ment for a display of activity in the bis mustard (**D-5**). Further alteration in which a tertiary nitrogen was attached to the nucleus caused profound changes in that the monofunctional nitrogen mustard was inactive (**D-1**) and the bis form was active only at high dosage levels (**D-3**).

Although the monofunctional mustards of 6-methoxy- and 7-chloroquinoline obtained previously[2,4] had displayed no activity, we decided to explore a variety of nuclear substituted and unsubstituted two-ring structures to determine whether the acridine ring system was specifically required for an increase in the molar and biological activities of the nitrogen mustard moiety. Although not strictly comparable in structure, **E-1** was inactive. The quinazoline monofunctional nitrogen mustard (**F-1**) and the bis mustards (**F-3** and **G-1**) were active against the ascites tumor but only at high molar dosages. In the 6-methoxy-8-aminoquinoline series, moderate activity was shown by the monofunctional mustard (**H-1**) and high activity by the bis form (**H-3**). Replacement of the terminal benzene ring in 2-methoxyacridine by two methyl groups to give a 2,3-dimethyl-6-methoxyquinoline (**I-1**) resulted in loss of activity in the monofunctional mustard. On the other hand, the presence of a phenyl group at the 2-position of quinoline led to moderate antitumor activity in the N-ethyl monofunctional mustard (**J-3**) and the N-methyl analog (**J-1**). A similar degree of effectiveness against the Ehrlich tumor was noted with the *p*-chlorophenyl analogs (**K-1** and **K-3**) at higher dosage ranges.

The bis mustards (**L-1**, **L-3**, **L-5**, **M-1**, and **M-5**) of a series of 3,7-dichloroquinolines displayed exceptionally broad, effective ranges but the monofunctional mustard (**M-3**) had only slight activity. The effects of structural variations in the side chain of 7-chloroquinoline were also studied and it was found that the bis forms (**P-7** and **P-9**) were active but the monofunctional mustards (**P-1**, **P-3**, and **P-5**) were ineffective. A similar situation prevailed in the 6-methoxyquinoline series (**Q-1** and **Q-3**) with toxicity, however, being evident at low dosage levels.

In contrast, a methyl group in the 7-chloroquinoline nucleus occasionally conferred moderate activity on the monofunctional mustards (**N-3**, and **S-5** to **S-11**). In the case of the last four compounds, it is interesting that the greatest molar activity was imparted by N-isopropyl, followed in turn by N-propyl, N-ethyl, and N-methyl. The aromatic-type bis nitrogen mustard (**S-19**) and the monofunctional mustard with the secondary amine structure (**T-1**) were found to be inactive, in keeping with our earlier observations[2] on these types of structures.

The miscellaneous series of compounds (**U–X**), the components of some of which are effective against certain types of tumors, were found to be inactive in our tests with ascites tumors. The presence of quinacrine in the compounds **U-1** and **U-2** did not confer activity on the two chloro side chains; the methanesulfonate and ethyleneimino derivative (**V-2** and **W-1**) also displayed no activity. The simple alkylamino nitrogen "half-mustard" **Y**, which was prepared to complete the series described previously,[1,2] was moderately effective against the ascites tumor at a relatively high molar dosage similar to that of its N-ethyl reference compound.

Thus, although a moderate degree of antitumor activity at high molar dosage is retained in certain monofunctional nitrogen mustard derivatives of quinazoline and methylquinolines, the only powerful activator at the moment seems to be the intact acridine nucleus. This suggests the possibility that acridines may be unique in their ability to impart bifunctional character to nitrogen "half-mustard." The observations of Lerman[12] indicate that the spatial configuration of this heterocyclic nucleus plays an important role in the special reactivity of various acridines with deoxyribonucleic acid.

(12) L. S. Lerman, *J. Mol. Biol.*, **3**, 18 (1961).

Acridine and Quinoline Analogs of Nitrogen Mustard with Amide Side Chains[1]

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Several mono- and bifunctional nitrogen mustards attached to aminoalkyl derivatives of some acridines and quinolines through an amide linkage were synthesized and studied with the use of ascites tumors. Since the acridine nucleus was again found to exert a powerful activating influence on both the bis and mono nitrogen mustard moieties, the amide linkage was apparently not hydrolyzed to yield glycine mustard during these *in vivo* tests. The presence of a hydrazine linkage in the side chain led to considerably decreased antitumor effectiveness.

