was kept at −70° for 1 hr, at 0° for 1 hr, and at room temperature overnight, the solids were collected by filtration and washed with Et_2O. The filtrate and washings were combined and treated in the same manner as described for the preparation of **10**. The filter cake was washed with H_2O and the insoluble solids were combined with the residue from the Et_2O filtrate to give 3.13 g of products. Glpc analysis showed that the mixture consisted of 1.67 g (27.9%) of **11** and 1.46 g (27.6%) of **10**. The mixture was stirred in 40 ml of 0.1 *N* HCl, and the acid-insoluble product was collected by filtration. A second treatment with acid and recrystallization of the insoluble product from cyclohexane gave 0.9 g of **11**, mp 182.5–184.5°. *Anal.* ($C_9H_{14}N_6O_2$) C, H, N.

B.—A mixture of 500 mg (2.75 mmoles) of **4** and 3 ml of formamide was heated at 180° for 3 hr under reduced pressure (315 mm). The melt was poured into 20 ml of H_2O and 330 mg of insoluble solids was collected by filtration (21.3% of **11** by glpc). The solids were triturated with 10 ml of 1 *N* HCl, and the acid-insoluble product was collected by filtration, washed with H_2O, and dried. Recrystallization from cyclohexane gave 106 mg of **11**.

C.—Oxidation of 336 mg of **9** with aqueous $KMnO_4$ and extraction of the solids with $CHCl_3$ gave 1.1 mg of **10** and 9.9 mg of **11**; compound **2** could not be detected among the products.

N,N′-[6-(Methylamino)-*s*-triazin-2,4-diyl]bis(N-methylformamide) (12).—A mixture of 7 g (0.042 mole) of **6** and 18 ml of formamide was heated at 185° for 2 hr. The melt was poured into 40 ml of H_2O and the mixture was chilled in an ice bath. The insoluble products were collected by filtration and triturated with 40 ml of 1 *N* HCl.[10] The acid-insoluble product was collected by filtration, washed with H_2O, and dried. Recrystallization from CCl_4 gave 0.26 g (3%) of **12**, mp 207.5–209°. *Anal.* ($C_8H_{12}N_6O_2$) C, H, N.

N-[4,6-Bis(dimethylamino)-*s*-triazin-2-yl]formamide was prepared in 28% yield from **3** by method B. The product was recrystallized from EtOH, mp 182.5–184.5°. *Anal.* ($C_8H_{14}N_6O$) C, H, N.

Acknowledgments.—We thank Mr. Robert Brouillette for valuable technical assistance and Mr. E. L. Gooden for the pmr spectra.

(10) The major product of the reaction which was soluble in HCl was identified as N-[4,6-bis(methylamino)-*s*-triazin-2-yl]-N-methylformamide, mp 168–171° (analytical sample). *Anal.* ($C_7H_{12}N_6O$) C, H, N.

Potential Antitumor Agents. IX. Bisquaternary Salts

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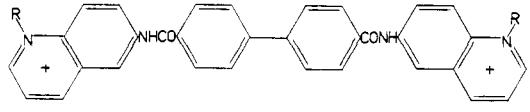
The postulate that a close approach to over-all planarity in bisquaternary ammonium heterocycles is essential for maximum activity when tested against the L1210 system has been further investigated. The preparation of L1210 active quaternary salts containing a diphenyl system suggests that complete planarity in this type of molecule is not an essential requirement.

In an earlier paper[2] we demonstrated that a close approach to over-all planarity of certain quaternary heterocycles was apparently essential for significant activity in the L1210 system in mice. Proceeding from this point we prepared[3,4] active agents whose length exceeded 30 Å. This paper details an investigation into these longer molecules in which deliberate attempts have been made to introduce a small degree of twist in the central area of the molecules.

Using the biphenyl moiety as a central fragment having the desired degree of twist about the pivot bond, activity was first found in series I. Here, convincing



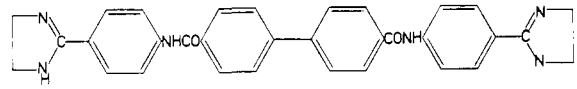
I

activity against the L1210 system could be demonstrated in the series from methyl through *n*-butyl quaternary salts (Table I).

Higher activity was shown by the bis(ethyl and bis-(*n*-propyl quaternary) salts as compared to the other homologs, but the precision of the test system does not allow a clear cut distinction between these two molecules.

