A. **Ethyl N-propioloyl-11-aminoundecanoate**, yield 40%, was recrystallized from ethyl ether as needles: mp 68–69°; ν^{CHCl_3} 2120 (C≡C), 1725 (C=O ester), 1660 and 1515 (C=O amide) cm^{-1}. *Anal.* Calcd for $C_{16}H_{27}NO_3$: C, 68.29; H, 9.67. Found: C, 68.26; H, 9.59.

Preparation of Enamine Mustards (3).—Bis(2-chloroethyl)-amine hydrochloride was dissolved in cold water and neutralized with 1 *N* NaOH as fast as possible under constant stirring and cooling. The aqueous mixture was exhaustively but rapidly extracted with ethyl ether, and the ether extract was washed with water and saturated NaCl solution and dried by passing through a Drierite bed. The ether solution was concentrated in the cold and used immediately in the following reaction. It was estimated that about 90% of the free amine was recovered.

Bis(2-fluoroethyl)amine[14] and (2-fluoro-2-chlorodiethyl)-amine[14] were liberated from their hydrochlorides by similar methods.

The following is a general method for the reaction of acetylenic compounds with bis(2-chloroethyl)amine or its fluoro analogs.

A solution of the bis(2-haloethyl)amine **2** in ether or DMF was added to a solution or suspension of the acetylene derivative **1** in the appropriate solvent in the cold. After stirring for 2 hr the reaction mixture was placed in the refrigerator and allowed to stand. The progress of the reaction was examined by withdrawing aliquots from the reaction mixture and determining the infrared spectra. When the reaction was completed, the solvent was evaporated and the residue was recrystallized or purified by distillation. The reaction of **2** with amino acid esters was slow and the reaction mixture was allowed to stand at room temperature for 5–10 days. The properties of the products are listed in Table II.

Methyl *p*-Nitrophenylpropiolate.—*p*-Nitrophenylpropiolic acid was prepared by the method of Perkin and Bellenot[16] from ethyl *p*-nitrocinnamate. It was decarboxylated in 86% yield to *p*-nitrophenylacetylene by the method of Drewson[17] (mp 149–152°, lit.[17] mp 152°) and esterified by heating for 16 hr with methanol and concentrated H_2SO_4 to yield 61% of product, mp 109–111°.

Bis(2-hydroxyethyl)aminostyrene Derivatives (5).—Diethanolamine was added to a solution of an equimolar quantity of the acetylenic compound in DMF (in the case of **5a** no solvent was used). The mixture was heated to 60–80° and the progress of the reaction was followed by examining the infrared spectra of aliquots. The reaction was completed in 1–2 hr. The solvent was removed under very high vacuum in a rotary evaporator and the residue was recrystallized. In the case of ethyl phenylpropiolate, the product was liquid and could not be purified by distillation. Any basic materials were removed by passing the reaction mixture through a bed of Amberlite IRC-50 (weakly acidic ion-exchange resin). The properties of the products are in Table III.

Methanesulfonates (6).—A solution of methanesulfonyl chloride (1 mole) in $CHCl_3$ was added to a solution of 0.5 mole of **5** in $CHCl_3$ and pyridine. The mixture was stored at 0° for 20 hr and it was then washed with water and saturated solution of NaCl and dried ($MgSO_4$). Evaporation of the solvent and recrystallization of the residue yielded the compounds listed in Table III.

Chlorides (7).—A solution of the methanesulfonates **6** and an excess of anhydrous LiCl in DMF was stirred for 20 hr at room temperature. The solvent was evaporated under high vacuum and the residue was triturated with CH_2Cl_2. The CH_2Cl_2 extract was decolorized with charcoal and concentrated to dryness, and the residue was recrystallized. The properties of the products are listed in Table III.

Hydrolysis Studies.—Alcoholic stock solutions of the enamine mustards were mixed with equal volumes of Clark and Lubs buffer solutions (0.1 *M* KH_2PO_4 and 0.1 *N* NaOH solutions) at pH 6, 7, and 7.9. The mixtures were allowed to stand at room temperature and their ultraviolet absorbance was determined at various times. Compounds **3h–j**, **6a** and **c**, and **7a** and **c** were stable in the three pH's examined. Compounds **3a–g**, **m**, **o**, and **p** hydrolyzed with rates very similar to that shown in Figure 1 for **3n**. The hydrolysis rates of **7b** were identical with those of **6b** shown in Figure 2.

Acknowledgment.—We thank Dr. Wilson M. Whaley for his contributions and encouragement in the initial phases of our program, Mrs. Frances Potts Fernandes for technical assistance, and Dr. G. Richard Handrick for helpful discussions.

Potential Antitumor Agents. V. Bisquaternary Salts

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Received October 31, 1966
Revised Manuscript Received February 16, 1967

A series of bis quaternary ammonium heterocycles has been prepared and evaluated against the L1210 leukemia system.

The observation of Ambrose and co-workers[2,3] that tumor cells apparently have a higher negative surface charge suggests that basic compounds could be concentrated in such cells. Moreover, if these compounds were also cytotoxic it should be possible to demonstrate selective toxicity toward the tumor cells. The antitumor properties of a recently prepared series of bisimidazolines[4,5] convincingly illustrate this point. The remarkable life extension obtained in leukemia with these drugs, coupled with the well-authenticated similarity of pharmacologically active compounds containing either amidinium or quaternary ammonium functions[6,7] prompted us to investigate a series of bisquaternary ammonium heterocyles.

