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# Antitumor and Mutagenic Properties of a Variety of Heterocyclic Nitrogen and Sulfur Mustards†

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Nitrogen and sulfur mustard derivatives of a variety of quinolines, acridines, azaacridines, benzacridines, and azabenzacridines have been synthesized for studies of the roles played by their polycyclic and alkylating components in the development of antitumor and mutagenic properties. For a display of exceptionally high activity in both the bis- and monoalkylating series of compounds, it appears that the polynuclear component must be an aromatic fused 3- or 4-ring system connected to the mustard moiety by an aminoalkyl linkage. Such nitrogen half-mustards with an ethyl substituent on the amino nitrogen containing the 2-chloroethyl group (the ICR 170 type) display a pronounced degree of activity against ascites tumors and are highly mutagenic for *Drosophila* and *Neurospora*, but not for *Salmonella*. Homologs in which the ethyl group is replaced by hydrogen to give a secondary amine (the ICR 191 type) are extremely potent frameshift mutagens for *Salmonella* and *Escherichia coli*, but not for *Drosophila* and *Neurospora*, and are relatively ineffective as antitumor agents. The sulfur mustard derivatives are active against ascites tumors, but rarely display mutagenic activity. It is considered that activity is imparted to the half-mustards because of the ability of an appropriate polynuclear component to intercalate into the nucleic acids of the ascites tumors or of the bacteria accompanied by the characteristic base alkylating reaction of the 2-chloroethyl group. The ability to exert a bifunctional action is apparently a necessary requirement for both antitumor and frameshift mutagenic activity.

It has been generally assumed[1] that the manifestation of antitumor activity by alkylating agents requires the presence of two alkylating groups, as in nitrogen mustard [methylbis(2-chloroethyl)amine], to permit cross-linking of two nucleophilic centers of biological macromolecules, *e.g.*, deoxyribonucleic acids. In our initial studies involving replacement of the methyl group of nitrogen mustard, it was discovered that compounds containing certain heterocycles joined through an aminoalkyl chain to the bisnitrogen mustard moiety were considerably more active on a molar basis than nitrogen mustard itself in tests with ascites tumor.[2,3] This demonstration that certain heterocyclic components have a potentiating influence on the nitrogen mustard moiety led us to synthesize a series of compounds containing a single 2-chloroethyl group (the nitrogen half-mustards).[4-6] Several of these compounds were found to be almost as active as their corresponding bisnitrogen mustards, thus indicating that certain heterocyclic nuclei are essentially equivalent to a 2-chloroethyl group in providing antitumor activity. Hence, some of the heterocyclic nitrogen half-mustards possess a mixed, or hybrid, bifunctionality. In many instances, treatment of mice bearing ascites tumors with these compounds caused at least a fivefold increase in survival time over that of the control mice. Since, in several long-term experiments, the tumors did not recur, it is clear that all of the ten million ascites tumor cells present in the mouse were destroyed by certain compounds. It was found that a definite degree of complexity is apparently required in the heterocyclic structure, namely, at least a 3-ring linear or angular structure, such as acridine or phenanthridine. In addition, the length of the aminoalkylamino side chain and the type of amine to which the 2-chloroethyl group is attached are important. It appears probable that the active heterocyclic nitrogen half-mustards exert their antitumor influence because of the intercalation of the heterocyclic component into the nucleic acids of the ascites tumor cells accompanied by the usual chemical reaction of the single 2-chloroethyl group. This view is supported by the finding

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by Lerman[7] of the exceptional affinity of acridines for deoxyribonucleic acid. In subsequent work,[8-10] sulfur mustard derivatives of various heterocyclic compounds as well as nitrogen and sulfur mustards derived from polynuclear aromatic hydrocarbons were prepared and examined.

During the course of these studies, investigations of the mutagenic properties of these compounds were undertaken in other laboratories. Oster and coworkers[11,12] first described the mutagenic activity in *Drosophila* of ICR 170, 2-methoxy-6-chloro-9-[3-(ethyl-2-chloroethyl)aminopropylamino]acridine dihydrochloride. Brockman and Goben[13] found that ICR 170 was also a mutagen for *Neurospora*.

Ames and Whitfield[14] demonstrated that some of the ICR compounds were potent frameshift mutagens for *Salmonella* and presented evidence that they specifically caused frameshift mutations due to additions or deletions involving one or two bases of the DNA of this organism. In their initial studies to determine the optimum structural specificities responsible for these mutations, it was found that the secondary amine (ICR 191), 2-methoxy-6-chloro-9-[3-(2-chloroethyl)aminopropylamino]acridine dihydrochloride, was many times more effective against *Salmonella* than the tertiary amine (ICR 170).

Malling[15] and Malling and deSerres[16] found that both the acridine ring system and the alkylating nitrogen half-mustard moiety must be present in the same molecule (ICR 170) for good mutagenesis in *Neurospora*.

In further work with ICR 191 on *Salmonella* and *E. coli* by Martin,[17] Brammar, *et al.*,[18] and Yourno,[19] it was clearly demonstrated that this mutagen causes additions and deletions of nucleotides. When used at low concentrations, ICR 191 appears specifically to cause deletions and additions, and not base pair substitution, in *Salmonella* (Oeschger and Hartman[20] and Hartman, *et al.*[21]).

Chromosome breakage in *Vicia falba* due to ICR 170 has been reported[22] and Caspersson, *et al.*,[23] have employed the acridine bis- and mononitrogen mustards for the fluorescent labeling of chromosomes. Kao and Puck[24] have utilized ICR 191 to produce forward mutations in Chinese hamster cells for quantitative studies of the relationships among cell killing, chromosomal aberration, single gene mutations, and carcinogenesis. Attempts to produce pulmonary tumors[25] in strain A mice by intraperitoneal injection of several bis- and mononitrogen mustard derivatives of acridine were not successful. It is probable that these highly reactive compounds do not reach the lungs by this method of administration. In our laboratories, repeated intramuscular injection of an acridine bisnitrogen mustard into rabbits led to the development of fibrosarcomas at the site of injection.[26]

Some 350 laboratories are currently utilizing various ICR compounds for studies of mutagenesis in *Drosophila, Neurospora, Salmonella, E. coli*, viruses, bacteriophages, yeasts, molds, and plants, and for investigations of the reactions of these compounds with chromosomes, nucleic acids, enzymes, etc. Although some simple acridines are mutagens for bacteriophage, they have been found to exert little or no mutagenic activity in bacteria;[27] thus, the specific frameshift mutagenic activity shown by the ICR compounds in bacteria has made them especially useful in studies of biochemical genetics.

With a view to defining in greater detail the types of heterocyclic and homocyclic structures required for the production of physiologically active nitrogen and sulfur half-mustards, the series of quinoline, acridine, azaacridine, benzacridine, and azabenzacridine derivatives described in this paper has been synthesized and studied. Information on the interesting correlations of structure and biological activity was obtained with the use of the sensitive quantitative ascites tumor system and with *Salmonella* for the mutagenic observations.

## Experimental Section‡

**Synthesis.** The 2-chloroethyl derivatives listed in Table I were prepared by the reaction of $SOCl_2$ on their 2-hydroxyethyl precursors (Table II), which had generally been obtained by the direct condensation of the appropriate chloroheterocyclic nucleus with the preformed side chain as described previously.[3-6,8-10] Occasionally, modifications in the general procedure were necessary to obtain pure products as illustrated in the following examples.

**10-[2-(2-Chloroethylamino)ethylamino]-2-methoxy-7-chlorobenzo[*b*][1,5]naphthyridine Dihydrochloride (ICR 364).** Attempts to prepare this compound by stirring a slurry of the hydroxy precursor in $SOCl_2$ at 35° were unsuccessful. A modified procedure involved the addition of 100 ml of $SOCl_2$ to 1.25 g of the 10-[2-(2-hydroxyethylamino)ethylamino]-2-methoxy-7-chlorobenzo[*b*]-[1,5]naphthyridine which had been suspended in 150 ml of freshly distilled dioxane and 250 ml of $CHCl_3$. The turbid solution was maintained at reflux for 2 hr and then kept overnight at room temperature. After concentration *in vacuo* and the addition of 100 ml of $SOCl_2$ followed by further concentration, the residue was crystallized from 400 ml of EtOH containing 80 ml of 2 *N* HCl to give 1.0 g of product, mp 252–253°.