It is interesting to observe that the relative R_f values for the ethyl and *n*-propyl quaternary salts of I lie on either side of the figure noted for the optimum members in a series prepared earlier.[2-4] Thus it would appear that, even with the structural changes introduced into I, the R_f values can still serve as a reliable guide to the relative hydrophilic–lipophilic balance.[2]

A marked contrast exists between the active series I and the completely inactive biphenyl analog II reported



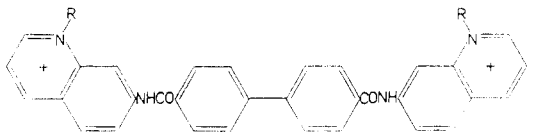
II

by Bennett.[5] Our results so far are now able to resolve this apparent discrepancy. In our lead series, the quaternary salts from N,N′-bis(6-quinolyl)terephthalamide, optimum activity is associated with the bis-*n*-butyl salt, the higher *n*-hexyl homolog being inactive. In variant I, where biphenyl replaces phenylene, in our lead series, a lower quaternary salt (ethyl or *n*-propyl) exhibits maximum activity. The change from phenyl to biphenyl has thus increased the lipophilic character of the resultant series by a factor equivalent to several methylene groups. Thus, if in the active 4′,4″-bis-(2-imidazolin-2-yl)terephthalanilide[5] the imidazoline as

(1) Author to whom correspondence should be addressed.
(2) G. J. Atwell and B. F. Cain, *J. Med. Chem.*, **10**, 706 (1967).
(3) G. J. Atwell and B. F. Cain, *ibid.*, **11**, 295 (1968).
(4) B. F. Cain, G. J. Atwell, and R. N. Seelye, *ibid.*, **11**, 300 (1968).
(5) L. L. Bennett, Jr., *Progr. Exp. Tumor Res.*, **7**, 259 (1965).

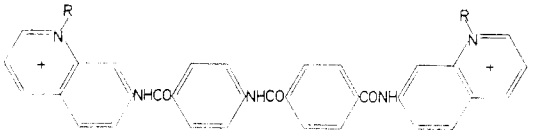
the basic function gives close to the optimum lipophilic-hydrophilic balance, then in variant II, the molecule will be too lipophilic by the equivalent of several methylene groups. As we have previously shown,[2] the cut-off in biological activity on homologation past the optimum is extremely rapid; hence it is not surprising that no activity was observed in this latter case.[6]

Activity occurring in the similarly linked biphenyl-7-aminoquinoline series (III) contrasts with the inactivity



III

of the previously described N,N′-bis(7-quinolyl)terephthalamide series.[2] These results considered in conjunction with our earlier work[2-4] and the extensive investigations in the bisimidazoline series[5,7] could be taken as indicating a minimum charge separation of approximately 18 Å as being necessary for activity in this type of compound. This apparent dependence on charge separation is supported by a similar level of activity appearing in the amide linked 7- aminoquinoline series IV.

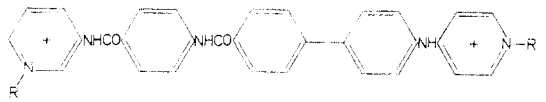


IV

Of two further series using previously investigated terminal basic functions but linked through a biphenyl center (V and VI), one (VI) displayed modest but unequivocal activity. Of three additional variants within the same general type but with smaller charge separation, two (VII and VIII) were inactive while the third (IX) demonstrated modest activity. This contrasts

V

VI



VII

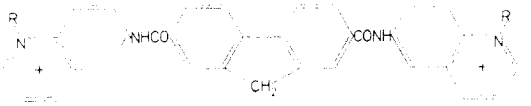
VIII

IX

with the examples previously described[2-4,8] in which interchange of the terminal bases (3-benzamidopyridine, 4-phenylpyridine, and 4-anilinopyridines) resulted in little change in over-all activity.

We have previously demonstrated[4] that in this type of quaternary salt biological activity appears to parallel the electron-donating properties of a series of substituents, when due allowance is made for changes in lipophilic-hydrophilic balance. It is interesting that in the series VII–IX only the latter, containing a *p*-phenylenediamine unit and presumably having the highest summation of electron density over the three noncharged rings, is active.[9]

If the somewhat lower activity of members containing a biphenyl unit was due to departure from over-all planarity by rotation at the central biphenyl bond then the fluorene-linked series X, in which rotation of the

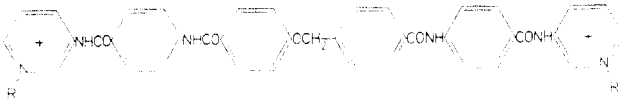


X

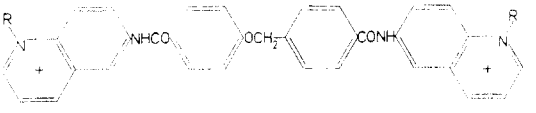
biphenyl moiety is constrained by the methylene bridge, could decide this issue. In fact these compounds proved inactive.