The first demonstration of unequivocal activity against the L1210 mouse leukemia in this laboratory was provided by the bisquinolinium salts I (R′ = H, Table I). While the bismethyl (I, R′ = H; R = CH_3) and bisethyl quaternary salts showed only borderline inhibition against this tumor, the *n*-propyl homolog showed decided inhibition. Maximum activity was reached with the *n*-butyl derivative and dropped off rapidly in the higher homologs, the *n*-hexyl compound being inactive. Our results, with an extensive range of quaternary salts, have led us to the conclusion that there is a marked dependence of biological properties on the relative lipophilic–hydrophilic balance of these compounds. For each structural type it is necessary to construct a homologous series of quaternary salts with a range of lipophilic–hydrophilic properties. Only by selecting the member of each homologous series

(1) Author to whom inquiries should be addressed.
(2) E. J. Ambrose, A. M. James, and J. H. B. Lovich, *Nature*, **177**, 576 (1956).
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(5) See *Cancer Chemotherapy Rept.*, **19**, 1 (1962), for preliminary biological test data.
(6) R. Jensch, *Angew. Chem.*, **60**, 248 (1948).
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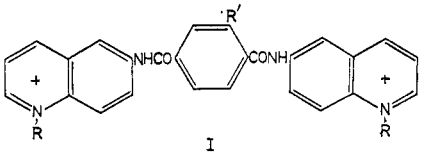
TABLE I[a]

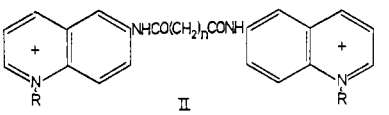


I

R	R′	Mp, °C	Formula	Calcd, % C	H	N	S	Found, % C	H	N	S	R_D[c]	L1210[d]
CH_3	H	326 dec	$C_{42}H_{38}N_4O_8S_2 \cdot H_2O$	62.3	5.0	6.9	7.9	62.0	4.9	7.0	8.1	0.61	±
C_2H_5	H	299 dec	$C_{44}H_{42}N_4O_8S_2 \cdot 2H_2O$	61.8	5.4		7.5	61.4	5.4		7.5	0.68	±
$(CH_2)_2CH_3$	H	282 dec	$C_{46}H_{46}N_4O_8S_2 \cdot 2H_2O$	62.6	5.7		7.3	62.7	5.9		7.4	0.80	+
$(CH_2)_3CH_3$	H	284 dec	$C_{48}H_{50}N_4O_8S_2 \cdot H_2O$	64.6	5.9		7.2	64.1	6.0		7.4	0.82	+
$(CH_2)_4CH_3$	H	302 dec	$C_{50}H_{54}N_4O_8S_2 \cdot 1.5H_2O$	64.6	6.2		6.9	64.4	6.0		6.7	0.87	±
$(CH_2)_5CH_3$	H	302 dec	$C_{52}H_{58}N_4O_8S_2 \cdot H_2O$	65.7	6.4		6.5	65.7	6.4		6.5	0.92	−
$(CH_2)_2OCH_3$	H	282 dec	$C_{46}H_{46}N_4O_{10}S_2 \cdot 2H_2O$	61.6	5.4		7.1	61.5	5.4		7.4	0.78	+
$(CH_2)_2OC_2H_5$	H	274 dec	$C_{48}H_{50}N_4O_{10}S_2 \cdot 1.5H_2O$	62.4	5.8		6.9	62.0	5.6		7.1	0.87	−
$(CH_2)_2O(CH_2)_3CH_3$	H	282 dec	$C_{52}H_{58}N_4O_{10}S_2 \cdot 2H_2O$	63.4	6.2		6.5	63.6	6.4		6.4	0.95	−
b	NO_2	297–299	$C_{26}H_{17}N_5O_4$	67.4	3.7	15.1		67.3	4.1	14.6			
C_2H_5	NO_2	322 dec	$C_{44}H_{41}N_5O_{10}S_2 \cdot H_2O$	60.0	4.9		7.3	60.3	5.1		7.4	0.81	−
$(CH_2)_2CH_3$	NO_2	312 dec	$C_{46}H_{45}N_5O_{10}S_2 \cdot 1.5H_2O$	60.1	5.3		7.0	60.1	5.4		6.9	0.91	−
$(CH_2)_3CH_3$	NO_2	308 dec	$C_{48}H_{49}N_5O_{10}S_2 \cdot H_2O$	61.5	5.5		6.8	61.3	5.6		6.9	1.00	−
$(CH_2)_4CH_3$	NO_2	306 dec	$C_{50}H_{53}N_5O_{10}S_2 \cdot 0.5H_2O$	62.8	5.7		6.7	63.1	5.9		6.8	1.08	−
C_2H_5	NH_2	300 dec[e]	$C_{44}H_{43}N_5O_8S_2 \cdot 2H_2O$	60.7	5.4		7.4	60.4	5.6		7.1	0.74	−
$(CH_2)_2CH_3$	NH_2	270 dec[e]	$C_{46}H_{47}N_5O_8S_2 \cdot H_2O$	62.8	5.6		7.3	62.4	5.7		6.9	0.83	+
$(CH_2)_3CH_3$	NH_2	280 dec[e]	$C_{48}H_{51}N_5O_8S_2 \cdot 1.5H_2O$	62.8	5.9		7.0	62.4	6.0		6.9	0.94	±
$(CH_2)_4CH_3$	NH_2	275 dec[e]	$C_{50}H_{55}N_5O_8S_2 \cdot 2H_2O$	62.9	6.2		6.7	62.5	6.1		6.8	1.05	±

[a] The anion used is *p*-toluenesulfonate. [b] Free base. [c] R_f relative to internal standard; see Experimental Section. [d] Results according to our experimental procedure against the tumor system increase in life span of 25–50%, ±; 50–100%, +; 100%, ++ (see Experimental Section for full details). [e] With previous darkening and sintering

TABLE II[a]



II

R	*n*	Mp, °C	Formula	Calcd, % C	H	N	S	Found, % C	H	N	S	R_D[c]	L1210[d]
...	3[b]	229–230	$C_{23}H_{20}N_4O_2$	71.1	6.2	14.4		71.3	5.9	14.2			
C_2H_5	3	264–265	$C_{41}H_{44}N_4O_8S_2 \cdot H_2O$	61.3	5.8		8.0	60.9	5.6		8.1	0.91	−
$(CH_2)_3CH_3$	3	258–259	$C_{45}H_{52}N_4O_8S_2 \cdot H_2O$	62.9	6.3		7.5	62.7	6.4		7.4	0.98	−
$(CH_2)_5CH_3$	3	238–240	$C_{49}H_{60}N_4O_8S_2$	65.6	6.7		7.1	65.5	6.9		7.3	1.06	−
...	4[b]	250–251	$C_{24}H_{22}N_4O_2$	72.3	5.6	14.1		72.1	5.9	14.2			
C_2H_5	4	262–264	$C_{42}H_{46}N_4O_8S_2$	63.1	5.8	7.0	8.0	63.0	6.0	6.7	7.8	0.96	−
$(CH_2)_3CH_3$	4	264 dec	$C_{46}H_{54}N_4O_8S_2$	64.6	6.4		7.5	64.4	6.5		7.7	1.03	−
$(CH_2)_5CH_3$	4	276 dec	$C_{50}H_{62}N_4O_8S_2$	65.8	6.9		7.0	65.7	7.0		7.3	1.12	−

[a–d] See corresponding footnotes in Table I.

showing maximum biological activity can a comparison of structural types be made. Then it appears that biological activity is determined to a greater extent by structural features rather than by physical properties.