**10-[2-(Ethyl-2-chloroethylamino)ethylamino]-2-methoxy-7-chlorobenzo[*b*][1,5]naphthyridine Dihydrochloride (ICR 339).** To a solution of 4.2 g (0.015 mole) of 10-chloro-2-methoxy-7-chlorobenzo[*b*][1,5]naphthyridine§ in 150 ml of methyl cellosolve were added 4.7 g (0.035 mole) of 2-hydroxyethylethylaminoethylamine and a trace of NaI. After being refluxed for 30 hr, the mixture was poured into 500 ml of cold 3 *N* AcOH. The resultant solution was made alkaline with 20% NaOH and extracted with $CHCl_3$. The yellow solid obtained by concentration of the extract *in vacuo* was dissolved in alcoholic HCl and $Et_2O$. Upon standing, the solution gave 2.8 g of crystalline product, mp 245–247°. A 2.4-g sample was added to 125 ml of $SOCl_2$ and kept at room temperature for 8 hr. The residue from concentration *in vacuo* was crystallized from EtOH-$Et_2O$ to give 1.9 g of pure product, mp 224–226°.

**Antitumor Studies.** A hypotetraploid clone of Ehrlich ascites tumor cells (EF) transplanted into albino mice (ICR Swiss) has been utilized in this laboratory since 1953 for the quantitative evaluation of the antitumor activities of bifunctional and monofunctional alkylating agents. This system is uniquely suitable for the nitrogen and sulfur mustards because of the extensive prolongation of the survival time of mice bearing ascites tumors that is brought about by these compounds.[28,29] Although the administration of a variety of other cancer chemotherapeutic agents may produce substantial reductions in the ascites cell count in the mouse, only a few have any significant effect on survival time.[28]

The procedures for the determination and interpretation of the results of our antitumor studies which have been described in detail[2,5,28] involve comparison of the survival times of the treated and control ascites tumor-bearing mice. Mice weighing 24–27 g were inoculated intraperitoneally with $7 \times 10^6$ cells of the EF ascites tumor freshly taken from donor mice. On each of the following 3 days, the test compound, dissolved or suspended in physiologic saline, was injected intraperitoneally into the mice. The control ascites tumor-bearing mice, which were injected with saline on days 2–4, have consistently shown a mean survival time of 16 ± 1 days, over more than a 10-year period. Survival data were recorded daily and the degree of antitumor activity of the compounds was calculated statistically from these graphs. The tests on each compound usually involved 100–200 mice. Routine experiments were terminated at the end of a period that was three times the mean survival time of the control mice in order to conserve space in our animal quarters. Treated mice surviving for this length of time rarely had any recurrence of their ascites tumors if kept for an additional 10–20 weeks.

A value of 3.0 for the degree of antitumor activity is at-

‡Melting points were taken in open capillary tubes in Hershberg apparatus using total immersion thermometers and are reported as uncorrected values.

§This compound is available from Aldrich Chemical Company as 2-methoxy-7,10-dichloropyrido[3,2-*b*]quinoline, as is also the corresponding 2-butoxy derivative.

tained when every mouse in an experimental group given a certain dosage of compound lives to the time of sacrifice at about 48 days. Values of 1.0–1.4 indicate that the compound has no significant influence on the survival time of the tumor-bearing mice. A substantial increase in survival time is considered to have occurred when the degree of activity reaches 1.8, which represents an 80% increase over the control survival time. In the tabular expressions of results for the compounds, values between 1.8 and 2.1 indicate moderate antitumor activity; those between 2.2 and 2.6 indicate pronounced activity. The expressed degree of activity for any compound can never reach 3.0 because this calculated average includes two minimum values of 1.8 (one at the lowest effective dosage and one at the maximum effective dosage at which there is concomitant toxicity) along with the higher values that may reach 3.0 at intermediate dosages. The other criteria of effectiveness are the minimum effective dosage and the dosage range, which are expressed in terms of the number of μmoles of compound injected per kg of body weight of the mouse on each of the 3 days. In the case of active compounds, the dosage range covers the lowest level producing an 80% increase of survival time, through the more effective levels, and finally again to a degree value of 1.8. In the case of inactive compounds, the dosage range listed is that employed up to the toxic level for the compound. The ratio of the high to the low dosages in the active range is an expression of therapeutic usefulness.

**Mutagenic Studies.** The compounds were screened for mutagenicity by examination of the reversion to growth on minimal medium of *hisC207*, a histidine-requiring frameshift mutant of *Salmonella typhimurium* LT-2.[14] This mutant is one of several mutant tester strains of this organism which show different responses to various mutagens depending on whether they add or delete bases and whether the frameshift occurs in a sequence of adenine-thymine base pairs or in a sequence of guanine-cytosine base pairs.[30-34] It is suspected that *hisC207* has suffered a deletion of a base pair in a guanine-cytosine sequence in the aminotransferase gene of the histidine operon.[20,21,30,34,35] This mutant is reverted by several ICR compounds that appear able to add a base to the DNA in the region of the missing base pair, but is not reverted by alkylating agents in general or by mutagens that cause base pair substitutions. Several other mutant strains of *Salmonella* are also reverted by ICR 191. It is considered that almost all of the ICR-induced mutations are true frameshift mutations caused by the addition or deletion of one or two base pairs from the DNA of the organisms. Since a multiple of three bases is not involved in this process, the translation of the genetic message is shifted out of frame.

A uniform lawn of the histidine-requiring frameshift mutant of *Salmonella typhimurium* LT-2 was obtained by pouring 0.1 ml of the nutrient broth culture of *hisC207* and 2 ml of molten 0.6% agar containing 0.5% NaCl at 45° into a Petri plate containing minimal agar medium. A trace (0.1 μmole) of histidine was added to the top agar so that the bacteria could grow slightly to form a background lawn and thus enable one to see any inhibition of growth caused by subsequent addition of the compound under test. The suspected mutagen was added to the plate after the top agar had hardened. For qualitative results, a few crystals (1 mg or less) of compound were added; for quantitative results, 5 μl of a 1 mg/ml aqueous solution of compound was added. A control to determine the spontaneous mutation rate of the organism was run on a separate plate. Plates were incubated in the dark for 2 days at 37°. If a compound causes an occasional bacterium of the *hisC207* mutant to revert, the reverted bacterium will grow and form a colony. As the mutagen diffuses from the point of application and forms a concentration gradient, a clear central area of inhibition of bacterial growth surrounded by a circle of mutant colonies often appears.

The mutagenic activity listed in Table I refers to the number of revertant colonies produced by the addition of 5 μg of compound in aqueous solution. Several experiments were averaged and the spontaneous blank was subtracted in each case. The use of a + sign indicates that a response was obtained only by the use of crystals of the compound. No response is shown by the use of zero; a blank means that no test was made.

## Results

The half-mustard derivatives of 6-methoxyquinoline (nucleus A, Table I) had no significant action against ascites tumors but did exhibit wide differences in toxicity. The upper level of the dosage range in the table represents $LD_{20-30}$; the $LD_{100}$ values were 500 μmoles/kg for **1**, 300 for **2**, 5 for **3**, and 50 for **4**. The bisnitrogen mustard **5** was effective against the ascites tumors at dosage levels of 0.5–2.5 μmoles/kg; the $LD_{100}$ was 4.0 μmoles/kg. Generally similar results were observed with the 7-trifluoromethylquinoline mustards **6-9**. It is evident, therefore, that the combination of these quinoline components with either a monofunctional nitrogen mustard or a sulfur mustard moiety does not produce active antitumor compounds; these results substantiate the findings with other quinoline derivatives.[5] A trace of mutagenic activity in *Salmonella* was noted with **2**.

Enlargement of the nuclear component to a fused 3-ring aromatic type (nucleus C) led to the development of antitumor and mutagenic properties, which also were dependent on the nature of the mustard moiety of the molecule. The secondary amine **10** displayed no antitumor activity, but had pronounced mutagenic properties, whereas the tertiary amine **11** was highly effective against ascites tumors, but only slightly mutagenic. This relationship is a general finding. Although the sulfur mustards **12**, **13**, and the bisnitrogen mustard **14** were effective against ascites tumors, they had no mutagenic capability in the *Salmonella* system. This is a second generalization. Lethal mutations may be produced in *Salmonella* by the bisnitrogen mustards; the inactivity of the sulfur mustards may be partly

