144°, was obtained; λ_{max} (10% EtOH), 242, 268–293 (plateau); pH 1, 299; pH 13, 277 (plateau) mμ. *Anal.* ($C_{16}H_{19}N_3O_2$) C, H, N.

Deamination of 1-Phenoxypropyl-5-phenylcytosine (21) to 27d.—To a gently stirred solution of 200 mg (0.62 mmole) of **21** in 5 ml of HOAc cooled in an ice bath was added dropwise a solution of 175 mg (2.48 mmoles) of $NaNO_2$ in 5 ml H_2O. The solution was allowed to stand at ambient temperature for 24 hr during which time the product separated. The solid was collected on a filter and washed with H_2O; yield 113 g (57%) of **27d**, mp 178–180°, that was identical with **27d** prepared *via* **26a** (Table II).

Potential Antitumor Agents. VIII. Bisquaternary Salts

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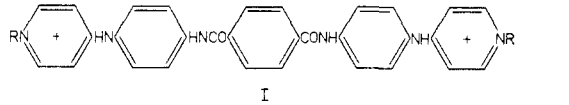
An investigation of types of quaternary ammonium heterocycles acceptable as basic functions in experimental antileukemic bisquaternary salts is described. Some aspects of the dependence for activity on charge separation and on certain steric features are discussed.

Since the initial observation of experimental antileukemic activity in quaternary salts of N,N¹-(6-quinolyl)terephthalamide[2] we have demonstrated that acceptable basic functions in this type of molecule are the quaternary salts of 6-acylaminoquinolines,[2,3] 3- and 4-(*p*-acylamino)phenylpyridines,[2,3] and 3-benzamidopyridines.[3,4] The researches described in this paper detail investigations of further acceptable basic functions as well as the effect of certain steric factors on biological activity.

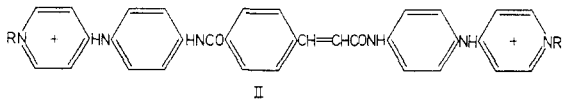
The alteration of basic functions in many cases changes charge separation in the resultant molecules. If biological activity was critically dependent on such separation, acceptable bases might be overlooked due to the resultant molecules possessing an unacceptable charge separation.

Fortunately in our series of bisquaternary compounds activity has been observed where distances between the quaternary nitrogen atoms are as low as 18 Å (as in the parent N,N′-(6-quinolyl)terephthalamide series) which can be increased by small increments to a maximum of 27 Å in the extended amide series described in our previous paper.[4]

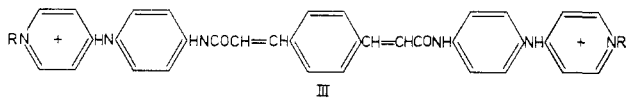
A further example of the permissibility of variable charge separation is provided by the three series I–III which all show convincing experimental antileukemic effectiveness in mice.



I



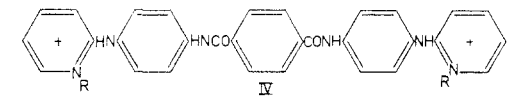
II



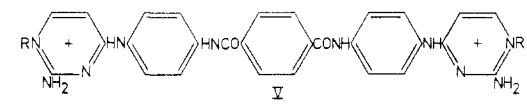
III

These three series utilize the new basic function 4-anilinopyridine; this function is somewhat more lipophilic than those previously used; for example, antileukemic effectiveness drops from I (R = CH_3) to higher members. This could have been predicted from the relative R_f values if these were taken as giving a measure of lipophilic–hydrophilic balance.[2]

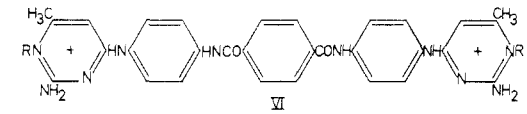
In contrast, the corresponding 2-anilinopyridine series IV, covering a similar range of R_f values to the 4-anilinopyridines, contained no active members. Further variants of the 4-anilino heterocycle system have been examined. The 2-amino-4-anilinopyrimidines V gave life extensions in the L1210 system similar to the corresponding pyridines but were less active on a molar basis. The 2-amino-4-anilino-6-methylpyrimidine series (VI) utilizing the pyrimidine function present in the trypanocides antrycide[5] and prothidium[6] also contained active members but these were even less active than the pyrimidines V on a molar basis; a dose of several hundred mg/kg being required to demonstrate an effect.