The quaternizing function may carry substituents, and the resultant molecules show antileukemic effectiveness if, presumably, the lipophilic–hydrophilic properties are in the correct range [*e.g.*, I (Table I), R′ = H; R = $(CH_2)_2OCH_3$].

Examination of a series of related molecules where the terephthaloyl backbone was replaced by flexible aliphatic dicarboxylic acid (II, Table II) of approximately the same linear dimensions and in which a range of quaternizing functions were used showed no activity. Similar results were obtained when aralkyldicarboxylic acids were used (III and IV, Table III).

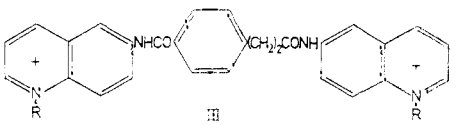
However, the isosteric series where pyridine-2,5-dicarboxyl replaced terephthaloyl contained active members (V, Table III), but activity did not appear to be as high as in the parent series.

In a series of nitro-substituted derivatives (I, R′ = NO_2, Table I), no activity was observed although the corresponding primary amines (I, R′ = NH_2, Table I) were active.

In an attempt to examine the effect of reducing charge separation the quaternary salts from tere- and isophthaloyl derivatives of 5- and 7-aminoquinoline were examined (VI–X, Tables III and IV), but no active compounds were found. Conversely, when intercharge separation was increased by a variety of means, augmented activity resulted. Both the cinnamoyl series (XI, Table V) and the phenylquinolines (XII, Table V) contained active members. Also, certain of the phenoxyacetic derivatives (XIII, Table V) were active although at a reduced level; this lower activity may be a consequence of a departure from planarity about the ether–methylene linkage.

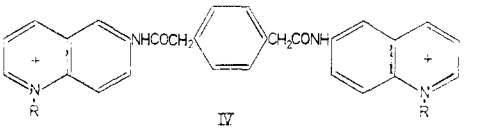
Since the bismethyl quaternary salt (XII, R = CH_3) was active while the corresponding ethyl derivative (XII, R = C_2H_5) was inactive, it was assumed that

TABLE III[a]



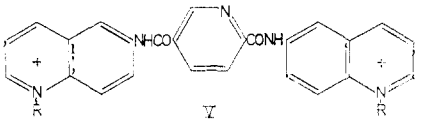
III

R	Mp, °C	Formula	Calcd, % C	H	N	S	Found, % C	H	N	S	R_D[c]	LI210[d]
b	308–309	$C_{28}H_{22}N_4O_2$	75.3	5.0	12.6		75.1	5.3	12.6			
CH_3	302–303	$C_{44}H_{32}N_4O_8S_2 \cdot H_2O$	63.2	5.3		7.7	63.5	5.6		7.7	0.90	–
C_2H_5	291–293	$C_{46}H_{46}N_4O_8S_2$	65.3	5.5		7.6	65.5	5.6		7.3	0.98	–
$(CH_2)_2CH_3$	289–291	$C_{48}H_{50}N_4O_8S_2 \cdot H_2O$	64.6	5.7		7.2	64.7	5.6		7.1	1.06	–



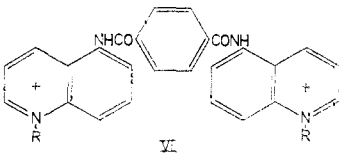
IV

b	>360	$C_{28}H_{22}N_4O_2$	75.3	5.0	12.6		75.5	5.1	12.3			
C_2H_5	292–294	$C_{46}H_{46}N_4O_8S_2$	65.3	5.5		7.6	65.5	5.6		7.3	0.92	–
$(CH_2)_3CH_3$	282–284	$C_{50}H_{54}N_4O_8S_2 \cdot H_2O$	65.2	6.1		7.0	65.4	6.2		6.9	1.07	–



V

b	320–322	$C_{25}H_{17}N_5O_2$	71.6	4.1	16.7		71.8	4.0	16.8			
CH_3	306–307	$C_{41}H_{37}N_5O_8S_2 \cdot H_2O$	60.8	4.9		7.9	60.5	4.7		8.0	0.60	±
C_2H_5	235–240	$C_{43}H_{41}N_5O_8S_2 \cdot 2H_2O$	60.3	5.3		7.5	60.1	5.5		7.3	0.73	±
$(CH_2)_2CH_3$	272–274	$C_{45}H_{45}N_5O_8S_2 \cdot 2H_2O$	61.1	5.6		7.25	61.2	5.7		7.4	0.80	±
$(CH_2)_3CH_3$	288–290	$C_{47}H_{49}N_5O_8S_2 \cdot 2H_2O$	63.2	5.75		7.2	62.9	5.6		7.1	0.82	+
$(CH_2)_4CH_3$	281–284	$C_{49}H_{53}N_5O_8S_2 \cdot H_2O$	63.8	6.0		7.0	63.9	6.1		6.9	0.86	–



VI

b	>360	$C_{28}H_{18}N_4O_2$	74.6	4.3	13.4		74.3	4.55	13.7			
C_2H_5	298–299	$C_{44}H_{42}N_4O_8S_2 \cdot 2H_2O$	61.8	5.4		7.5	62.1	5.3		7.1	0.81	–
$(CH_2)_3CH_3$	286 dec	$C_{48}H_{56}N_4O_8S_2$	64.7	5.9		7.2	64.7	6.0		7.8	0.98	–

[a–d] See corresponding footnotes in Table I.

these molecules were close to the permissible upper limit for fat–water partition, consonant with antitumor properties. Accordingly, a more water-soluble series of isomeric phenylpyridines (XIV–XVII, Tables V and VI) were prepared.