Chart I. Heterocyclic Nuclei Listed in Table I

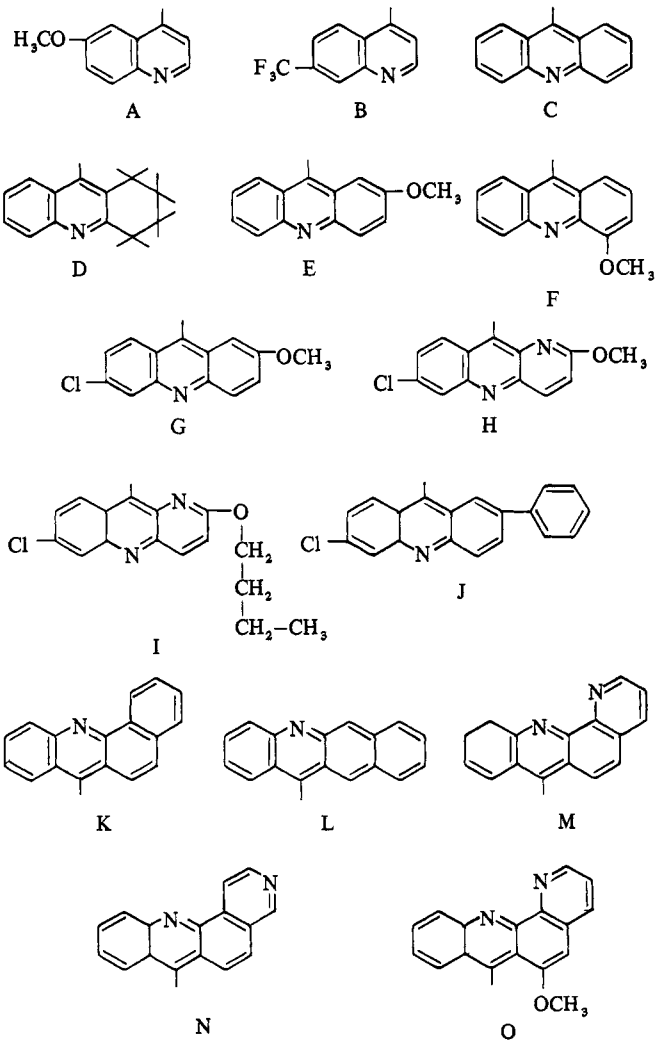

Table I. Structure and Antitumor and Mutagenic Activity

| No. | ICR code | Nucleus | Side chain | Formula | Mp, °C | Yield,[a] % | Antitumor activity: Range, μmoles/kg | Antitumor activity: Degree[b] | Mutagenic activity[c] | Analysis[a] |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 190 | A | $NHCH_2CH_2NHCH_2CH_2Cl$ | $C_{14}H_{18}ClN_3O \cdot 2HCl$ | 266–268 | a-4 | 100–400 | 1.1 | 0 | a |
| 2 | 180 | A | $NH(CH_2)_3NHCH_2CH_2Cl$ | $C_{15}H_{20}ClN_3O \cdot 2HCl$ | 238–240 | a-4 | 10–250 | 1.1 | 10 | a |
| 3 | 174 | A | $NH(CH_2)_3N(C_2H_5)CH_2CH_2Cl$ | $C_{17}H_{24}ClN_3O \cdot 2HCl \cdot H_2O$ | 112–114 | a-4 | 0.5–4.0 | 1.2 | 0 | a |
| 4 | 353 | A | $NHCH_2CH_2SCH_2CH_2Cl$ | $C_{14}H_{17}ClN_2OS \cdot HCl$ | 167–168 | 80 | 20–40 | 1.4 | | C, H, Cl, N |
| 5 | 58 | A | $NH(CH_2)_3N(CH_2CH_2Cl)_2$ | $C_{17}H_{23}Cl_2N_3O \cdot 2HCl \cdot H_2O$ | 140–142.5 | a-3 | 0.5–2.5 | 2.3 | | a |
| 6 | 348 | B | $NHCH_2CH_2NHCH_2CH_2Cl$ | $C_{14}H_{15}F_3ClN_3 \cdot 2HCl \cdot 0.5H_2O$ | 242–244 | 97 | 100–200 | 1.2 | 0 | C, H, Cl, N |
| 7 | 380 | B | $NH(CH_2)_3NHCH_2CH_2Cl$ | $C_{15}H_{17}F_3ClN_3 \cdot 2HCl$ | 233–235 | 81 | 50–250 | 1.0 | 0 | C, H, Cl, N |
| 8 | 336 | B | $NHCH_2CH_2SCH_2CH_2Cl$ | $C_{14}H_{14}F_3ClN_2S \cdot HCl$ | 163–165 | 85 | 75–250 | 1.4 | | C, H, Cl, N, S |
| 9 | 328 | B | $NH(CH_2)_3SCH_2CH_2Cl$ | $C_{15}H_{16}F_3ClN_2S \cdot HCl$ | 150–151 | 70 | 80–200 | 1.0 | | C, H, Cl, N |
| 10 | 449 | C | $NH(CH_2)_3NHCH_2CH_2Cl$ | $C_{18}H_{20}ClN_3 \cdot 2HCl \cdot H_2O$ | 266–268 | 95 | 200–600 | 1.0 | 150–300 | C, H, Cl, N |
| 11 | 217 | C | $NH(CH_2)_3N(C_2H_5)CH_2CH_2Cl$ | $C_{20}H_{24}ClN_3 \cdot 2HCl$ | 238–240 | a-5 | 1.5–4.0 | 2.6 | 20 | a |
| 12 | 316 | C | $NHCH_2CH_2SCH_2CH_2Cl$ | $C_{17}H_{17}ClN_2S \cdot HCl$ | 192.5–193.5 | 73 | 3.5–12 | 2.4 | 0 | C, H, Cl, S |
| 13 | 317 | C | $NH(CH_2)_3SCH_2CH_2Cl$ | $C_{18}H_{19}ClN_2S \cdot HCl$ | 184.5–186 | 64 | 3.0–9.0 | 2.0 | 0 | C, H, Cl, S |
| 14 | 220 | C | $NH(CH_2)_3N(CH_2CH_2Cl)_2$ | $C_{20}H_{23}Cl_2N_3 \cdot 2HCl$ | 225–228 | a-5 | 0.2–1.0 | 2.6 | 0 | a |
| 15 | 445 | D | $NH(CH_2)_3NHCH_2CH_2Cl$ | $C_{18}H_{24}ClN_3 \cdot 2HCl \cdot H_2O$ | 196–198 | 38 | 40–120 | 1.2 | 35 | C, H, Cl, N |
| 16 | 360 | D | $NH(CH_2)_3N(C_2H_5)CH_2CH_2Cl$ | $C_{20}H_{28}ClN_3 \cdot 2HCl \cdot 0.5H_2O$ | 188–190 | 88 | 5–15 | 1.1 | 0 | C, H, Cl, N |
| 17 | 338 | D | $NHCH_2CH_2SCH_2CH_2Cl$ | $C_{17}H_{21}ClN_2S \cdot HCl$ | 181–182 | 48 | 10–50 | 1.3 | 0 | C, H, Cl, N, S |
| 18 | 324 | D | $NH(CH_2)_3SCH_2CH_2Cl$ | $C_{18}H_{23}ClN_2S \cdot HCl$ | 141–142 | 76 | 10–30 | 1.0 | | C, H, Cl, N, S |
| 19 | 249 | D | $N(CH_3)CH_2CH_2N(CH_3)CH_2CH_2Cl$ | $C_{19}H_{26}ClN_3 \cdot 2HCl$ | 232–233.5 | a-5 | 10–30 | 1.0 | 0 | a |
| 20 | 287 | D | $NHCH_2CH_2N(CH_2CH_2Cl)_2$ | $C_{19}H_{25}Cl_2N_3 \cdot 2HCl$ | 235–237 | 96 | 10–40 | 2.5 | | C, H, Cl, N |
| 21 | 262 | D | $NH(CH_2)_3N(CH_2CH_2Cl)_2$ | $C_{20}H_{27}Cl_2N_3 \cdot 2HCl \cdot 0.5H_2O$ | 207–209 | a-5 | 1.2–6.0 | 2.6 | | a |
| 22 | 411 | E | $NHCH_2CH_2NHCH_2CH_2Cl$ | $C_{18}H_{20}ClN_3O \cdot 2HCl$ | 220–222 | 93 | 75–125 | 2.0 | 30 | C, H, Cl, N |
| 23 | 376 | E | $NH(CH_2)_3NHCH_2CH_2Cl$ | $C_{19}H_{22}ClN_3O \cdot 2HCl$ | 258–260 | 74 | 20–60 | 2.1 | 60 | C, H, Cl, N |
| 24 | 459 | E | $NHCH_2CH_2N(C_2H_5)CH_2CH_2Cl$ | $C_{20}H_{24}ClN_3O \cdot 2HCl$ | 246–248 | 86 | 2.5–6.0 | 2.0 | | C, H, Cl, N |
| 25 | 224 | E | $NH(CH_2)_3N(C_2H_5)CH_2CH_2Cl$ | $C_{21}H_{26}ClN_3O \cdot 2HCl$ | 229.5–230 | a-5 | 0.6–2.5 | 2.4 | 25 | a |
| 26 | 378 | E | $NH(CH_2)_3SCH_2CH_2Cl$ | $C_{19}H_{21}ClN_2OS \cdot HCl$ | 130–133 | 59 | 4–10 | 2.5 | | C, H, Cl, N, S |
| 27 | 442 | E | $NHCH_2CH_2NHCH_2CH(CH_3)Cl$ | $C_{19}H_{22}ClN_3O \cdot 2HCl \cdot 0.5H_2O$ | 202–204 | 80 | 100–150 | 2.1 | 10 | C, H, Cl, N |
| 28 | 230 | E | $NH(CH_2)_3N(CH_2CH_2Cl)_2$ | $C_{21}H_{25}Cl_2N_3O \cdot 2HCl \cdot 0.5H_2O$ | 219.5–221 | a-5 | 0.15–1.0 | 2.6 | | a |
| 29 | 410 | F | $NHCH_2CH_2NHCH_2CH_2Cl$ | $C_{18}H_{20}ClN_3O \cdot 2HCl$ | 233–235 | 87 | 75–100 | 1.9 | 30 | C, H, Cl, N |
