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Synthesis of Potential Anti-Cancer Agents. XVI. Nitrogen Mustards from 1-Aminophenazine and 8-Aminoquinoline*

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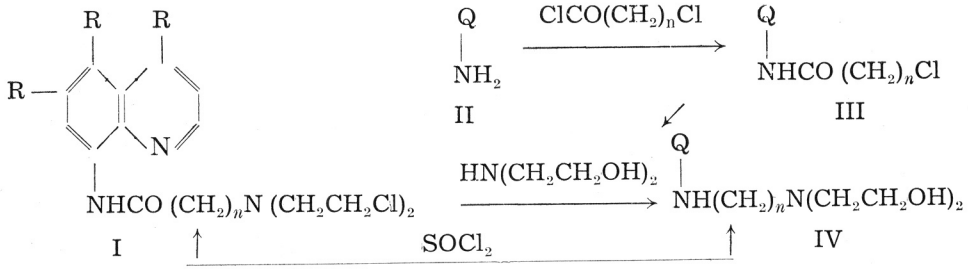
The synthesis of nitrogen mustard amides from 1-aminophenazine and 8-aminoquinoline is described.

In an earlier communication[1] the synthesis of a number of derivatives of 8-[3-bis-(2'-chloroethylamino)-propionamido]-quinoline (I, $n = 2$) as potential anti-cancer compounds was described. Preliminary evaluation of these substances against experimental animal tumors indicated a high degree of activity[2]. It therefore seemed desirable to prepare further substances of this general type in which the side chain in the 8-position of the quinoline ring was varied since it is common knowledge, based largely on studies of the 8-aminoquinoline antimalarials,[3] that the pharmacological action of this group of compounds varies quite widely both qualitatively and quantitatively as the nature of the side chain is altered.



Q = the substituted quinoline

However, the preferred synthetic route to these substances as shown in II—IV—I severely limits the choice in variations of n. Thus, when $n = 1$, possible cyclization of the intermediate (III) onto the quinoline ring nitrogen becomes a factor to be taken into account and when $n = 3$ cyclization of III onto the amide nitrogen also must be considered. In order to obtain some evidence regarding the effect of increasing the length of the side chain in

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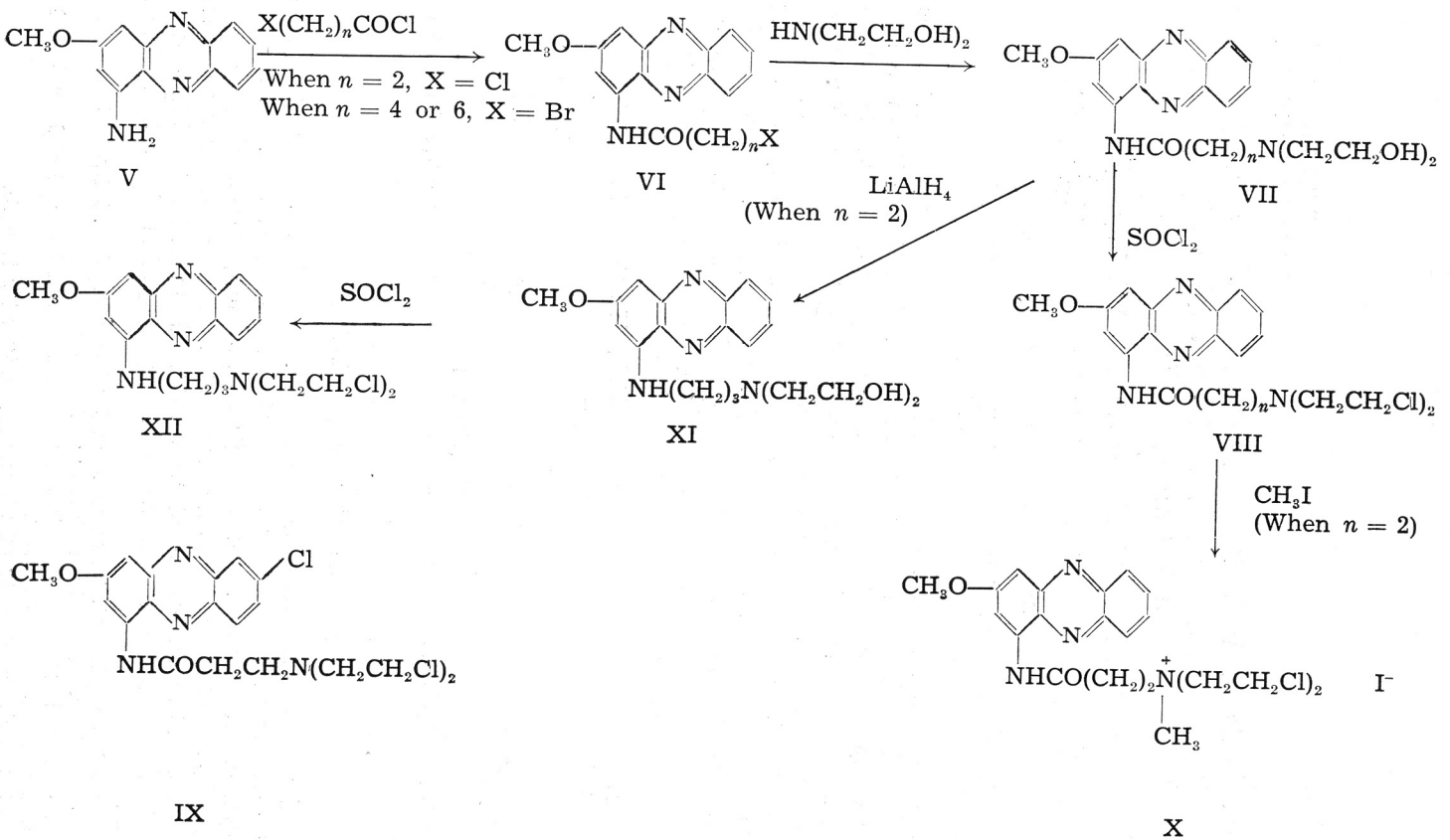