The biphenyl systems discussed here may be regarded as the previously described extended amide systems[2-4] in which one amide function is deleted. From this viewpoint it could be considered that activity might be dependent upon the spacing of the aromatic nuclei along the linear backbone of the molecule.

Examination of Courtald models suggests that a benzyl ether in a planar conformation maintains the spacing and positioning of the benzene rings very close to that of a benzanilide while still allowing some degree of rotation about the benzyl ether. Therefore series XI and XII were prepared; these may be considered



XI



XII

(6) It is possible to predict from these results that the bisamidine corresponding to II should be active in the L1210 system.

(7) R. Hirt in Chemotherapy of Cancer, P. A. Plattner, Ed., Elsevier Publishing Co., Amsterdam, 1964, p 228.

(8) G. J. Atwell, B. F. Cain, and R. N. Seelye, *J. Med. Chem.*, **11**, 690 (1968), part VIII of this series.

(9) Similar reasoning could be applied to explain the differences in biological activity between V and VI.

TABLE I

Drug	R	Mp, °C	Formula	Analyses[f]	R_D[a]	L1210[b]
I	c	>360	$C_{32}H_{22}N_4O_2$	C, H, N		
I	CH_3[d]	>360	$C_{48}H_{42}N_4O_8S_2 \cdot H_2O$	C, H, S	0.59	+
I	C_2H_5	320–321	$C_{50}H_{46}N_4O_8S_2 \cdot H_2O$	C, H, S	0.76	++
I	$CH_3(CH_2)_2$	290–291	$C_{52}H_{50}N_4O_8S_2 \cdot H_2O$	C, H, S	0.85	++
I	$CH_3(CH_2)_3$	300–301	$C_{54}H_{54}N_4O_8S_2 \cdot 2H_2O$	C, H, S	0.96	+
III	c	338–339	$C_{32}H_{22}N_4O_2$	C, H, N		
III	CH_3	>360	$C_{48}H_{42}N_4O_8S_2$	C, H, S	0.65	+
III	C_2H_5	328–329	$C_{50}H_{46}N_4O_8S_2 \cdot 0.5H_2O$	C, H, S	0.84	++
IV	c	349–350	$C_{33}H_{23}N_5O_3$	C, H, N		
IV	CH_3	301–303	$C_{49}H_{43}N_5O_9S_2$	C, H, S	0.61	+
IV	C_2H_5	270–271	$C_{51}H_{47}N_5O_9S_2 \cdot H_2O$	C, H, S	0.80	+
V	c	>360	$C_{38}H_{28}N_6O_4$	C, H, N		
V	C_2H_5	353–354	$C_{56}H_{50}N_6O_{10}S_2 \cdot 0.5H_2O$	C, H, S	0.67	−
V	$CH_3(CH_2)_2$	331–332	$C_{58}H_{54}N_6O_{10}S_2$	C, H, S	0.83	−
V	$CH_3(CH_2)_3$	330–331	$C_{60}H_{58}N_6O_{10}S_2$	C, H, S	0.90	−
VI	c	355–356	$C_{36}H_{28}N_6O_2$	C, H, N		
VI	CH_3	194–196	$C_{52}H_{48}N_6O_8S_2 \cdot 0.5H_2O$	C, H, S	0.92	+
VII	c	>360	$C_{30}H_{23}N_5O_2$	C, H, N		
VII	CH_3[e]	270–271	$C_{32}H_{29}N_5O_2I_2$	C, H, I	0.88	−
VIII	c	341–342	$C_{29}H_{22}N_4O$	C, H, N		
VIII	CH_3	281–283	$C_{45}H_{42}N_4O_7S_2 \cdot H_2O$	C, H, S	0.825	−
IX	c	251–252	$C_{29}H_{23}N_5O$	C, H, N		
IX	CH_3[e]	203–206	$C_{31}H_{29}N_5OI_2$	C, H, I	0.90	+
X	c	344–345	$C_{33}H_{22}N_4O_2$	C, H, N		
X	C_2H_5[e]	309–310	$C_{37}H_{32}N_4O_2I_2 \cdot H_2O$	C, H, I	0.65	−
X	$CH_3(CH_2)_2$[e]	317–319	$C_{39}H_{38}N_4O_2I_2 \cdot H_2O$	C, H, I	0.79	−
X	$CH_3(CH_2)_3$[e]	252–253	$C_{41}H_{40}N_4O_2I_2$	C, H, I	0.87	−
XI	c	>360	$C_{39}H_{30}N_6O_5$	C, H, N		
XI	CH_3	338–339	$C_{55}H_{50}N_6O_9S_2$	C, H, S	0.58	−
XI	C_2H_5	320–321	$C_{57}H_{54}N_6O_{11}S_2$	C, H, S	0.72	−
XI	$CH_3(CH_2)_3$	307–308	$C_{59}H_{58}N_6O_{11}S_2$	C, H, S	0.89	−
XII	c	297–298	$C_{33}H_{24}N_4O_3$	C, H, N		
XII	CH_3	336–337	$C_{49}H_{44}N_4O_9S_2$	C, H, S	0.79	−
XII	C_2H_5	294–295	$C_{51}H_{48}N_4O_9S_2$	C, H, S	0.88	−
XII	$CH_3(CH_2)_2$	228–230	$C_{53}H_{52}N_4O_9S_2$	C, H, S	0.96	−