IV

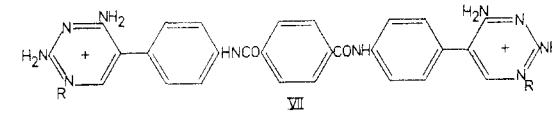


V



VI

Consideration of the above results coupled with the activity of the 3-phenylpyridine series described earlier[2] led to the preparation of the 2,4-diamino-5-phenylpyrimidines VII. These compounds proved to be extremely potent experimental antileukemic drugs with the ethyl quaternary salt (VII, R = C_2H_5) in early treatment groups giving a proportion of 100-day survivors.

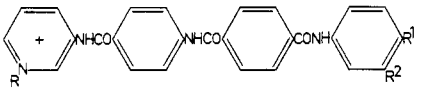


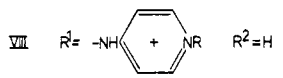
VII

(1) Author to whom inquiries should be addressed.
(2) Part V: G. J. Atwell and B. F. Cain, *J. Med. Chem.*, **10**, 706 (1967).
(3) Part VI: G. J. Atwell and B. F. Cain, *ibid.*, **11**, 295 (1968).
(4) Part VII: G. J. Atwell, B. F. Cain, and R. N. Seelye, *ibid.*, **11**, 300 (1968).
(5) A. D. Ainley, F. H. S. Curd, W. Hepworth, A. G. Murray, and C. H Vasey, *J. Chem. Soc.*, 59 (1953).
(6) T. I. Watkins and G. Woolfe, *Nature*, **178**, 368, 727 (1956).

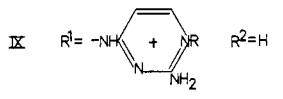
As the majority of the compounds so far prepared had indentical terminal basic functions it became desirable to examine a series of asymmetric analogs. The order of activity found for these (VIII, IX) could readily have been predicted from a consideration of the activity of the corresponding symmetrical series and their relative R_f values.

An attempt to assess the effects of steric features led to an examination of the *meta*-fused series X and XI

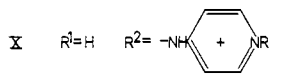




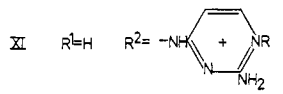
VIII R^1= -NH- R^2=H



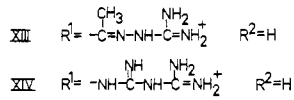
IX R^1= -NH- R^2=H



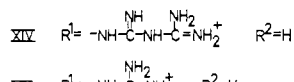
X R^1=H R^2= -NH-



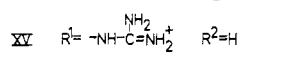
XI R^1=H R^2= -NH-



XIII R^1= R^2=H

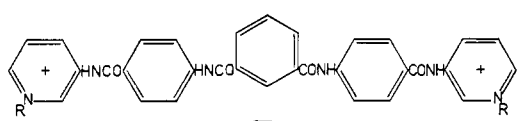


XIV R^1= R^2=H



XV R^1= R^2=H

which showed greatly reduced activity when compared with their *para*-fused analogs. However, consonant with this change there is an increase in lipophilic–hydrophilic balance, as measured by R_f values, past the figure that we consider to be optimum in these compounds. Since the cut-off of biologic activity on homologation past the optimum is quite rapid[2] it is conceivable that the same *meta*-fused system in a more hydrophilic system could be of comparable activity to the *para* isomers. An alternative mode of branching through an isophthaloyl unit gave a less active compound (XII) than the corresponding linear terephthaloyl isomer. Here again an increase in lipophilic character associated with the nonlinear isophthaloyl group makes difficult the assessment of the intrinsic activity of a *meta*-fused system.



XII

One heterocyclic basic function in these materials may be replaced by an open-chain analog as exemplified in the guanylhydrazone XIII, the biguanide XIV, and the guanidine XV. Demonstrable activity can be observed with all three compounds; the laborious task of preparing series in this area to cover a range of lipophilic–hydrophilic properties has not been undertaken.

From our totalled experiences thus far we can state that there is a marked dependence of antileukemic effectiveness on a physical property which appears to be the hydrophilic–lipophilic balance. The range of permissible hydrophilic–lipophilic properties is quite restricted. In the quaternary salts from N,N^1-(6-quinolyl)terephthalamide only the *n*-propyl, *n*-butyl, and *n*-amyl homologs show activity, lower and higher homologs being inactive.[2] Provided the agents have the correct lipophilic–hydrophilic balance, activity can be observed over an intercharge separation from 18 to 27 Å; these figures do not represent outside limits but merely the area where activity has so far been recorded.