CH3O
NH2
V
X(CH2)nCOCl
When n = 2, X = Cl
When n = 4 or 6, X = Br
NHCO(CH2)nX
VI
HN(CH2CH2OH)2
NHCO(CH2)nN(CH2CH2OH)2
VII
LiAlH4
(When n = 2)
SOCl2
NHCO(CH2)nN(CH2CH2Cl)2
VIII
NH(CH2)3N(CH2CH2OH)2
XI
SOCl2
NH(CH2)3N(CH2CH2Cl)2
XII
CH3I
(When n = 2)
Cl
NHCOCH2CH2N(CH2CH2Cl)2
IX
NHCO(CH2)2N+(CH2CH2Cl)2
CH3
I−
X

substances of the type of I, we have prepared 6-methoxy-8-[7-bis-(2-chloroethylamino-heptanamido]-quinoline (I, n = 6) from the readily available 7-bromoheptanoyl chloride.

Several quaternary salts of phenazine derivatives have shown evidence of activity against experimental animal neoplasms[4]. However, the only unquaternized phenazine which has displayed activity in this regard is 3-methoxy-1-(3-diethylaminopropylamino)-phenazine previously described by one of us.[5] The synthesis of nitrogen mustard derivatives of selected phenazines thus appeared to be warranted. We have therefore prepared derivatives of 1-amino-3-methoxyphenazine of the type shown in VIII in which n = 2,4 or 6 by the sequence V — VIII as well as one derivative (IX) of 1-amino-3--methoxy-6-chlorophenazine.

In view of the activity reported for the quaternary phenazinium compounds, the methiodide (X) was prepared from VIII, $n = 2$. On treatment of X with methanolic hydrogen chloride, the side chain was cleaved at the amido group. Basification of the reaction mixture resulted in recovery of 83 per cent of the calculated amount of 3-methoxy-1-aminophenazine. Mc Ilwain[6] reports that *N*-methylphenazonium hydroxide disproportionates to phenazine and *N*-methyl-dihydrophenazine in equal amounts with formation of one equivalent each of formaldehyde and water. From this we conclude that in all probability, the more basic side-chain (mustard) nitrogen of VIII, $n = 2$, undergoes quaternization.

Reduction of VII, $n = 2$, gave XI, characterized as the picrate, which in turn apparently gave the mustard (XII) on reaction with thionyl chloride. The hydrochloride of XII was an extremely hygroscopic substance for which no satisfactory method of purification could be found.

Results of the evaluation of these compounds against experimental animal tumors will be presented elsewhere.

EXPERIMENTAL

All melting points are uncorrected.

Microanalyses by Spang Microanalytical Laboratory, Ann Arbor, Michigan.

7-Bromoheptanoyl chloride

To a cooled solution of 26.6 g. (0.127 mole) of 7-bromoheptanoic acid[7] was added gradually 30.3 g. (0.254 mole) of thionyl chloride. After stirring at 38—40° for 3 hours, the excess of thionyl chloride was removed at the water pump and the residue was distilled to give 26.6 g. (91%) of the colorless acid chloride, b.p. 90° (0.3 mm.).

6-Methoxy-8-(7-bromoheptanamido)-quinoline

This was prepared in 76% yield by the general method previously described.[1] It melted at 121—122° after recrystallization from 2-propanol.