| 30 | 391 | F | $NH(CH_2)_3NHCH_2CH_2Cl$ | $C_{19}H_{22}ClN_3O \cdot 2HCl \cdot 0.5H_2O$ | 219–221 dec | 98 | 15–40 | 2.2 | 45 | C, H, Cl, N |
| 31 | 401 | F | $NHCH_2CH_2N(C_2H_5)CH_2CH_2Cl$ | $C_{20}H_{24}ClN_3O \cdot 2HCl \cdot 0.5H_2O$ | 221–222.5 | 74 | 0.6–3.0 | 2.2 | 0 | C, H, Cl, N |
| 32 | 377 | F | $NH(CH_2)_3N(C_2H_5)CH_2CH_2Cl$ | $C_{21}H_{26}ClN_3O \cdot 2HCl \cdot 0.5H_2O$ | 190–192 | 95 | 0.6–3.0 | 2.1 | 10 | C, H, Cl, N |
| 33 | 427 | F | $NHCH_2CH_2SCH_2CH_2Cl$ | $C_{18}H_{19}ClN_2OS \cdot HCl \cdot 0.5H_2O$ | 188–190 | 50 | 3–20 | 1.9 | | C, H, Cl, N, S |
| 34 | 375 | F | $NH(CH_2)_3N(CH_2CH_2Cl)_2$ | $C_{21}H_{25}Cl_2N_3O \cdot 2HCl \cdot H_2O$ | 194–196 | 75 | 0.1–0.8 | 2.4 | | C, H, Cl, N |
| 35 | 171 | G | $NHCH_2CH_2NHCH_2CH_2Cl$ | $C_{18}H_{19}Cl_2N_3O \cdot 2HCl \cdot 1.5H_2O$ | 249–251 dec | a-4 | 50–400 | 1.2 | 20 | a |
| 36 | 191 | G | $NH(CH_2)_3NHCH_2CH_2Cl$ | $C_{19}H_{21}Cl_2N_3O \cdot 2HCl$ | 274–276 dec | a-4 | 45–90 | 2.0 | 300–500 | a |
| 37 | 191-OH | G | $NH(CH_2)_3NHCH_2CH_2OH$ | $C_{19}H_{22}ClN_3O_2 \cdot 2HCl$ | 252–254 | a-4 | | 1.0 | 0 | a |
| 38 | 170 | G | $NH(CH_2)_3N(C_2H_5)CH_2CH_2Cl$ | $C_{21}H_{25}Cl_2N_3O \cdot 2HCl \cdot H_2O$ | 236–238 | a-4 | 1.5–4.0 | 2.5 | 10 | a |
| 39 | 443 | G | $NHCH_2CH_2NHCH_2CH(CH_3)Cl$ | $C_{19}H_{21}Cl_2N_3O \cdot 2HCl \cdot H_2O$ | 209–210 | 75 | 100–300 | 1.6 | 10 | C, H, Cl, N |
| 40 | 283 | G | $NHCH_2CH_2OCH_2CH_2Cl$ | $C_{18}H_{18}Cl_2N_2O_2 \cdot HCl$ | 208–209 | 95 | 50–150 | 1.0 | 0 | C, H, Cl, N |
| 41 | 290 | G | $NH(CH_2)_3NHCOCH_2N(C_2H_5)CH_2CH_2Cl$ | $C_{23}H_{28}Cl_2N_4O_2 \cdot 2HCl \cdot 0.5H_2O$ | 221–223 | 31 | 4.0–10 | 2.3 | 0 | C, H, Cl, N |
| 42 | 397 | G | $NH(CH_2)_2O(CH_2)_2N(C_2H_5)CH_2CH_2Cl$ | $C_{22}H_{27}Cl_2N_3O_2 \cdot 2HCl$ | 225–226.5 | 77 | 0.8–1.6 | 1.9 | 10 | C, H, Cl, N |
| 43 | 364 | H | $NHCH_2CH_2NHCH_2CH_2Cl$ | $C_{17}H_{18}Cl_2N_4O \cdot 2HCl$ | 252–253 | 80 | 300–600 | 1.7 | 25 | C, H, Cl, N |
| 44 | 364-OH | H | $NHCH_2CH_2NHCH_2CH_2OH$ | $C_{17}H_{19}ClN_4O_2 \cdot 2HCl \cdot H_2O$ | 250–252 | 58 | | 1.0 | 15 | C, H, Cl, N |
| 45 | 372 | H | $NH(CH_2)_3NHCH_2CH_2Cl$ | $C_{18}H_{20}Cl_2N_4O \cdot 2HCl \cdot H_2O$ | 257–258 | 63 | 60–200 | 2.4 | 800–1200 | C, H, Cl, N |
| 46 | 372-OH | H | $NH(CH_2)_3NHCH_2CH_2OH$ | $C_{18}H_{21}ClN_4O_2 \cdot 2HCl \cdot 0.5H_2O$ | 250–252 | 29 | | 1.0 | 40 | C, H, Cl, N |
| 47 | 339 | H | $NHCH_2CH_2N(C_2H_5)CH_2CH_2Cl$ | $C_{19}H_{22}Cl_2N_4O \cdot 2HCl \cdot H_2O$ | 224–226 | 80 | 4–20 | 2.1 | 0 | C, H, Cl, N |
| 48 | 340 | H | $NH(CH_2)_3N(C_2H_5)CH_2CH_2Cl$ | $C_{20}H_{24}Cl_2N_4O \cdot 2HCl \cdot H_2O$ | 245–247 | 80 | 2.0–7.5 | 2.3 | 100–150 | C, H, Cl, N |
| 49 | 340-OH | H | $NH(CH_2)_3N(C_2H_5)CH_2CH_2OH$ | $C_{20}H_{25}ClN_4O \cdot 2HCl$ | 250–252 | 74 | | 1.0 | 10 | C, H, Cl, N |
| 50 | 346 | H | $NHCH_2CH_2SCH_2CH_2Cl$ | $C_{17}H_{17}Cl_2N_3OS \cdot HCl$ | 238–239 | 85 | 10–60 | 2.2 | | C, H, Cl, N, S |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| 51 | 342 | H | $NH(CH_2)_3SCH_2CH_2Cl$ | $C_{18}H_{19}Cl_2N_3OS \cdot HCl$ | 217-219 | 86 | 2.5-25 | 2.4 | 0 | C, H, Cl, N, S |
| 52 | 438 | H | $NHCH_2CH_2NHCH_2CH(CH_3)Cl$ | $C_{18}H_{20}Cl_2N_4O \cdot 2HCl$ | 238-240 | 85 | 100-300 | 1.4 | + | C, H, Cl, N |
| 53 | 415 | H | $NH(CH_2)_2O(CH_2)_2N(C_2H_5)CH_2CH_2Cl$ | $C_{21}H_{26}Cl_2N_4O_2 \cdot 2HCl$ | 236 dec | 91 | 0.8-2.0 | 2.3 | 65 | C, H, Cl, N |
| 54 | 400 | H | $NHCH_2CH_2N(CH_2CH_2Cl)_2$ | $C_{19}H_{21}Cl_3N_4O \cdot 2HCl$ | 215.5-217 | 82 | 3.0-30 | 2.3 | | C, H, Cl, N |
| 55 | 349 | H | $NH(CH_2)_3N(CH_2CH_2Cl)_2$ | $C_{20}H_{23}Cl_3N_4O \cdot 2HCl$ | 206-208 | 73 | 0.4-2.0 | 2.3 | | C, H, Cl, N |
| 56 | 359 | H | $NHCH(CH_3)(CH_2)_3N(CH_2CH_2Cl)_2$ | $C_{22}H_{27}Cl_3N_4O \cdot 2HCl$ | 210-211.5 | 92 | 1.0-4.5 | 2.4 | | C, H, Cl, N |
| 57 | 447 | I | $NHCH_2CH_2NHCH_2CH_2Cl$ | $C_{20}H_{24}Cl_2N_4O \cdot 2HCl$ | 248-250 | 61 | 100-400 | 1.7 | 0 | C, H, Cl, N |
| 58 | 371 | I | $NH(CH_2)_3NHCH_2CH_2Cl$ | $C_{21}H_{26}Cl_2N_4O \cdot 2HCl \cdot 0.75H_2O$ | 245-247 | 97 | 50-100 | 2.2 | 0 | C, H, Cl, N |
| 59 | 381 | I | $NHCH_2CH_2N(C_2H_5)CH_2CH_2Cl$ | $C_{22}H_{28}Cl_2N_4O \cdot 2HCl$ | 233-235 | 89 | 2-12 | 1.0 | | C, H, Cl, N |