[a] R_f relative to internal standard; see ref 2. [b] L1210 results according to our experimental procedure. Increase in life span 25–50%, ±; 50–100%, +; >100%, ++. [c] Free base. [d] Anion throughout this paper is *p*-toluenesulfonate unless otherwise stated. [e] Anion iodide. [f] Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.

equivalent to our earlier polyamides[2-4] but with an amide function replaced by a benzyl ether group. Although the R_f values for this series showed that the balance of lipophilic–hydrophilic properties was in the correct range,[2] no activity against the L1210 leukemia could be demonstrated.

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Analyses were performed by Dr. A. D. Campbell, Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal melting point apparatus with the makers supplied stem corrected thermometer, a 2°/min heating rate from 20° below the melting point was used. Methods of preparation of symmetrical bisbases and quaternary salts, details for chromatography and purification, etc., have been described adequately.[2-4,8]

In the preparation of the acid chloride of diphenyl-4,4′-dicarboxylic acid previous investigators have used organic solvent–PCl_5 mixtures; it has been found that the use of $POCl_3$ as solvent gives superior results. This method can be used advantageously with fluorene-2,7-dicarboxylic acid also. The bisbases prepared from the above two acid chlorides are extremely insoluble and difficulty is experienced in crystallizing these; for example, I (R = H) is best crystallized by dissolving in boiling phenol and adding boiling DMF until crystallization begins; slow cooling yields a highly crystalline specimen. For quaternization of these insoluble bases it is essential to use N-methyl-2-pyrrolidone as solvent.

3-{*p*-[*p*-(*p*-Nitrophenyl)benzamido]benzamido}pyridine.—A solution of 4-(*p*-nitrophenyl)benzoyl chloride (2 g) in pyridine (10 ml) at 20° was added in one portion to a solution of 3-(*p*-aminobenzamido)pyridine (1.63 g) in pyridine (15 ml). The mixture was heated at 100° for 15 min and cooled, then crude product precipitated with a large volume of 2 *N* NH_4OH. Crystallization from DMF–MeOH gave pale yellow prisms (2.95 g), mp 350–352°. *Anal.* ($C_{25}H_{18}N_4O_4$) C, H, N.

3-{*p*-[*p*-(*p*-Aminophenyl)benzamido]benzamido}pyridine was prepared by Fe reduction[3,4] of the preceding nitro compound in 80% DMF–H_2O. Repeated crystallization from DMF–MeOH gave pure material as colorless prisms, mp 334.5–335°. *Anal.* ($C_{25}H_{20}N_4O_2$) C, H, N.