Anal. $C_{17}H_{21}BrN_2O_2$ (365.28) calc'd.: C 55.90; H 5.78; Br 21.88; N 7.67%
found: C 56.01; H 5.91; Br 21.80; N 7.64%

6-Methoxy-8-[7-bis-(2-hydroxyethylamino)-heptanamido]-quinoline dihydrochloride

This was prepared by the procedure previously described[1]. The yield of yellow crystalline material, m.p. 140—143°, was 69%.

Anal. $C_{21}H_{31}N_3O_4 \cdot 2HCl$ (472.41) calc'd.: C 54.55; H 7.20; N 9.09%
found: C 54.48; H 7.27; N 9.11%

6-Methoxy-8-[7-bis-(2-chloroethylamino)-heptanamido]-quinoline dihydrochloride (I, $n = 6$)

To a cooled solution of 2.3 g. (0.0046 mole) of the above diol in 75 ml. of chloroform (previously dried over calcium chloride) was added 3 ml. of purified thionyl chloride in one portion with vigorous stirring. A brown oily mass separated. After allowing it to come to room temperature was stirred for 3 hr. The chloroform was removed at 40° and the residue was concentrated twice with 20 ml. portions of benzene leaving 2.8 g. of yellow powder, m.p. 85—87°, after crystallization from a large volume of acetone with an amount of decolorizing carbon. The yield was 70%.

When the crude product was heated or allowed to stand with alcoholic hydrogen chloride, the dihydrochloride of 6-methoxy-8-aminoquinoline, m.p. 235—237°, was formed.

Anal $C_{21}H_{29}Cl_2N_3O_2 \cdot 2HCl$ (499.30) calc'd.: C 50.50; H 6.26; Cl 28.41; N 8.42%
found: C 50.28; H 6.46; Cl 28.19; N 8.21%

The Phenazine Derivatives

With a few exceptions these were prepared by the general procedure described in an earlier publication for the 8-aminoquinoline analogs[1]. Deviations from this procedure will be noted. Yields, m.p. 's. and analytical data are given in Table I. Unless otherwise noted, all compounds were recrystallized from absolute ethanol. In general, they formed yellow or brownish yellow crystals.

1-[3-bis-(2-chloroethylamino)-propionamido]-3-methoxyphenazine (VIII, $n = 2$)

Ice-cold purified thionyl chloride (10 ml.) was added all at once to 9.6 g. of VII,$n = 2$, chilled in ice and the mixture was kept in the ice bath an hour with occasional swirling. Within a few minutes most of the diol had dissolved with the formation of a deep red solution. After allowing the mass to come to room temperature overnight, the thionyl chloride was removed at reduced pressure leaving 11.1 g. of the mustard hydrochloride as a red solid, m.p. 181—183° (dec.). An ice-cold suspension of the hydrochloride in 50 ml. of water was basified and stirred for 30 min. Collection of the precipitate and recrystallization from absolute ethanol gave 8 g. (76%) of the free base as golden yellow needles, m.p. 134—135°.

1-[3-bis-(2-hydroxyethylamino)-propylamino]-3-methoxyphenazine (XI)

To a well-stirred suspension of 1.5 g. of lithium aluminum hydride in 150 ml. of dry tetrahydrofuran was gradualy added 3 g. of VII,$n = 2$. After addition of 50 ml. of solvent, the mixture was refluxed gently for 9 hr. To the cooled stirred reaction mixture ethanol was cautiously added followed by 100 ml. of water and finally, 200 ml. of a 10% potassium hydroxide solution. After thorough stirring the organic layer was separated and alkaline solution was extracted with ether. Removal of the solvent from the combined washed and dried organic extracts left 2.8 g. of red viscous material. For isolation 1.4 g. of the crude product was converted to the hydrochloride in warm absolute ethanol. The yield of the light blue salt, m.p. 198—200° (dec.) was 1.5 g. (95%).

The picrate, prepared in absolute ethanol and recrystallized from the same solvent, formed small chocolate-colored needles, m.p. 172.5—173°.

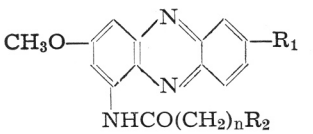
Anal. $C_{26}H_{29}N_7O_{10}$ (599.55) calc'd.: C 52.06; H 4.88; N 16.36%
found: C 52.13; H 4.89; N 16.18%

1-[3-bis-(2-chloroethylamino)-propylamino]-3-methoxyphenazine (XII)

To 1.5 g. of XI purified through the hydrochloride and cooled in an ice bath was added 3 ml. of cold purified thionyl chloride. After one hour in the ice bath the mixture was allowed to stand overnight and the excess thionyl chloride was removed under reduced pressure. The free base was liberated with sodium hydroxide and extracted into chloroform. Conversion to the hydrochloride gave a tacky, hygroscopic material which could not be purified.