| 60 | 355 | I | $NH(CH_2)_3N(C_2H_5)CH_2CH_2Cl$ | $C_{23}H_{30}Cl_2N_4O \cdot 2HCl \cdot H_2O$ | 212-214 | 63 | 8.0-30 | 2.7 | 0 | C, H, Cl, N |
| 61 | 363 | I | $NHCH_2CH_2SCH_2CH_2Cl$ | $C_{20}H_{23}Cl_2N_3OSHCl$ | 248-248.5 | 83 | 80-300 | 2.6 | | C, H, Cl, N, S |
| 62 | 369 | I | $NH(CH_2)_3SCH_2CH_2Cl$ | $C_{21}H_{25}Cl_2N_3OSHCl$ | 225-226 | 88 | 16-80 | 2.7 | | C, H, Cl, N, S |
| 63 | 446 | I | $NHCH_2CH_2NHCH_2CH(CH_3)Cl$ | $C_{21}H_{26}Cl_2N_4O \cdot 2HCl$ | 245-247 | 71 | 100-400 | 1.1 | 0 | C, H, Cl, N |
| 64 | 406 | I | $NH(CH_2)_2O(CH_2)_2N(C_2H_5)CH_2CH_2Cl$ | $C_{24}H_{32}Cl_2N_4O_2 \cdot 2HCl \cdot H_2O$ | 195-196.5 | 96 | 2.5-6.0 | 2.2 | 0 | C, H, Cl, N |
| 65 | 351 | I | $NH(CH_2)_3N(CH_2CH_2Cl)_2$ | $C_{23}H_{29}Cl_3N_4O \cdot 2HCl \cdot H_2O$ | 208-210 | 79 | 1-20 | 2.7 | | C, H, Cl, N |
| 66 | 386 | I | $NHCH(CH_3)(CH_2)_3N(CH_2CH_2Cl)_2$ | $C_{25}H_{33}Cl_3N_4O \cdot 2HCl$ | 214-216 | 73 | 1.4-20 | 2.6 | | C, H, Cl, N |
| 67 | 472 | J | $NH(CH_2)_3NHCH_2CH_2Cl$ | $C_{24}H_{23}Cl_2N_3 \cdot 2HCl$ | 294-296 | 80 | 80-150 | 2.0 | | C, H, Cl, N |
| 68 | 471 | J | $NH(CH_2)_3N(C_2H_5)CH_2CH_2Cl$ | $C_{26}H_{27}Cl_2N_3 \cdot 2HCl \cdot 0.75H_2O$ | 216-218 | 94 | 10-30 | 2.2 | | C, H, Cl, N |
| 69 | 470 | J | $NH(CH_2)_3N(CH_2CH_2Cl)_2$ | $C_{26}H_{26}Cl_3N_3 \cdot 2HCl \cdot 0.5H_2O$ | 207-208 | 50 | 2-20 | 2.2 | | C, H, Cl, N |
| 70 | 312 | K | $NHCH_2CH_2NHCH_2CH_2Cl$ | $C_{21}H_{20}ClN_3 \cdot 2HCl \cdot H_2O$ | 152-154 | 68 | 80-400 | 1.2 | 50-80 | C, H, Cl, N |
| 71 | 370 | K | $NH(CH_2)_3NHCH_2CH_2Cl$ | $C_{22}H_{22}ClN_3 \cdot 2HCl \cdot 1.5H_2O$ | 255-257 | 95 | 25-150 | 1.6 | 200-375 | C, H, Cl, N |
| 72 | 370-OH | K | $NH(CH_2)_3NHCH_2CH_2OH$ | $C_{22}H_{23}N_3O \cdot 2HCl \cdot 0.5H_2O$ | 155-157 | 77 | | 1.0 | + | C, H, Cl, N |
| 73 | 311 | K | $NHCH_2CH_2N(C_2H_5)CH_2CH_2Cl$ | $C_{23}H_{24}ClN_3 \cdot 2HCl \cdot 1.5H_2O$ | 233-235 | a-5 | 2.0-8.0 | 2.0 | 0 | a |
| 74 | 292 | K | $NH(CH_2)_3N(C_2H_5)CH_2CH_2Cl$ | $C_{24}H_{26}ClN_3 \cdot 2HCl \cdot H_2O$ | 181-186 | 84 | 1.5-6.0 | 2.2 | 65 | C, H, Cl, N |
| 75 | 299 | K | $NHCH_2CH_2N(CH_2CH_2Cl)_2$ | $C_{23}H_{23}Cl_2N_3 \cdot 2HCl \cdot H_2O$ | 205-209 | 83 | 1.5-4.0 | 2.6 | | C, H, Cl, N |
| 76 | 426 | L | $NH(CH_2)_3N(C_2H_5)CH_2CH_2Cl$ | $C_{24}H_{26}ClN_3 \cdot 2HCl \cdot H_2O$ | 195-197 | 91 | 1.0-2.0 | 2.1 | | C, H, Cl, N |
| 77 | 424 | L | $NH(CH_2)_3SCH_2CH_2Cl$ | $C_{22}H_{21}ClN_2S \cdot HCl$ | 186.5-187.5 | 57 | 75-120 | 2.0 | | C, H, Cl, N, S |
| 78 | 417 | L | $NH(CH_2)_3N(CH_2CH_2Cl)_2$ | $C_{24}H_{25}Cl_2N_3 \cdot 2HCl \cdot H_2O$ | 183-185 | 55 | 0.3-1.2 | 2.2 | | C, H, Cl, N |
| 79 | 395 | M | $NH(CH_2)_3NHCH_2CH_2Cl$ | $C_{21}H_{21}ClN_4 \cdot 2HCl$ | 210-212 | 87 | 15-40 | 2.0 | 200 | C, H, Cl, N |
| 80 | 368 | M | $NH(CH_2)_3N(C_2H_5)CH_2CH_2Cl$ | $C_{23}H_{25}ClN_4 \cdot 2HCl$ | 241-242 | 97 | 0.4-2.0 | 2.4 | 0 | C, H, Cl, N |
| 81 | 394 | M | $NH(CH_2)_3N(C_3H_7)CH_2CH_2Cl$ | $C_{24}H_{27}ClN_4 \cdot 3HCl$ | 220-221 | 92 | 0.2-2.2 | 2.4 | + | C, H, Cl, N |
| 82 | 428 | N | $NHCH_2CH_2NHCH_2CH_2Cl$ | $C_{20}H_{19}ClN_4 \cdot 2HCl \cdot H_2O$ | 267-269 | 25 | 45-150 | 1.1 | | C, H, Cl, N |
| 83 | 455 | N | $NH(CH_2)_3NHCH_2CH_2Cl$ | $C_{21}H_{21}ClN_4 \cdot 2HCl \cdot H_2O$ | 262-264 | 58 | 30-150 | 1.0 | 150 | C, H, Cl, N |
| 84 | 434 | N | $NH(CH_2)_3N(C_2H_5)CH_2CH_2Cl$ | $C_{23}H_{25}ClN_4 \cdot 3HCl \cdot H_2O$ | 180 dec | 39 | 6.0-15 | 2.1 | | C, H, Cl, N |
| 85 | 441 | N | $NH(CH_2)_3N(CH_2CH_2Cl)_2$ | $C_{23}H_{24}Cl_2N_4 \cdot 2HCl \cdot 1.5H_2O$ | 258-260 | 47 | 1.0-6.0 | 2.2 | | C, H, Cl, N |
| 86 | 423 | O | $NHCH_2CH_2NHCH_2CH_2Cl$ | $C_{21}H_{21}ClN_4O \cdot 2HCl \cdot 2.5H_2O$ | 193-195 | 71 | 20-60 | 2.0 | + | C, H, Cl, N |
| 87 | 463 | O | $NH(CH_2)_3N(C_2H_5)CH_2CH_2Cl$ | $C_{24}H_{27}ClN_4O \cdot 3HCl \cdot 2H_2O$ | 145-147 | 62 | 0.5-5.0 | 2.1 | | C, H, Cl, N |
| 88 | 425 | O | $NH(CH_2)_3N(CH_2CH_2Cl)_2$ | $C_{24}H_{26}Cl_2N_4O \cdot 2HCl \cdot 2H_2O$ | 118-120 | 83 | 0.1-1.5 | 2.4 | | C, H, Cl, N |
| 89 | 430 | O | $NH(CH_2)_6N(CH_2CH_2Cl)_2$ | $C_{27}H_{32}Cl_2N_4O \cdot 2HCl \cdot 2H_2O$ | 114-116 | 10 | 0.2-1.0 | 2.1 | | C, H, Cl, N |