The quaternary salts from the 2-phenylpyridines (XIV, Table V) were completely inactive while those from the 3 (XV) and 4 (XVI) isomers (Table VI) contained the most powerful antileukemic agents listed in this paper. The optimum members of these series are those with an ethyl quaternary function and these, at optimal drug levels, give life extensions of 250–300% while significant results can be obtained over a fivefold range of doses.

Introduction of one-2-phenylpyridyl residue (XVII, Table VI) is sufficient to completely abolish activity. This result lends support to the thesis that over-all coplanarity of these molecules is essential for antitumor effectiveness. A quaternary function of a 2-phenylpyridine would certainly give rise to steric overcrowding which could be relieved by a rotation of the plane of the pyridine ring from that of the phenyl substituent.

The marked changes induced by variation of the quaternary functions in the compounds listed in this paper suggest that biological properties are dependent on some physical parameter, probably the partition coefficient at a lipid–water barrier. Many workers have drawn attention to the importance of such coefficients in a diverse series of drugs, *inter alia* hypnotics,[8–10] bacteriostatic phenols,[11,12] and naphthoquinone antimalarials.[13]

Many score of passing comments on the importance of this property can be found in the literature; many examples can be seen in biologically active series where attention has not been drawn to it. In a series where this property is of importance, random synthesis of structural variants without adjustment of lipophilic–hydrophliic properties could certainly give rise to anomalous structure–activity relationships.

It is not as yet possible to measure the partition coefficient at a lipid interface in a living cell, but it has been shown that there is a parallelism between partition

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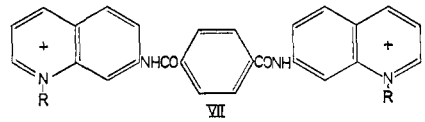
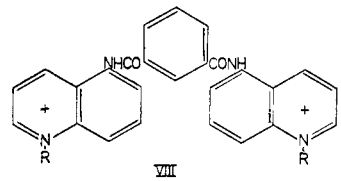
TABLE IV[a]



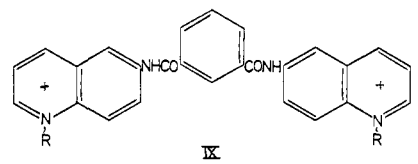
VII

R	Mp, °C	Formula	Calcd, % C	H	N	S	Found, % C	H	N	S	R_D[c]	L1210[d]
b	328–329	$C_{26}H_{18}N_4O_2$	74.6	4.3	13.4		74.65	4.6	13.4			
C_2H_5	293–294	$C_{44}H_{42}N_4O_8S_2 \cdot 2H_2O$	61.8	5.4		7.5	62.0	5.7		7.3	0.80	–
$(CH_2)_3CH_3$	277–278	$C_{48}H_{50}N_4O_8S_2 \cdot H_2O$	64.7	5.9		7.2	64.3	6.3		7.2	0.84	–



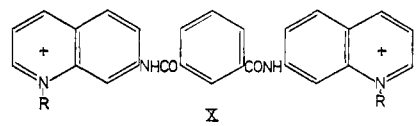
VIII

b	312–313	$C_{26}H_{18}N_4O_2$	74.6	4.3	13.4		74.5	4.5	13.9			
C_2H_5	154–157	$C_{44}H_{42}N_4O_8S_2 \cdot 2H_2O$	61.8	5.4		7.5	61.6	5.7		7.2	1.04	–
$(CH_2)_3CH_3$	142–144	$C_{48}H_{50}N_4O_8S_2 \cdot H_2O$	64.7	5.9		7.2	64.4	6.1	7.3		1.24	–



IX

b	293–294	$C_{26}H_{18}N_4O_2$	74.6	4.3	13.4		75.0	4.7	13.2			
C_2H_5	144–148	$C_{44}H_{42}N_4O_8S_2 \cdot 2H_2O$	61.8	5.4		7.5	61.4	5.5		7.3	0.91	–
$(CH_2)_3CH_3$	128–132	$C_{48}H_{50}N_4O_8S_2 \cdot 3H_2O$	62.85	5.9		7.0	62.6	6.1		6.8	1.02	–



X

b	301–302	$C_{26}H_{18}N_4O_2$	74.6	4.3	13.4		74.65	4.6	13.6			
C_2H_5	132–136	$C_{44}H_{42}N_4O_8S_2 \cdot 2H_2O$	61.8	5.4		7.5	61.4	5.6		7.1	0.94	–
$(CH_2)_3CH_3$	122–125	$C_{48}H_{50}N_4O_8S_2 \cdot 3H_2O$	62.85	5.9		7.0	62.6	6.1		6.5	1.03	–

[a–d] See corresponding footnotes in Table I.

coefficient in a water-immiscible solvent mixture and diverse biological properties. Immiscible solvents that have been utilized are olive oil,[8–12] diethyl ether,[13] heptane,[14] benzene,[14] and $CHCl_3$.[14] It is possible to criticize these *in vitro* systems in that the solvent used probably has little structural relationship to the lipid material in cellular membranes. We suggest, however, that if the partition coefficients of a series of drugs were measured in different water–aliphatic solvent systems, although different numerical values would be obtained in each solvent system, the *order* of values for the series of drugs would remain the same from solvent to solvent.[15] Therefore, in a series of drugs where partition at a cellular lipid barrier was a prime determinant of activity, it would not be surprising to find a correlation between partition coefficients in water–aliphatic solvent systems and biological activity.

It is possible to check some of the above statements since a very large array of partition coefficients in organic solvent–water systems exist as R_f values from paper chromatographic data, partition coefficient being proportional to $(1 - R_f)/R_f$ (ignoring, momentarily, absorption efforts on the paper). Reference to the many compilations of R_f values shows that the *order* of the R_f values obtained does not change markedly from solvent to solvent,[15] although very different numerical values are obtained in different solvents. Relative partition coefficients can be easily and conveniently measured by paper chromatography in immiscible solvents, but undoubtedly adsorption effects on the paper influence the results. The macromolecular cellulose has an array of functions capable of binding in diverse ways with an impingent molecule. But, in the cell, many other macromolecules are present to influence partition across a cellular barrier in a similar way. R_f values measured for the drugs mentioned in this paper in an aqueous 1-butanol system shows that the peak members of each biologically active homologous series have extremely similar values, the range being from 0.81 to 0.88. It has been found that with this system it is possible to predict accurately what changes in the quaternizing function are necessary to reach the peak member in any series that has been prepared.[16]

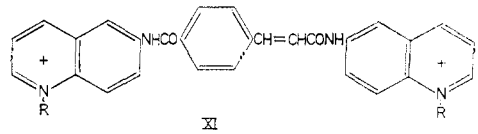
Examination of the more active members of the compounds described against a variety of rodent tumors has shown that there is no inhibition of Sarcoma 180 or the spontaneous mammary tumors in C3H females.