TABLE I

Derivatives of 1-Aminophenazine



					Analysis							
					Calculated				Found			
n	R_1	R_2	m.p., °C	Yield (%)	C	H	N	Cl	C	H	N	Cl
2	H	Cl	172—173	85	60.83	4.47	13.32	11.23	60.84	4.72	13.35	11.60
2	H	$N(CH_2CH_2OH)_2$	145—146	79	62.46	6.29	14.58		62.53	6.08	14.75	
2	H	$N(CH_2CH_2Cl)_2$	134—135	76	56.99	5.27	13.30	16.84	56.74	5.54	13.20	17.01
4	H	Br	173.5	90	55.68	4.67	10.82	20.58[a]	55.90	4.77	10.92	20.69[a]
4	H	$N(CH_2CH_2OH)_2$	139—140	85	64.06	6.84	13.58		63.96	6.86	13.68	
4	H	$N(CH_2CH_2Cl)_2$	96—98[b]	55	58.80	5.83	12.47	15.78	58.98	5.98	12.52	15.98
6	H	Br	180	90	57.68	5.33	10.09	19.21[a]	57.85	5.38	9.96	19.06[a]
6	H	$N(CH_2CH_2OH)_2$	104—105	96	65.43	7.32	12.72		65.34	7.24	12.59	
6	H	$N(CH_2CH_2Cl)_2$	71—73[b]	31	60.37	6.33	11.74	14.85	60.21	6.22	11.85	14.27
2	Cl	Cl	210.5	77	54.87	3.74	12.00	20.25	54.79	3.87	12.02	20.24
2	Cl	$N(CH_2CH_2OH)_2$	147.5—148	76	57.34	5.53	13.38	8.46	57.10	5.43	13.25	8.64
2	Cl	$N(CH_2CH_2Cl)_2$	172—173	71	52.70	4.64	12.29	23.34	52.66	4.77	12.15	23.46

(a) Calcd. and found for bromine
(b) Recrystallized from methanol

Methiodide of 1-[3-bis-(2-chloroethylamino)-propionamido]-3-methoxyphenazine (X)

The free base (1.5 g.) was refluxed with methyl iodide (5 ml.) in ethanol for 5 hr. The methiodide (80%) separated as golden yellow plates, m.p. 118.5°.

Anal. $C_{21}H_{25}Cl_2JN_4O_2$ (563.26) calc'd.: C 44.75; H 4.48; N 9.95%
found: C 44.69; H 4.38; N 10.21%

Preparation of the methiodide was particularly sensitive to variation of reaction conditions and solvent. When the reaction time was changed, lower yields resulted. With benzene as solvent, unreacted starting material was recovered.

When 300 mg. of the methiodide was heated with 50 ml. of a saturated solution of hydrogen chloride in methanol, the initial red color of the solution changed to greenish blue within 20 min. After cooling, the solution was diluted and made basic with sodium hydroxide. A red solid (100 mg., 83%) precipitated. This was identified by m.p., mixture m.p. and infrared spectrum as 1-amino-3-methoxyphenazine.

5-Bromovaleryl chloride

5-Bromovaleric acid was prepared by hydrolysis of 5-bromovaleronitrile (Columbia Organic Chemicals, Inc.) with 48-% hydrobromic acid and purified by distillation under reduced pressure, b.p. 121—125° (3 mm.). The acid chloride, b.p. 80° (3.5 mm.) was prepared with thionyl chloride.

1-[5-bis-(2-chloroethylamino)-valeramido]-3-methoxyphenazine (VIII, $n = 4$)

The semi-solid mass obtained by the standard procedure was dissolved in water, the solution was basified and extracted with chloroform. Removal of the solvent from the washed and dried extracts left a red viscous mass which, after recrystallization from methanol, gave brownish yellow crystals (55% yield), m.p. 96—98°.

1-Amino-3-methoxy-7-chlorophenazine

This was prepared according to Elderfield, *et al*[5]. This compound has now been found to melt at 204—205° after recrystallization from a large volume of absolute ethanol. Reported m.p. 187—190°.

Anal. $C_{13}H_{10}ClN_3O$ (259.69) calc'd.: C 60.12; H 3.88; Cl 13.65; N 16.18%
found: C 60.23; H 4.03; Cl 13.72; N 16.08%

Acknowledgment. We acknowledge the valuable assistance of J. Hudson in the preparation of certain intermediates.

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IZVOD

Sinteza potencijalnih antikancerogenih supstancija. XVI. Derivati bis-(2-kloretil)-amina od 1-aminofenazina i 8-aminokinolina

R. C. Elderfield, D. Kolbah i *J. Roy*

Derivati diklor-dietilamina su još uvijek u centru pažnje na području pronalaženja sredstava za sprečavanje rasta tumora. Kao prilog tim istraživanjima priređen je niz novih spojeva iz toga reda polazeći od 1-aminofenazina i 8-aminokinolina.

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