[a]Under yield and analysis, "a" indicates that the preparation and analyses of the compound have been described previously; the number is the reference to the literature. Where analyses are indicated only by symbols of the elements, the analytical results were within ±0.4% of the theoretical values. The yield is based on the hydroxy precursor. [b]The numbers under degree of antitumor activity have the following meaning: 1.0–1.4, inactive; 1.5–1.7, slight activity; 1.8–2.1, moderate activity; 2.2–2.6, high activity. (See text for additional information.) [c]The numbers under mutagenic activity refer to the number of revertant colonies produced by addition of the compounds under standardized conditions to cultures of *Salmonella hisC207*. Since observations on many of these mutagens are currently appearing in the biological literature under the ICR code number only, without any accompanying chemical name or structural formula, the ICR code numbers for these compounds have been listed in this table.

the result of their limited solubility in the culture medium.

The requirement of aromaticity in the polycyclic component for antitumor activity in the half-mustards became evident when the series of tetrahydroacridine mustards (nucleus D) was examined. Compounds **16**, **17**, **18**, and **19** containing the customarily active monofunctional mustard moieties were ineffective against the ascites tumors (*cf.* **11**, **12**, and **13**). This suggests that the nonplanar tetrahydroacridine nucleus, unlike the acridine nucleus, does not undergo the extent of intercalation into the deoxyribonucleic acid of the ascites tumor cells necessary for a demonstration of antitumor activity. This finding is reminiscent of the results quoted by Albert[34] indicating that a minimum area of flatness in the order of 38 Å is required for an