(14) B. B. Brodie in Absorption and Distribution of Drugs, AMAPI Symposium, E. and S. Livingstone, Ltd., Edinburgh, Scotland, 1964.

(15) This argument is only advanced for the aliphatic solvents and not for aromatics; phenol, collidine, etc., do give rise to changes of order in a series, presumably though solvent–solute interactions.

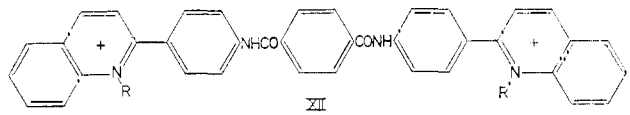
(16) Further values from more than 200 compounds will be published later.

TABLE V[a]



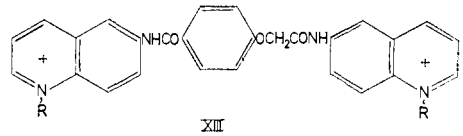
XI

R	Mp, °C	Formula	Calcd, % C	H	N	S	Found, % C	H	N	S	R_D[c]	L1210[d]
b	357–358	$C_{28}H_{20}N_4O_2$	75.65	4.5	12.6		75.8	4.4	12.9			
CH_3	348–349	$C_{44}H_{40}N_4O_8S_2 \cdot 2H_2O$	61.9	5.2		7.5	61.7	5.3		7.2	0.74	−
C_2H_5	318–321	$C_{46}H_{44}N_4O_8S_2 \cdot H_2O$	64.0	5.4		7.4	63.9	5.6		7.3	0.82	++
$(CH_2)_2CH_3$	281–282	$C_{48}H_{48}N_4O_8S_2 \cdot H_2O$	64.7	5.6		7.2	64.4	6.2		7.3	0.90	++
$(CH_2)_3CH_3$	278–281	$C_{50}H_{52}N_4O_8S_2 \cdot 2H_2O$	64.1	6.0		6.8	63.9	6.2		6.7	0.97	−



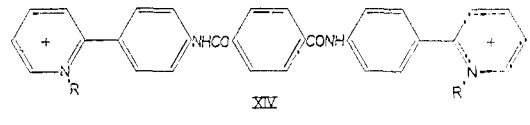
XII

b	>360	$C_{38}H_{26}N_4O_2$	80.0	4.6	9.8		79.8	4.4	9.9			
CH_3	343–345	$C_{54}H_{46}N_4O_8S_2 \cdot 2H_2O$	66.5	4.6		6.6	66.3	4.7		6.4	0.88	++
C_2H_5	323–328	$C_{56}H_{50}N_4O_8S_2$	69.5	4.8		6.6	69.1	4.6		6.4	1.01	−



XIII

b	>360°	$C_{27}H_{20}N_4O_3$	72.3	4.5	12.5		71.9	4.1	12.1			
CH_3	301 dec	$C_{43}H_{40}N_4O_9S_2$	62.9	4.9		7.8	62.8	5.4		7.6	0.66	−
C_2H_5	279 dec	$C_{45}H_{44}N_4O_9S_2 \cdot H_2O$	62.3	5.35		7.4	62.5	5.4		7.2	0.78	−
$(CH_2)_2CH_3$	262 dec	$C_{47}H_{48}N_4O_9S_2 \cdot 2H_2O$	62.1	5.8		7.1	62.0	6.1		6.8	0.83	±
$(CH_2)_3CH_3$	235–237	$C_{49}H_{52}N_4O_9S_2 \cdot 2H_2O$	62.55	6.0		6.8	62.3	5.5		6.7	0.86	+
$(CH_2)_4CH_3$	228–232	$C_{51}H_{56}N_4O_9S_2 \cdot H_2O$	64.3	6.15		6.7	63.7	6.0		6.6	0.94	±



XIV

b	>360	$C_{30}H_{22}N_4O_2$	76.6	4.7	11.9		76.1	4.9	11.8			
CH_3	306–308	$C_{46}H_{42}N_4O_8S_2 \cdot H_2O$	63.9	5.3		7.4	63.7	5.5		7.2	0.85	−
C_2H_5	302–322	$C_{48}H_{46}N_4O_8S_2 \cdot H_2O$	64.7	5.65		7.2	64.5	5.5		7.3	0.96	−
$(CH_2)_2CH_3$	326–328	$C_{50}H_{50}N_4O_8S_2 \cdot H_2O$	65.4	5.9		7.0	65.5	5.7		6.9	1.07	−

[a–d] See corresponding footnotes in Table I.

Marked inhibition of a methylcholanthrene-induced lymphosarcoma has been obtained, the results paralleling those obtained with the L1210 leukemia.

Experimental Section

The following examples outline the general methods used for the preparation of the bulk of the compounds described in this paper.

N,N'-(6-Quinolyl)terephthalamide.—A solution of terephthaloyl chloride (0.1 mole) in dioxane (100 ml) was added dropwise with stirring to a solution of anhydrous 6-aminoquinoline (0.22 mole) in toluene (500 ml) on the H_2O bath. Heating and stirring were continued for 2 hr. When cold the precipitated HCl was collected, washed with petroleum ether (60–80°), and dried *in vacuo*. The crude HCl was suspended in 50% aqueous methanol (500 ml) and concentrated NH_4OH was added with vigorous stirring until the suspension was strongly alkaline. The mixture was stirred vigorously for a further hour and the free base was collected, washed well with H_2O, and dried. Crystallization from dimethylformamide (DMF)–methanol gave the pure base, mp >360°.