One of the initial reasons for our study of quinoline and acridine nitrogen mustards was based on the observation[2] that related carrier molecules (antimalarial drugs) exhibited preferential localization in different tissues dependent on the chemical structure of the heterocyclic base. Thus, the use of a variety of substituted quinoline and acridine carriers might permit the accumulation of the mustard moiety in specific tissues and presumably also in tumors of these tissues. The exceptionally great chemical and biological activities shown by the acridine mono- and bifunctional

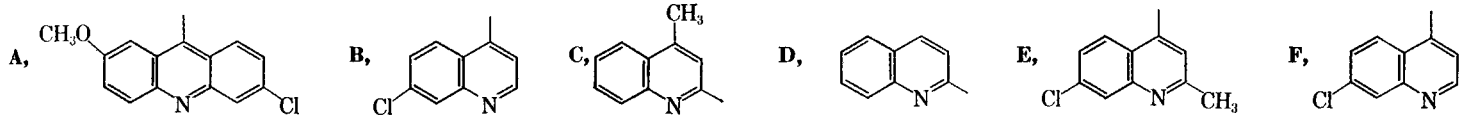
(1) Supported by research Grants CA 02975 and CA 06927 from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service.

(2) L. H. Schmidt, "A Survey of Antimalarial Drugs," F. Y. Wiselogle, Ed., Edwards Bros., Ann Arbor, Mich., 1946.

TABLE I: ANALYTICAL INFORMATION AND ANTITUMOR ACTIVITY

Compd. no.[c]	Side chain	Salt	Yield, %	M.p., °C.	Calcd., % C	Calcd., % H	Calcd., % N	Calcd., % Cl	Found, %[a] C	Found, %[a] H	Found, %[a] N	Found, %[a] Cl	Antitumor activity μmoles/kg.	Antitumor activity Degree
A-1	$NHCH_2CH_2NHCOCH_2N(C_2H_5)(C_2H_4Cl)$	$2HNO_3$	52	128–130 dec.	45.92	4.91	14.60	12.32	45.20	5.39	14.71	12.29	3–10	2.3
A-2	$NHCH_2CH_2NHCOCH_2N(C_2H_5)(C_2H_4OH)$	$B\cdot0.5H_2O$	90	133–138	60.13	6.41	12.73		60.31	6.71	12.50			
A-3	$NHCH_2CH_2NHCOCH_2N(C_2H_4Cl)_2$	2HCl	52	210–212 dec.	47.40	5.08	10.06	31.85	47.43	5.07	10.06	29.84	1.5–12	2.6
A-4	$NHCH_2CH_2NHCOCH_2N(C_2H_4OH)_2$	...	70	180–184	59.12	6.09	12.54		58.87	6.29	12.43			
B-1	$NHCH_2CH_2NHCOCH_2N(C_2H_5)(C_2H_4Cl)$	$2HCl\cdot2.5H_2O$	48	93–96	41.90	6.00	11.50	29.10	42.28	5.93	12.23	28.53	36–60	2.0
B-2	$NHCH_2CH_2NHCOCH_2N(C_2H_5)(C_2H_4OH)$	...	86	168–170	58.20	6.61	15.97		58.04	6.69	15.75			
B-3	$NHCH_2CH_2NHCOCH_2N(C_2H_4Cl)_2$	2HCl	72	225–226	42.80	4.83	11.76	37.20	42.59	5.05	11.53	36.71	4–40	2.7
B-4	$NHCH_2CH_2NHCOCH_2N(C_2H_4OH)_2$	...	54	157.5–160	55.65	6.32	15.27		55.53	6.28	15.22			
C-1	$NHCH_2CHNHCOCH_2N(C_2H_4Cl)_2$	$2HCl\cdot0.5H_2O$	59	178–181	46.51	5.85	12.06	30.50	46.60	5.87	11.98	29.56	15–120	2.7
C-2	$NHCH_2CH_2NHCOCH_2N(C_2H_4OH)_2$	...	63	129–130.5	62.41	7.51	16.17		62.14	7.65	16.33			
D-1	$NHCH_2CH_2NHCOCH_2N(C_2H_4Cl)_2$	$2HCl\cdot0.25H_2O$	51	179–184	45.74	5.54	12.55	31.72	45.87	5.61	12.51	31.47	30–200	2.7
D-2	$NHCH_2CH_2NHCOCH_2N(C_2H_4OH)_2$	...	82	97.2–99	61.45	7.28	16.85		61.85	7.44	16.97			
D-3	$NHNHCOCH_2N(C_2H_5)(C_2H_4Cl)$	2HCl	72	195–196.5	47.45	5.57	14.75	28.02	47.62	5.91	14.78	27.51	40–180	1.3
D-4	$NHNHCOCH_2N(C_2H_5)(C_2H_4OH)$	...	76	135–137	62.49	7.00	19.43		62.56	7.05	19.68			
D-5	$NHNHCOCH_2N(C_2H_4Cl)_2$	2HCl	79	175–177 dec.	43.55	4.87	13.54	34.28	43.87	5.24	13.49	32.36	30–175	2.4
D-6	$NHNHCOCH_2N(C_2H_4OH)_2$	...	62	144–144.7	59.21	6.62	18.40		59.27	6.75	18.55			
E-1	$NHNHCOCH_2N(C_2H_5)(C_2H_4Cl)$	$2HCl\cdot0.75H_2O$	65	211–212.5 dec.	43.52	5.30	12.69	32.12	43.49	5.47	12.96	32.40	25–90	1.1
E-2	$NHNHCOCH_2N(C_2H_5)(C_2H_4OH)$	...	70	155.5–156.8 dec.	57.06	6.29	16.64		56.93	6.53	16.73			
F-1	$NHCH_2CONHCH_2CH_2N(C_2H_4Cl)_2$	2HCl	56	179–181 dec.	42.80	4.83	11.76	37.20	42.65	5.16	11.44	36.72	1.25–7.5	2.5
F-2	$NHCH_2CONHCH_2CH_2N(C_2H_4OH)_2$[b]	...	85	165–168	55.65	6.32	15.27		55.83	6.40	14.75			