**Table II.** Precursors to Compounds in Table I

| Nucleus | Side chain | Formula | Mp, °C | Yield, % | Analysis[a] |
|---|---|---|---|---|---|
| B | $NHCH_2CH_2NHCH_2CH_2OH$ | $C_{14}H_{16}F_3N_3O\cdot 2HCl$ | 216-217 | 80 | C, H, Cl, N |
| B | $NH(CH_2)_3NHCH_2CH_2OH$ | $C_{15}H_{18}F_3N_3O\cdot 2HCl$ | 107-109 | 61 | C, H, Cl, N |
| B | $NHCH_2CH_2SCH_2CH_2OH$ | $C_{14}H_{15}F_3N_2OS\cdot HCl$ | 143-145 | 55 | C, H, Cl, N |
| B | $NH(CH_2)_3SCH_2CH_2OH$ | $C_{15}H_{17}F_3N_2OS\cdot HCl$ | 170-171 | 48 | C, H, Cl, N |
| C | $NH(CH_2)_3NHCH_2CH_2OH$ | $C_{18}H_{21}N_3O\cdot 2HCl\cdot H_2O$ | 252-253 | 9 | C, H, Cl, N |
| C | $NHCH_2CH_2SCH_2CH_2OH$ | $C_{17}H_{18}N_2OS\cdot HCl$ | 217-219 | 29 | C, H, Cl, S |
| C | $NH(CH_2)_3SCH_2CH_2OH$ | $C_{18}H_{20}N_2OS$ | 114.5-118 | 49 | C, H, S |
| D | $NH(CH_2)_3NHCH_2CH_2OH$ | $C_{18}H_{25}N_3O\cdot 2HCl$ | 179-181 | 50 | C, H, Cl, N |
| D | $NH(CH_2)_3N(C_2H_5)CH_2CH_2OH$ | $C_{20}H_{29}N_3O\cdot 2HCl\cdot H_2O$ | 179-180 | 42 | C, H, Cl, N |
| D | $NHCH_2CH_2SCH_2CH_2OH$ | $C_{17}H_{22}N_2OS\cdot 2HCl$ | 207-209 | 52 | C, H, N, S |
| D | $NH(CH_2)_3SCH_2CH_2OH$ | $C_{18}H_{24}N_2OS\cdot HCl\cdot 0.25H_2O$ | 151-152 | 27 | C, H, Cl, N, S |
| D | $NHCH_2CH_2N(CH_2CH_2OH)_2$ | $C_{19}H_{27}N_3O_2\cdot 2HCl$ | 242-243 | 69 | C, H, Cl, N |
| E | $NHCH_2CH_2NHCH_2CH_2OH$ | $C_{18}H_{21}N_3O_2\cdot 2HCl\cdot 1.5H_2O$ | 208-210 | 56 | C, H, Cl, N |
| E | $NH(CH_2)_3NHCH_2CH_2OH$ | $C_{19}H_{23}N_3O_2\cdot 2HCl$ | 238-240 | 80 | C, H, Cl, N |
| E | $NHCH_2CH_2N(C_2H_5)CH_2CH_2OH$ | $C_{20}H_{25}N_3O_2\cdot 2HCl$ | 220-222 | 43 | C, H, Cl, N |
| E | $NH(CH_2)_3SCH_2CH_2OH$ | $C_{19}H_{22}N_2O_2S\cdot HCl\cdot 0.75H_2O$ | 96-98 | 68 | C, H, Cl, S |
| E | $NHCH_2CH_2NHCH_2CH(CH_3)OH$ | $C_{19}H_{23}N_3O_2\cdot HCl$ | 212-214 | 35 | C, H, Cl, N |
| F | $NHCH_2CH_2NHCH_2CH_2OH$ | $C_{18}H_{21}N_3O_2\cdot 2HCl$ | 186-188 | 15 | C, H, Cl, N |
| F | $NH(CH_2)_3NHCH_2CH_2OH$ | $C_{19}H_{23}N_3O_2\cdot 2HCl$ | 195-198 | 77 | C, H, Cl, N |
| F | $NHCH_2CH_2N(C_2H_5)CH_2CH_2OH$ | $C_{20}H_{25}N_3O_2\cdot 2HCl$ | 219.5-221 | 51 | C, H, Cl, N |
| F | $NH(CH_2)_3N(C_2H_5)CH_2CH_2OH$ | $C_{21}H_{27}N_3O_2\cdot 2HCl$ | 205-207 | 49 | C, H, Cl, N |
| F | $NHCH_2CH_2SCH_2CH_2OH$ | $C_{18}H_{20}N_2O_2S\cdot HCl$ | 162.5-165 | 65 | C, H, Cl, N, S |
| F | $NH(CH_2)_3N(CH_2CH_2OH)_2$ | $C_{21}H_{27}N_3O_3\cdot 2HCl\cdot 0.5H_2O$ | 221-222 | 64 | C, H, Cl, N |
| G | $NHCH_2CH_2NHCH_2CH(CH_3)OH$ | $C_{19}H_{22}ClN_3O_2\cdot 2HCl\cdot H_2O$ | 220-222 | 52 | C, H, Cl, N |
| G | $NHCH_2CH_2OCH_2CH_2OH$ | $C_{18}H_{19}ClN_2O_3$ | 142-143.5 | 92 | C, H, N |
| G | $NH(CH_2)_3NHCOCH_2N(C_2H_5)CH_2CH_2OH$ | $C_{23}H_{29}ClN_4O_3\cdot H_2O$ | 90-94 | 31 | C, H, N |
| G | $NH(CH_2)_2O(CH_2)_2N(C_2H_5)CH_2CH_2OH$ | $C_{22}H_{28}ClN_3O\cdot 2HCl$ | 217.5-221 | 26 | C, H, Cl, N |
| H | $NHCH_2CH_2N(C_2H_5)CH_2CH_2OH$ | $C_{19}H_{23}ClN_4O_2\cdot 2HCl$ | 245-247 | 73 | C, H, Cl, N |
| H | $NHCH_2CH_2SCH_2CH_2OH$ | $C_{17}H_{18}ClN_3O_2S\cdot HCl$ | 254-256 | 62 | C, H, Cl, N |
| H | $NH(CH_2)_3SCH_2CH_2OH$ | $C_{18}H_{20}ClN_3O_2S\cdot HCl$ | 221-223 | 44 | C, H, Cl, N |
| H | $NHCH_2CH_2NHCH_2CH(CH_3)OH$ | $C_{18}H_{21}ClN_4O_2\cdot 2HCl\cdot H_2O$ | 243-244 | 27 | C, H, Cl, N |
| H | $NH(CH_2)_2O(CH_2)_2N(C_2H_5)CH_2CH_2OH$ | $C_{21}H_{27}ClN_4O_3\cdot 2HCl\cdot 0.25H_2O$ | 243-245 | 72 | C, H, Cl, N |
| H | $NHCH_2CH_2N(CH_2CH_2OH)_2$ | $C_{19}H_{23}ClN_4O_3\cdot 2HCl$ | 244-245 | 78 | C, H, Cl, N |
| H | $NH(CH_2)_3N(CH_2CH_2OH)_2$ | $C_{20}H_{25}ClN_4O_3\cdot 2HCl$ | 241-242 | 49 | C, H, Cl, N |
| H | $NHCH(CH_3)(CH_2)_3N(CH_2CH_2OH)_2$ | $C_{22}H_{29}ClN_4O_3\cdot 2HCl$ | 231-232 | 59 | C, H, Cl, N |
| I | $NHCH_2CH_2NHCH_2CH_2OH$ | $C_{20}H_{25}ClN_4O_2\cdot 2HCl\cdot H_2O$ | 240-241 | 45 | C, H, Cl, N |
| I | $NH(CH_2)_3NHCH_2CH_2OH$ | $C_{21}H_{27}ClN_4O_2\cdot 2HCl\cdot H_2O$ | 236-237 | 51 | C, H, Cl, N |
| I | $NHCH_2CH_2N(C_2H_5)CH_2CH_2OH$ | $C_{22}H_{29}ClN_4O_2\cdot 2HCl\cdot H_2O$ | 226-228 | 41 | C, H, Cl, N |
| I | $NH(CH_2)_3N(C_2H_5)CH_2CH_2OH$ | $C_{23}H_{31}ClN_4O_2\cdot 2HCl\cdot H_2O$ | 211-213 | 62 | C, H, Cl, N |
| I | $NH(CH_2CH_2SCH_2CH_2OH$ | $C_{20}H_{24}ClN_3O_2S\cdot HCl$ | 210-211 | 45 | C, H, Cl, N |
| I | $NH(CH_2)_3SCH_2CH_2OH$ | $C_{21}H_{26}ClN_3O_2S\cdot HCl$ | 220-222 | 49 | C, H, Cl, N |
| I | $NH(CH_2)_2O(CH_2)_2N(C_2H_5)CH_2CH_2OH$ | $C_{24}H_{33}ClN_4O_3\cdot 2HCl\cdot 0.5H_2O$ | 194-195 | 51 | C, H, Cl, N |
| I | $NH(CH_2)_3N(CH_2CH_2OH)_2$ | $C_{23}H_{31}ClN_4O_3\cdot 2HCl\cdot H_2O$ | 221-222 | 34 | C, H, Cl, N |
| I | $NHCH(CH_3)(CH_2)_3N(CH_2CH_2OH)_2$ | $C_{25}H_{35}ClN_4O_3\cdot 2HCl\cdot 0.5H_2O$ | 158-160 | 12 | C, H, Cl, N |
| J | $NH(CH_2)_3NHCH_2CH_2OH$ | $C_{24}H_{24}ClN_3O\cdot 2HCl$ | 160-162 | 10 | C, H, Cl, N |
| J | $NH(CH_2)_3N(C_2H_5)CH_2CH_2OH$ | $C_{26}H_{28}ClN_3O\cdot 2HCl\cdot H_2O$ | 199-201 | 49 | C, H, Cl, N |
| J | $NH(CH_2)_3N(CH_2CH_2OH)_2$ | $C_{26}H_{28}ClN_3O_2\cdot 2HCl\cdot 1.5H_2O$ | 155-157 | 11 | C, H, Cl, N |
| K | $NHCH_2CH_2NHCH_2CH_2OH$ | $C_{21}H_{21}N_3O\cdot 2HCl\cdot H_2O$ | 210-212 | 30 | C, H, Cl, N |
| K | $NHCH_2CH_2N(CH_2CH_2OH)_2$ | $C_{23}H_{25}N_3O_2\cdot 2HCl\cdot H_2O$ | 243-245.5 | 64 | C, H, Cl, N |
| L | $NH(CH_2)_3N(C_2H_5)CH_2CH_2OH$ | $C_{24}H_{27}N_3O\cdot 2HCl$ | 211-213 | 52 | C, H, Cl, N |
| L | $NH(CH_2)_3SCH_2CH_2OH$ | $C_{22}H_{22}N_2OS\cdot HCl$ | 199-201 | 87 | C, H, Cl, N |
| L | $NH(CH_2)_3N(CH_2CH_2OH)_2$ | $C_{24}H_{27}N_3O_2\cdot 2HCl$ | 213-215 | 56 | C, H, Cl, N |
| M | $NH(CH_2)_3NHCH_2CH_2OH$ | $C_{21}H_{22}N_4O\cdot 2HCl$ | 212-214 | 79 | C, H, Cl, N |
| M | $NH(CH_2)_3N(C_2H_5)CH_2CH_2OH$ | $C_{25}H_{26}N_4O\cdot 2HCl\cdot 0.5H_2O$ | 243-245 | 67 | C, H, Cl, N |
| M | $NH(CH_2)_3N(C_2H_5)CH_2CH_2OH$ | $C_{24}H_{28}N_4O\cdot 2HCl\cdot 0.5H_2O$ | 225-226 | 66 | C, H, Cl, N[b] |
| N | $NHCH_2CH_2NHCH_2CH_2OH$ | $C_{20}H_{20}N_4O\cdot 3HCl\cdot 1.5H_2O$ | 209-211 | 53 | C, H, Cl, N |
| N | $NH(CH_2)_3NHCH_2CH_2OH$ | $C_{21}H_{22}N_4O\cdot 2HCl$ | 209-212 | 52 | C, H, Cl, N |
| N | $NH(CH_2)_3N(C_2H_5)CH_2CH_2OH$ | $C_{23}H_{26}N_4O\cdot 3HCl\cdot H_2O$ | 234-236 | 50 | C, H, Cl, N |
| N | $NH(CH_2)_3N(CH_2CH_2OH)_2$ | $C_{23}H_{26}N_4O_2\cdot 3HCl$ | 235-237 | 28 | C, H, Cl, N |
| O | $NHCH_2CH_2NHCH_2CH_2OH$ | $C_{20}H_{22}N_4O_2\cdot 2HCl\cdot 1.5H_2O$ | 129-132 | 28 | C, H, Cl, N |
| O | $NH(CH_2)_3N(C_2H_5)CH_2CH_2OH$ | $C_{24}H_{28}N_4O_2\cdot 2HCl\cdot 0.75H_2O$ | 138-140 | 63 | C, H, Cl, N |
| O | $NH(CH_2)_3N(CH_2CH_2OH)_2$ | $C_{24}H_{28}N_4O_3\cdot 3HCl\cdot H_2O$ | 118-121 | 61 | C, H, Cl, N |
| O | $NH(CH_2)_6N(CH_2CH_2OH)_2$ | $C_{27}H_{34}N_4O_3\cdot 3HCl\cdot H_2O$ | 108-110 | 65 | C, H, Cl, N |

[a] Where analyses are indicated only by symbols of the elements, the analytical results were within ±0.4% of the theoretical values. [b] Calcd: Cl, 15.08; N, 11.91; found: Cl, 15.75; N, 11.32.

expression of antibacterial activity by various aminoacridines. The bisnitrogen mustards, which do not depend on the polycyclic component for their activity, were effective against the ascites tumors; compound **21** containing the aminopropylamino side chain was more potent than the aminoethylamino analog **20**. Comparisons of the results with **10**, **11**, **15**, and **16** show that the aromatic acridine ring system is also needed for an expression of pronounced mutagenic activity.

Introduction of a methoxy group into the 2 position (nucleus E) or into the 4 position (nucleus F) of the acridine nucleus caused a significant increase in antitumor activity, particularly in terms of molar dosages, over that shown by the respective analogs in the C nucleus series. Definite activity was even exhibited by compounds **22**, **23**, **29**, and **30** containing a secondary amine in the monofunctional nitrogen mustard moiety, albeit at relatively high molar dosages. The effectiveness against ascites tumors was particularly striking with the tertiary amine forms **24**, **25**, **31**, and **32** which were active at very low molar dosages. A third generalization became evident, namely, that compounds containing the aminopropylamino side chain are considerably more effective on a molar basis than those with an aminoethylamino side chain; this applies to the half-mustards of the secondary and tertiary amine types as well as to the bismustards. There seem to be no significant differences in the antitumor activities of comparable compounds in the 2-methoxy and 4-methoxy series. No unusual properties were noted with a different mustard moiety **27** (*cf.* **22**).