Table II

Antitumor Tests

Drug	R	Dose, mg/kg/day	Wt change	Survivors	Av days survival Treated	Control	T/C, %
I	CH_3	50	−0.8	6	12.6	9.6	134
		33	−0.1	6	14.2	9.6	148
		22	+0.8	6	14.6	9.6	152
		15	+3.1	6	12.5	9.6	130
I	C_2H_5	30	−3.5	6	20.1	9.6	210
		20	+0.4	6	24.9	9.6	259
		13	+2.0	6	19.0	9.6	198
		8.9	+1.4	6	13.6	9.6	142
I	$CH_3(CH_2)_2$	60	−1.5	6	19.2	9.6	200
		40	+0.2	6	23.8	9.6	248
		27	+0.7	6	20.0	9.6	208
		18	+2.8	6	15.4	9.6	161
		12	+3.8	6	11.3	9.6	
I	$CH_3(CH_2)_3$	50	−2.4	6	12.8	9.8	131
		33	+0.6	6	15.0	9.8	153
		22	+2.2	6	14.3	9.8	146
		15	+4.8	6	10.6	9.8	
III	CH_3	75	−2.5	6	13.8	10.2	135
		50	+0.1	6	15.6	10.2	153
		33	+1.4	6	13.8	10.2	135
		22	+2.4	6	12.0	10.2	
III	C_2H_5	150	−2.6	6	15.6	10.2	153
		100	−1.5	6	20.8	10.2	204
		67	+0.6	6	18.4	10.2	181
		44	+1.2	6	14.4	10.2	141
		30	+3.0	6	11.2	10.2	
IV	CH_3	50		2			
		33	−2.8	6	13.4	9.6	140
		22	+0.6	6	14.8	9.6	154
		15	+1.7	6	13.6	9.6	142
		10	+2.4	6	11.6	9.6	121
IV	C_2H_5	50	−3.2	6	12.4	9.6	129
		33	−2.2	6	17.0	9.6	178
		22	−0.6	6	18.6	9.6	194
		15	+0.4	6	17.0	9.6	177
		10	+1.7	6	12.8	9.6	133
VI	CH_3	30	−4.5	5	11.0	9.9	
		20	−2.8	6	13.8	9.9	140
		13	+0.2	6	16.2	9.9	174
		8.9	+0.8	6	13.6	9.9	137
		5.9	+2.3	6	11.2	9.9	
IX	CH_3	2.0	−2.0	4	13.8	9.8	141
		1.3	+0.2	6	15.8	9.8	161
		0.89	+0.6	6	15.7	9.8	160
		0.59	+0.5	6	13.6	9.8	139
		0.39	+1.2	6	11.2	9.8	

Conversion of this amino compound to the anilinopyridine (VII, R = H) by reaction with N-pyridyl-4-pyridinium chloride hydrochloride was carried out by our previously described method.[5]

4-{*p*-[*p*-(*p*-Nitrophenyl)benzamido]phenyl}pyridine was obtained by reaction of 4-(*p*-nitrophenyl)benzoyl chloride and 4-(*p*-aminophenyl)pyridine in pyridine solution. The base separated from DMF–H_2O as pale yellow plates, mp 280–281°. *Anal.* ($C_{24}H_{17}N_3O_3$) C, H, N.

4-{*p*-[*p*-(*p*-Aminophenyl)benzamido]phenyl}pyridine was obtained by Fe reduction[3,4] of the corresponding nitro compound in 80% DMF–H_2O. The amine separated from small volumes of DMF as colorless prisms, mp 348–349°. *Anal.* ($C_{24}H_{19}N_3O$) C, H, N. Reaction of this amine with N-pyridyl-4-pyridinium chloride hydrochloride in the usual way[5] gave the corresponding anilinopyridine (VIII, R = H).

4-{*p*-[*p*-(*p*-Nitrophenyl)benzamido]anilino}pyridine from reaction of 4-(*p*-nitrophenyl)benzoyl chloride and 4-(*p*-aminoanilino)pyridine in pyridine solution crystallized from DMF–MeOH as yellow needles, mp 303–304°. *Anal.* ($C_{24}H_{18}N_4O_3$) C, H, N.

4-{*p*-[*p*-(*p*-Aminophenyl)benzamido]anilino}pyridine was obtained by Fe reduction[3,4] of the corresponding nitro compound in 70% DMF–H_2O. Pure material separated from DMF–H_2O in colorless plates, mp 306–308°. *Anal.* ($C_{24}H_{20}N_4O$) C, H, N.

Reaction of this amine with N-pyridyl-4-pyridinium chloride hydrochloride by the described method[5] gave the corresponding anilinopyridine (IX, R = H).

Biological Testing.—The screening test consisted 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 was initiated 24 hr later and continued for 5 days. An attempt was made to test all drugs from a level which was frankly toxic, giving either toxic deaths before control deaths or marked weight loss; serial dilutions were then tested until an obviously nontoxic dose was reached. Compounds which under these test conditions did not give T/C values greater than 125% were classed as negative and this is recorded in the requisite column in Table I. Full test data for these negative compounds has not been given.

All dosage was intraperitoneal in 0.2 ml of H_2O. Groups of six animals per dose level were used and one control group for every five tests. The weight change column in Table II 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. The work was supported by the Auckland Division, Cancer Society of New Zealand (Inc.).