5) D. G. Constantinescu, M. Constantinescu, M. Retezeanu, R. Oteleanu, and V. Stoenescu., *Compt. Rend.*, **253**, 176, 1061 (1961); **254**, 1665 (1962).

(6) P. Dustin, Jr., "Exposés Annuels de Biologie Cellulaire," Masson and Cie., Editeurs, Paris, 1956, pp 189–240.

(7) A. Gosselin *Compt. Rend.*, **210**, 544 (1940).

TABLE I[a]



No.	R	R′	Mp, °C[b]	Yield, %	Formula[d]	Analyses[c]	Act. appearing after 24 hr, μg/ml[e]
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, 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	$C_{15}H_{26}Br_2N_2O_2$	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

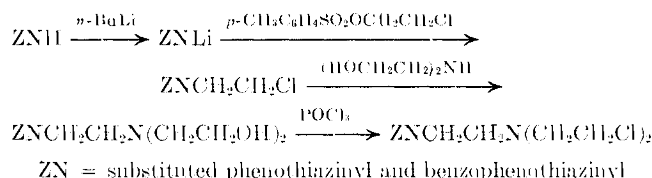
T. GERALD JACKSON AND DAVID A. SHIRLEY[1]

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

Received October 31, 1967

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.

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.

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.
(2) D. A. Shirley, K. Sen, and J. C. Gilmer, *J. Org. Chem.*, **26**, 3587 (1961).
(3) K. Sen, unpublished results, The University of Tennessee, Knoxville, Tenn.
(4) H. Gilman and D. A. Shirley, *J. Am. Chem. Soc.*, **66**, 888 (1944).