The methoxyacridine nuclei in combination with the secondary amine type of nitrogen mustard moiety provided moderately active mutagenic agents, particularly in the case of compounds with the aminopropylamino side chain **23** and **30**.

In agreement with previous observations on the bisnitrogen mustards,[5] the presence of a chloro substituent in the acridine nucleus was found to exert a depressing influence on antitumor activity as is shown by comparing the inactive **35** with **22** and **29**. Among the active compounds with nucleus G, **36** and **38** (ICR 191 and 170) were required in considerably higher molar dosages than their analogs **23**, **30** and **25**, **32**. Derivatives of 2-methoxy-6-chloroacridine with several different types of side chain and alkylating moiety were also prepared and examined. Attachment of the 2-chloroethyl group to the remainder of the side chain by an ether linkage, instead of an amino or a sulfur linkage, gave an inactive compound **40**. Moderately effective compounds were obtained, however, when the tertiary amine mustard moiety was joined to the rest of the side chain by an amide linkage **41** or an ether linkage **42**.

An exceptional degree of mutagenic activity in the *Salmonella* system was displayed by **36** (ICR 191) which has become the standard agent for producing frameshift mutations in *Salmonella, E. coli,* and certain other organisms. The hydroxy precursor **37** (ICR 191-OH) was ineffective as a mutagen, thus indicating the need for the 2-chloroethyl group in frameshift mutagenesis. The other alkylating derivatives of 2-methoxy-6-chloroacridine showed no more than a weak mutagenic capability in this organism. Although ICR 170 was found to be only weakly mutagenic in *Salmonella,* it is a potent mutagen in *Drosophila*[11,12] and in *Neurospora,*[13,15,16] whereas ICR 191 is only slightly mutagenic in these latter two organisms.

Studies by Glusker and coworkers[35] in this Institute using methods of X-ray diffraction have established the existence of significant differences in bond lengths, angles, and conformation among the hydroxy precursors of ICR 170, 171, and 191. These structural variations may be sufficient to influence the degree of intercalation into the different nucleic acids and thus account in part for the specificity of the antitumor and mutagenic responses.

Another important structural modification was introduced by utilizing the azaacridine nucleus as the polycyclic component. Comparisons of the respective pairs of analogs in the H and I nucleus series of methoxy- and *n*-butoxy-7-chlorobenzo[*b*][1,5]naphthyridines show that the *n*-butoxy derivatives are less active as antitumor agents. No activity was shown by **59** whereas **47** was highly effective against the ascites tumors. In the case of the butoxy derivatives that were active, it was found that significantly larger molar dosages were required for all types of the butoxy mustards than for the corresponding methoxy derivatives. In general, the methoxyazaacridine mustards (nucleus H) were somewhat less active than the corresponding compounds in the methoxyacridine series (nucleus G).

Major differences were observed in the mutagenic properties of the two series of azaacridines. Compound **45** (ICR 372), 10-[3-(2-chloroethylamino)propylamino]-2-methoxy-7-chlorobenzo[*b*][1,5]naphthyridine, is an extremely potent mutagen in *Salmonella* and even the tertiary amine **48** was found to be unusually active (*cf.* **11**, **25**, **38**). Definite mutagenic capability was also observed in the hydroxy precursors **44**, **46**; this indicates that the presence of a 2-chloroethyl group is not always mandatory for mutagenic activity provided the molecule contains an exceptionally effective polycyclic component joined through an aminopropyl side chain to a secondary amine containing the 2-hydroxyethyl group. The methoxy substituent on the azaacridine ring cannot be replaced by the *n*-butoxy group, however, as is shown by the inactivity of **57**, **58**, **63**, and **64** in the *Salmonella* system. The presence of the *n*-butoxy group probably decreases the antitumor activity of the compounds by interfering to some extent with the intercalation process involving the ascites cells; in the case of *Salmonella,* the *n*-butoxy substituent apparently prevents intercalation into the deoxyribonucleic acid of this organism. Slightly higher levels were needed for a demonstration of antitumor activity with compounds containing nucleus J in which a phenyl group replaced the methoxy or *n*-butoxy substituent at the 2 position of 6-chloroacridine.

The benz[*a*]acridine nucleus (K) was also found to be an effective polycyclic component in the tertiary amine type of nitrogen mustard (**73**, **74**) although no significant antitumor activity was shown by the secondary amine **70** and only slight activity by **71**. Strong mutagenic activity was displayed by **71**; **70** and **74** were good mutagens; when added as crystals, **72** showed a trace of activity in *Salmonella,* but **73** was inactive. Compounds containing the linear benzacridine nucleus (L) were effective against ascites tumors at levels that were lower than those for the angular form.

The presence of an additional nitrogen atom in the ring system caused significant changes in the antitumor and mutagenic properties dependent on its location. The benzo[*b*][1,10]phenanthrolines **79**, **80**, and **81** (nucleus M) were highly effective against ascites tumors at low molar dosages; the *n*-butyl group on the nitrogen containing the 2-chloroethyl group provided a somewhat greater degree of activity than the ethyl group. The corresponding benzo[*b*][1,8]-phenanthrolines **82**, **83** (nucleus N) were inactive against ascites tumors; **84** was active only at much greater molar dosage levels than those required for **80**, **81**. The mutagenic

responses in *Salmonella* were excellent with both **79** and **83** and were equal to that with the corresponding analog in the benzacridine series **71**. The influence of a methoxy substituent in reducing the dosage levels for antitumor activity became evident again upon comparison of the compounds in the nucleus O series with those in the nucleus N series.

## Discussion

It is evident from these studies that the antitumor and mutagenic activities displayed by the ICR compounds depend on a variety of structural components. In all instances, the nature of the polycyclic portion of the molecule is of great importance. No more than a trace of activity is shown by any of the nitrogen half-mustards or sulfur mustards that contain a methoxyquinoline or a trifluoroquinoline nucleus. A pronounced degree of antitumor and mutagenic effectiveness is displayed by compounds containing an acridine nucleus but not by those containing a tetrahydroacridine nucleus. Antitumor activity is increased by the presence of a 2- or a 4-methoxy substituent in the acridine nucleus and is depressed by an additional 6-chloro substituent. Although the presence of an additional nitrogen atom in the ring system (azaacridines) has a profound effect in increasing the mutagenic activity of the methoxy derivative, the antitumor properties are somewhat less pronounced. Replacement of the methoxy group by a *n*-butoxy group decreases the antitumor activity significantly and obliterates the mutagenic activity. Benzacridine half-mustards are effective mutagens for *Salmonella* and potent antitumor agents. Significant differences were noted in the mutagenic and antitumor properties of the two isomeric azabenzacridines.

The type of mutagenic response and the degree of activity against ascites tumors are also determined by the nature of the amine to which the 2-chloroethyl group is attached. The highly effective mutagens for *Salmonella* are all secondary amines with the exception of **48** (ICR 340) which, however, is still far less active than its homolog **45** (ICR 372). On the other hand, investigations being conducted by H. V. Malling# with *Neurospora* indicate that greater mutagenic responses are noted with the tertiary amines **38**, **25**, **74** than with the secondary amine type **36** (ICR 191). Furthermore, this organism is readily mutated by the bisnitrogen mustards **14**, **28** which do not produce mutations in *Salmonella*. The *E. coli* organism, a close relative of *Salmonella*, is also susceptible to the mutating influence of the ICR 191 type compounds whereas *Drosophila* is more sensitive to the mutagenicity of the tertiary amine form (ICR 170). Mutations due to ICR compounds appear to be highly specific in the *Salmonella hisC207* mutant in contrast to those in *Neurospora* and *Drosophila* which involve an undefined mixture of several types of mutation. The sulfur mustards have not displayed any mutagenic activity in *Salmonella* and only one has been effective in *Neurospora*.

Another determining factor in the activities of the mono- and bisnitrogen mustards and the sulfur mustards is the length of the carbon chain that connects the nitrogen of the polycyclic component with either the nitrogen or the sulfur of the mustard moiety. In all instances, the "propyl" and "methylbutyl" forms are far more active than the "ethyl" form. This observation applies to the antitumor tests with the bisnitrogen mustards, the nitrogen half-mustards, and the sulfur mustards and also to the mutagenic tests with the secondary amine half-mustards. Although some mutagenic activity was shown by the hydroxy precursors, **44** and **46** (ICR 364-OH and ICR 372-OH), containing the exceptionally active azaacridine nucleus, it is evident that alkylation plus intercalation are required for maximum mutagenic and antitumor activity in the heterocyclic nitrogen half-mustards.

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