Anal. Calcd for $C_{26}H_{18}N_4O_2 \cdot 0.5(CH_3)_2NCHO$: C, 72.6; H, 4.8; N, 13.9. Found: C, 72.8; H, 4.9; N, 13.7.

Crystallization from phenol gave a sample, mp >360°.

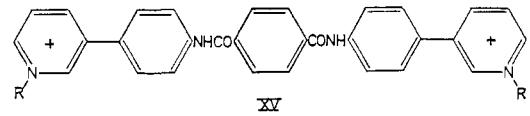
Anal. Calcd for $C_{26}H_{18}N_4O_2$: C, 74.6; H, 4.3; N, 13.4. Found: 74.4; H, 4.5; N, 13.3.

Most bis bases, prepared by similar methods, were obtained in virtually quantitative yield. Only when the acid was substituted did the yields drop, but generally they were better than 65%. Many of these compounds failed to melt below 360°. Solvents for crystallization are limited; occasionally 1-butanol could be used but more normally, pyridine, phenol, DMF, N-methylpyrrolidone, or dimethyl sulfoxide (DMSO) was used. Crystallization from DMF often yields solvates from which it is extremely difficult to remove the solvent by drying *in vacuo*. The bases were dried at 100° (vacuum) for analysis.

Quaternization.—In all cases alkyl *p*-toluenesulfonates were used as quaternizing agents. The following exemplifies the general method. N,N'-(6-Quinolyl)terephthalamide (1.0 g) was dissolved in boiling DMF (10 ml), the solution was cooled to 140°, and methyl *p*-toluenesulfonate (4 molar proportions) was added in one portion. The solution was then heated at 140–150° for 30 min. The mixture was cooled well and the crystalline salt was collected. The solid was suspended in boiling water (100 ml) and ethanol slowly was added until solution was complete. The solution was filtered and sodium *p*-toluenesulfonate (10 g) was added to the hot solution and the mixture was cooled slowly, the crystalline salt slowly separating in a pure condition, mp 326° dec.

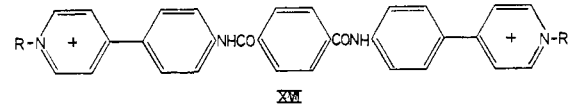
Difficulties were sometimes experienced in purifying quaternary salts prepared from the longer chain alkyl *p*-toluenesulfonates. This was traced to the competing elimination reaction giving rise to free *p*-toluenesulfonic acid which was bound by the heterocyclic base. To remove unquaternized bases the samples were recrystallized from aqueous solvents in the usual way with the inclusion of 2 molar porportions of pyridine. The more insoluble bases liberated from their salts by the pyridine were filtered from the hot solution.

TABLE VI[a]



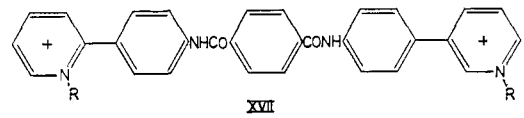
XV

R	Mp, °C	Formula	Calcd, % C	H	N	S	Found, % C	H	N	S	R_D[c]	L1210[d]
b	>360	$C_{30}H_{22}N_4O_2$	76.6	4.7	11.9		76.8	5.1	11.9			
CH_3	330–335	$C_{46}H_{42}N_4O_8S_2 \cdot 2H_2O$	62.9	5.3		7.3	63.1	5.6		6.7	0.69	+
C_2H_5	310–312	$C_{48}H_{46}N_4O_8S_2 \cdot H_2O$	63.6	5.6		7.1	63.4	5.5		6.9	0.81	++
$(CH_2)_2CH_3$	325–326	$C_{50}H_{50}N_4O_8S_2 \cdot 0.5H_2O$	66.2	5.7		7.0	66.4	5.7		6.9	0.93	++



XVI

b	>360	$C_{30}H_{22}N_4O_2$	76.6	4.7	11.9		76.2	4.9	11.8			
CH_3	335–343	$C_{46}H_{42}N_4O_8S_2 \cdot 3.5H_2O$	61.0	5.5		7.1	61.0	5.5		7.1	0.75	++
C_2H_5	230–233	$C_{48}H_{46}N_4O_8S_2 \cdot 3H_2O$	62.3	5.7		6.9	62.4	5.7		7.2	0.84	++
$(CH_2)_2CH_3$	337–339	$C_{50}H_{50}N_4O_8S_2 \cdot 2.5H_2O$	63.6	5.9		6.8	63.7	6.0		6.9	0.88	++



XVII

b	>360	$C_{30}H_{22}N_4O_2$	76.6	4.7	11.9		76.2	4.9	11.8			
CH_3	305–308	$C_{46}H_{42}N_4O_8S_2$	65.5	5.0		7.6	65.5	5.4		7.8	0.81	−
C_2H_5	290–294	$C_{48}H_{46}N_4O_8S_2 \cdot 3H_2O$	62.3	5.7		6.9	62.6	5.4		6.8	0.96	−
$(CH_2)_3CH_3$	290–291	$C_{50}H_{50}N_4O_8S_2$	65.4	5.9		7.0	65.5	5.7		6.9	0.99	−

[a–d] See corresponding footnotes in Table I.

Either DMF or N-methylpyrrolidone was used as solvent for quaternization. The latter gave better results when solubility problems were encountered. Care must be taken that all of the base is in solution before adding the alkyl *p*-toluenesulfonate. The alkyl *p*-toluenesulfonates should be shaken with sufficient dry $MgCO_3$ to neutralize free acid before use. If the quaternary salt does not separate from the reaction mixture it may be precipitated with ether or dry acetone. The salts were crystallized from aqueous sodium *p*-toluenesulfonate with the addition of methanol, ethanol, or DMF. Occasionally the anhydrous form of the salt could be crystallized from 1-butanol–methanol mixtures. The quaternary salts as crystallized from aqueous solvents were invariably hydrated. For analysis samples have been dried *in vacuo* over silica gel at room temperatures. Attempts to dry thoroughly at elevated temperatures gave extremely hygroscopic samples and in some cases a loss of crystallinity. Melting points have been determined on the samples dried and ready for analysis and are really decomposition points of either the hydrate or the quaternary salt and are dependent on the rate of heating. Careful attention to detail is necessary to reproduce the same melting point for different samples prepared at different times. Melting points have been determined on an Electrothermal melting point apparatus with the makers-supplied, stem-corrected thermometer and with a 2°/min heating rate from 20° below the melting point. Paper chromatography is a superior index of purity to melting point and compounds listed have been purified, where possible, to give only one spot.