[a] Values are either single analyses or averages of checks. [b] This compound was synthesized by the same method and from the same quinolyl intermediate as used in the preparation of N-(7-chloro-4-quinolyl)glycyldiethanolamine (4). [c] The letters A to F in the first column of Table I represent the heterocyclic group attached to the side chains. The heterocyclic structures are



nitrogen mustards,[3-6] however, might conceivably be detrimental to this objective, since any enhanced degree of activity displayed during transport would tend to limit the localization of effective compound in the target areas.

Consequently, it seemed desirable to synthesize and study compounds containing an enzymatically hydrolyzable linkage in the side chain between the heterocyclic nucleus and the mono- or bifunctional nitrogen mustard grouping. Our first series of such compounds contained an amide linkage which, upon cleavage, should yield glycine mustard, a potent alkylating agent which is effective against a variety of animal tumors.[7] One example is given of a different structure which, upon hydrolysis, should yield the highly active 2-bis(2-chloroethyl)aminoethylamine.[3] In the ideal case, such compounds would contain a tissue-directing heterocyclic nucleus, 2-chloroethyl groups of low chemical reactivity to decrease losses during transport, and an enzymatically hydrolyzable linkage which would release a potent alkylating agent in the target area. The beneficial effects achieved by regional perfusion techniques with present chemotherapeutic agents might thus be obtained by regular methods of administration of an improved compound.

Most of the compounds listed in Table I have an amide linkage involving a glycine skeleton with one or two 2-chloroethyl groups. The synthesis of their hydroxy precursors entailed reaction of the corresponding hydrazinoheterocyclic or 2-aminoethylaminoheterocyclic compound with either 4-(2-hydroxyethyl)-2-morpholinone[8] or 4-ethyl-2-morpholinone.[9]

Experimental[10]

The first two groups of compounds (**A, B**) in the table were synthesized from the appropriate morpholinone[8,9] and heterocyclic diamine. Only one diamine is previously unreported; its synthesis is given together with a representative condensation. Chlorination of the hydroxyamides was accomplished with excess thionyl chloride.[3]

2-(2-Aminoethylamino)lepidine.—A solution of 9.0 g. of 2-chlorolepidine in 25 ml. of ethylenediamine was refluxed for 1 hr., and taken up in dilute acetic acid. Overnight cooling and filtration gave 0.7 g. of N,N′-bis(2-lepidyl)ethylenediamine; recrystallization gave 0.5 g., m.p. 215.5–217°.