Chromatography.—The solvent used was the top phase from a mixture of 1-butanol (4 vol.) and 2% aqueous sodium *p*-toluenesulfonate (3 vol.), the paper being Whatman No. 1. The quaternary salts were applied as their *p*-toluenesulfonate salts in phenol or aqueous DMF. It is important that the applied spots are not completely dried on the paper, otherwise the salts crystallize on the surface of the paper and tail badly or fail to move. Development was horizontal and the quaternary salts were located either by their fluorescence or by spraying with Dragendorff's reagent. 3,8-Diamino-5-methyl-6-phenylphenanthridinium *p*-toluenesulfonate (dimidium *p*-toluenesulfonate) was used as a convenient colored internal standard having an R_f value in the median range (R_f 0.69). All R_f values were taken in reference to dimidium as one and are quoted as R_D values.

Reduction of Nitro Quaternary Salts.—The nitro salts, prepared by the standard methods outlined, were reduced in aqueous ethanol with freshly prepared $Fe(OH)_2$ essentially by the method used for the reduction of nitrophenanthridinium quaternary salts.[17]

Terephthalic Acid Monobenzyl Ester.—Terephthalic acid (15 g) and a solution of KOH (12 g) in H_2O (150 ml) were heated together until solution was complete. The pH of the solution was adjusted to 9 with dilute HCl. Ethanol (100 ml) and benzyl chloride (10.8 ml) were added and the mixture was heated under reflux for 2 hr. A solution of $KHCO_3$ (10 g) in H_2O (50 ml) was added to the thoroughly cooled reaction mix and the oily layer was separated by gravity filtration. The filtrate was acidified (concentrated HCl) and shaken with ethyl acetate (500 ml), and precipitated terephthalic acid was removed by filtration. The organic layer was separated, washed with saturated NaCl, dried (Na_2SO_4), and evaporated. The solid crystallized from aqueous ethanol as silky needles, mp 179–180° (7.0 g).

Anal. Calcd for $C_{15}H_{12}O_4$: C, 70.3; H, 4.7. Found: C, 70.1; H, 4.9.

4′-(2-Pyridyl)-4″-(3-pyridyl)terephthalanilide.—Terephthalic acid monobenzyl ester (5 g) was suspended in benzene (20 ml) and pyridine (1.6 ml) and $SOCl_2$ (20 ml) was added. The mixture was refluxed for 10 min and evaporated *in vacuo*, and the residue was extracted with boiling benzene. Evaporation of the extracts gave the acid chloride; needles from petroleum ether (40–60°), mp 29–30° (4.1 g). The acid chloride was immediately added to a solution of 3-(4-aminophenyl)pyridine (2.6 g) in dry pyridine (20 ml) and the mixture was heated on the H_2O bath for 2 hr. Precipitation with H_2O afforded the crude amide ester which was washed well with 2 *N* NH_4OH and dried. Crystallization from methanol gave pure material (5.7 g), mp 194–195°.

Anal. Calcd for $C_{26}H_{20}N_2O_3$: C, 76.5; H, 4.9; N, 6.9. Found: C, 76.2; H, 5.3; N, 6.8.

This ester (5 g) was hydrolyzed by suspending in boiling methanol (100 ml) and adding 2 *N* aqueous KOH (50 ml); after 10 min of boiling the solution was cooled and H_2O (150 ml) was added. The solution was filtered and the filtrate was adjusted to pH 6 with acetic acid. The precipitated acid crystallized from DMF–methanol; mp 330–331°.

Anal. Calcd for $C_{19}H_{14}N_2O_3$: C, 71.7; H, 4.4; N, 8.8. Found: C, 71.5; H, 4.8; N, 8.8.

A mixture of this acid (2.1 g) and 2-(4-aminophenyl)pyridine (1.17 g) in pyridine (20 ml) was stirred at 0° while PCl_3 (0.35 ml) was slowly added. After 1 hr at 0°, the mixture was heated in a

(17) L. P. Walls and J. Whittaker, *J. Chem. Soc.*, 41 (1950)

Table VII

Antitumor Activities

Compd	R	R'	Dose, mg/kg/day	Survivors	Wt change, g	Av survival days T	Av survival days C	T/C, %
I	CH_3	H	5.2	4	+3.3			
			2.6	6	+5.5	9.9	7.9	125
			1.3	6	+4.1	7.9	7.9	
I	C_2H_5	H	5.0	0				
			4.0	4	+2.6	10.4	8.3	126
			2.0	6	+3.7	8.4	8.3	
I	$(CH_2)_2CH_3$	H	25	1				
			20	6	0.0	13.9	7.9	176
			16	6	+1.9	10.7	7.9	136
			13	6	+2.0	10.1	7.9	128
			10	6	+2.0	11.7	7.9	148
I	$(CH_2)_3CH_3$	H	63	4	−3.5	10.7	8.3	129
			50	6	−2.8	13.7	8.0	171
			40	6	−2.0	14.5	8.0	181
			31	6	−0.1	10.6	8.0	132
			25	6	−0.5	11.5	8.0	144
			20	6	+1.1	9.8	8.0	122
I	$(CH_2)_4CH_3$	H	100	0				
			50	6	0.0	10.3	8.0	129
			40	6	+0.4	10.6	8.0	133
			25	6	+5.4	9.4	8.0	
I	$(CH_2)_2OCH_3$	H	20	0				
			16	6	+0.7	9.5	7.9	120
			13	6	+1.3	14.4	7.9	163
			10	6	+2.6	9.9	7.9	126
V	CH_3		4.0	0				
			2.0	6	+0.6	11.8	9.3	127
			1.0	6	+3.6	11.4	9.3	122
V	C_2H_5		8.0	2	+0.8	8.0	8.2	
			4.0	6	+1.8	10.8	8.2	132
			2.0	6	+3.2	10.4	8.2	126
V	$(CH_2)_2CH_3$		16	4	+0.6	10.2	8.2	124
			8.0	6	+2.1	10.5	8.2	128
			4.0	6	+3.8	10.2	8.2	124
V	$(CH_2)_3CH_3$		100	0				
			50	6	−3.3	15.4	9.3	165
			25	6	−0.6	11.2	9.3	121
I	$(CH_2)_2CH_3$	NH_2	15	4	−0.8	12.4	8.8	140
			7.5	6	+0.4	13.4	8.8	164
			3.8	6	+1.4	12.6	8.8	143
I	$(CH_2)_3CH_3$	NH_2	20	5	+0.6	10.8	8.2	132
			10	6	+2.1	11.3	8.2	138
			5.0	6	+4.6	10.2	8.2	124
I	$(CH_2)_4CH_3$	NH_2	30	1	−0.9	11.5	8.8	131
			15	6	+0.5	11.6	8.8	132
			7.5	6	+1.9	9.0	8.8	
XI	C_2H_5		250	1	+0.8	12.6	7.8	160
			125	6	+3.1	17.2	7.8	220
			62.5	6	+3.9	10.0	7.8	128
XI	$(CH_2)_2CH_3$		20	6	−3.8	8.4	8.6	
			10	6	−0.2	26.5	8.6	310
			5.0	6	+0.6	14.5	8.6	144
			2.5	6	+1.9	10.8	8.8	123
XI	$(CH_2)_3CH_3$		50	2	−4.0	8.5	8.6	
			25	6	+0.2	10.8	8.6	126
			13	6	+5.3	8.9	8.6	
XII	CH_3		500	4	−4.8	16.0	8.6	186
			250	6	−1.2	18.2	8.6	212
			125	6	+0.5	15.1	8.6	176
			63.5	6	+2.5	11.3	8.6	132
XIII	$(CH_2)_2CH_3$		12	0				
			6.0	6	+2.3	11.4	8.6	133
			3.0	6	+2.8	10.7	8.6	124
XIII	$(CH_2)_3CH_3$		300	2	−3.2	12.2	8.5	144
			150	6	−2.1	13.3	8.5	156
			75	6	−0.5	10.3	8.5	122

TABLE VII (*Continued*)

Compd	R	R′	Dose, mg/kg/day	Survivors	Wt change, g	Av survival days T	Av survival days C	T/C, %
XIII	$(CH_2)_4CH_3$		200	1	−6.2	8.0	8.5	
			100	6	−5.0	11.4	8.5	134
			50	6	+2.2	8.8	8.5	
XV	CH_3		60	6	−3.4	13.1	9.8	133
			40	6	−0.7	20.2	9.8	206
			27	6	+0.7	15.0	9.8	153
			18	6	+3.1	12.7	9.8	130
XV	C_2H_5		40	3	−2.6	19.3	10.6	180
			27	6	−0.9	20.8	10.6	290
			18	6	+1.5	22.3	10.6	210
			12	6	+1.8	21.2	10.6	200
			8	6	+2.0	17.4	10.6	164
			5.3	6	+2.2	13.9	9.8	142
XV	$(CH_2)_2CH_3$		80	6	−1.8	17.3	10.6	161
			54	6	−0.6	18.7	10.6	176
			36	6	+0.5	23.7	10.6	220
			24	6	+1.4	21.0	10.6	198
			16	6	+1.9	15.7	10.6	148
XVI	CH_3		150	6	−2.0	27.0	9.5	284
			100	6	−0.3	27.8	9.5	292
			67	6	+1.3	24.8	9.5	256
			45	6	+1.1	22.8	9.5	240
			30	6	+0.9	14.9	9.9	157
			20	6	+1.8	13.6	9.9	143
XVI	C_2H_5		25	6	−2.7	9.8	9.9	
			20	6	−2.3	16.9	9.5	178
			14	6	−1.9	24.8	9.5	262
			9.3	6	−1.1	29.4	9.5	310
			6.2	6	−0.4	27.8	9.5	282
			4.1	6	+1.4	22.3	9.9	235
			2.7	6	+1.4	17.2	9.9	181
			1.8	6	+2.3	12.8	9.9	134
XVI	$(CH_2)_2CH_3$		20	6	−3.7	9.4	9.5	
			14	6	−2.5	13.3	9.5	142
			9.3	6	−0.1	19.6	9.5	207
			6.2	6	+0.1	14.8	9.5	156
			4.1	6	+1.6	13.5	9.9	136

H_2O bath for 1 hr and cooled, and the crude product was precipitated with 2 *N* NH_4OH. The solid was washed with boiling 1% aqueous NaOH, H_2O, and methanol, dried at 110° and the 4′-(2-pyridyl)-4″-(3-pyridyl)terephthalanilide crystallized from N-methylpyrrolidone–methanol (2.6 g), mp >360°.

Biological Testing.—In general the L1210 testing has been patterned on the CCNSC protocols. We are greatly indebted to the CCNSC for their generous donation of animal and tumor strains. The routine test consists of intraperitoneal inoculation of 10^5 L1210 cells into 18.5–22.5-g C_3H/DBA_2F_1 hybrids on day 1; drug treatment is initiated 24 hr later and continued for 5 days. Average survivals are calculated in the usual way. An attempt has been made to test all drugs from a level which is frankly toxic, giving either toxic deaths before control deaths or marked weight loss; serial twofold dilutions have then been tested until an obviously nontoxic dose has been reached; this usually requires a total of three tests. Compounds which under these test conditions have not given T/C values greater than 125% have been classed as negative and this is recorded in the requisite column in Table I–VI. Full test data for these negative compounds has not been given. On retesting positives a two-thirds dosage schedule has been used, the levels ensuring tests from toxic levels to those which give less than 40% increase in life span.

Table VII shows the data obtained and is virtualy self-explanatory. All dosage has been intraperitoneal in 0.2 ml of H_2O. Groups of six animals per dose level have been used and one control group for every five tests. The weight-change column records the difference between initial weight and that at day 8 for survivors.

The number of animals surviving as long or longer than controls is listed under survivors. Doses have been rounded off to two significant figures.

Acknowledgments.—We are greatly indebted to Miss L. Armiger and her capable assistants for performance of the many biological tests. Analyses were performed by Dr. A. D. Campbell of the Microchemical Laboratory, University of Otago, New Zealand. This work was supported by the Auckland Division, Cancer Society of New Zealand (Inc.).