Anal. Calcd. for $C_{22}H_{22}N_4$: C, 77.19; H, 6.48; N, 16.34. Found: C, 77.00; H, 6.49; N, 16.39.

Addition of excess saturated sodium nitrate precipitated 15.0 g. (90%) of dinitrate, m.p. 203° dec. It was purified by recrystallization from aqueous alcohol; m.p. 198° dec.

Anal. Calcd. for $C_{12}H_{15}N_3 \cdot 2HNO_3$: C, 44.03; H, 5.24; N, 21.40. Found: C, 44.37; H, 5.52; N, 21.80.

N-(2-Lepidyl)-N′-[N,N-bis(2-hydroxyethyl)glycyl]ethylenediamine.—A mixture of 2.3 g. of 2-aminoethylaminolepidine, 1.7 g. of 4-(2-hydroxyethyl)-2-morpholinone, and 5 ml. of ethanol was heated on a steam cone for 1 hr., cooled, diluted with ether, and the product filtered. It weighed 3.0 g. (76%) and melted at 128–130°. Crystallization from ethanol gave an analytical sample reported in Table I (**C-2**).

Biological Results.—Results of the studies with ascites tumor-bearing mice are presented in the table. The testing procedure and the analysis of data were described in the preceding paper.[6] The reference compound for this series is glycine mustard, N,N-bis(2-chloroethyl)glycine hydrochloride, which displayed a degree of activity of 2.5 in the range 4–30 μmoles/kg.

Although 2-methoxy-6-chloro-9-ethylaminoacridine glycine "half-mustard" (**A-1**) displayed maximum activity within the same range as the bis form (**A-3**), the lowest dosage of the monofunctional nitrogen mustard producing the standard, minimum degree of antitumor effectiveness was twice that of the bis analog. The pronounced influence of the quinacrine nucleus in reducing the molar-dosage requirement was again evident when an amide linkage was present in the side chain between the nucleus and the nitrogen-mustard moiety. The results indicate that, in both instances, the entire molecule functioned as a unit in its action on the ascites tumors rather than through the formation of glycine mustard or glycine "half-mustard" by hydrolysis of the amide linkage. If rapid and complete hydrolysis had occurred in the peritoneal cavity, the dosage range for activity of the quinacrine bis analog would be 4–30 μmoles/kg. instead of 1.5–12 μmoles/kg. and the monofunctional form would be relatively inactive. It is possible, however, that *in vivo* hydrolysis of the amide linkage in these compounds might occur in other tissues and organs of the mouse; this is currently being explored.

Substitution of the ethylchloroquine nucleus into glycine mustard (**B-3**) caused no alteration in the molar and biological activities of the mustard moiety. In contradistinction to the lack of antitumor activity shown by the monofunctional aminoalkylamino mustards of chloroquine described in the preceding paper, the glycine "half-mustard" derivative (**B-1**) exhibited moderate activity, although at relatively high molar dosage. The two bis nitrogen mustards (**C-1, D-1**) were highly effective against ascites tumors when large dosages were administered to the mice; these activities are in keeping with previously observed relationships[5,6] in the alkylaminoalkyl mustards involving these two heterocyclic nuclei. When the union of glycine mustard to quinoline involved a hydrazine linkage, the bis mustard (**D-5**) remained active, but the monofunctional mustards (**D-3, E-1**) were ineffective against the ascites tumor.

Since earlier work[3] had shown that 2-bis(2-chloroethyl)aminoethylamine dihydrochloride had a degree of activity of 2.7 at 2–12 μmoles/kg., its ethylchloroquine derivative (**F-1**) was prepared and studied. It was found to be highly effective against the ascites tumor in a molar range similar to that of the simple aminoalkyl mustard component, thus paralleling the observations with the chloroquine glycine mustard derivative (**B-3**).

When the bis mustards of acridine and quinoline containing an amide linkage in the side chain are compared to those with an aminoalkylamino side chain,[3,6] it is seen that the former compounds have retained the broad activity range between low and high dosages which is characteristic of glycine mustard.

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(10) See preceding paper (ref. 6) for information on melting points.