For highest activity a close approach to planarity appears to be required.[2]

Linear fusion through terephthaloyl or *p*-aminobenzoate units appears to give higher activity materials than angular structures containing isophthaloyl or *m*-aminobenzoate units. This may only be a reflection of the higher lipophilic character of the angular structures. There is a wide array of functions that can serve as the terminal bases; the most notable exceptions are the quaternary salts from 2-substituted pyridines, *viz.*, 2-phenylpyridine, 2-benzamidopyridine, and 2-anilinopyridine.

Experimental Section

Analyses were by Dr. A. D. Campbell, Microchemical Laboratory, University of Otago, New Zealand. The symbol for the requisite element has been used to signify that analytical results were within ±0.4% of the calculated figure.

Meting points have been 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.

Details of preparations are given for only those intermediates which have not previously been described in the literature.

4-Anilinopyridines.—The convenient method of Jerchel and Jakob,[7] interacting the salt of an aromatic amine with N-pyridyl-4-pyridinium chloride hydrochloride,[8] has been used. Great care must be taken to ensure all reactants are scrupulously dry, otherwise markedly reduced yields result. The general method is exemplified by the preparation of 4-(*p*-aminoanilino)pyridine. *p*-Toluenesulfonic acid hydrate (19.0 g) was dried by suspending in C_6H_6 (500 ml) and refluxing under a H_2O separation head until no further water separated. To the clear solution *p*-acetamidoaniline (15 g), N-pyridyl-4-pyridinium chloride hydrochloride (23.5 g), and phenol (50 g) were added. The heterogenous mixture was again refluxed until all traces of H_2O had been removed. Benzene was then removed at steam bath temperature at 20 mm. The mixture was heated at 180° in an oil bath for 1 hr, a homogeneous melt resulting after approximately 10 min. The phenol was removed by steam distillation and the aqueous solution was evaporated dry *in vacuo*. The gum was dissolved in aqueous 2 *N* HCl (500 ml) and boiled for 1 hr to cleave the acetylamino function. After evaporation the solid hydrochloride was dissolved in H_2O (200 ml), stirred with charcoal (10 g) for 10 min, and crude product precipitated from the filtered solution with excess NH_3. Recrystallization from boiling H_2O (65 ml/g) gave pure product as colorless plates, mp 171–172° (14.6 g, 79%). *Anal.* ($C_{11}H_{11}N_3$) C, H, N.

The methods used for preparation of the bis bases and the quaternary salts listed in Tables I and II have been adequately described.[2,3]

2-Amino-4-(*p*-nitroanilino)pyrimidine.—2-Amino-4-chloropyrimidine (1.38 g) and *p*-nitroaniline (1.48 g) were dissolved in hot 2-ethoxyethanol (40 ml), HCl (4 ml) was added, and the clear solution refluxed for 1 hr. Product started to separate after 10 min. The crude product was collected from the well-cooled mixture and suspended in excess aqueous NH_3, and the resultant base crystallized from H_2O–MeOH. Pure base separated as yellow prisms, mp 273.5–274°. *Anal.* ($C_{10}H_9N_5O_2$) C, H, N.

(7) D. Jerchel and J. Jakob, *Chem. Ber.*, **91**, 1266 (1958).
(8) E. Koenigs and H. Greinar, *ibid.*, **64**, 1049 (1931).

Table I

Drug	R	Mp, °C	Formula	Analyses	R_f[b]	L1210[c]
I	*a*	>360	$C_{30}H_{24}N_6O_2$	C, H, N		
I	CH_3[d]	310–311	$C_{46}H_{44}N_6O_8S_2$	C, H, S	0.92	++
I	C_2H_5	295–296	$C_{48}H_{48}N_6O_8S_2 \cdot 0.5H_2O$	C, H, S	0.96	++
I	$CH_3(CH_2)_2$	303–304	$C_{50}H_{52}N_6O_8S_2 \cdot 0.5H_2O$	C, H, S	1.00	++
II	*a*	313–315	$C_{32}H_{26}N_6O_2$	C, H, N		
II	CH_3	248[e]	$C_{48}H_{46}N_6O_8S_2 \cdot 1.5H_2O$	C, H, S	0.91	++
II	C_2H_5	160–161	$C_{50}H_{50}N_6O_8S_2$	C, H, S	0.95	++
III	*a*	>360	$C_{34}H_{28}N_6O_2$	C, H, N		
III	CH_3	323–325	$C_{50}H_{48}N_6O_8S_2$	C, H, S	0.82	+
III	C_2H_5	188–191	$C_{52}H_{52}N_6O_8S_2$	C, H, S	0.85	+
III	$CH_3(CH_2)_2$	340–341[f]	$C_{40}H_{42}N_6O_2Br_2 \cdot H_2O$	C, H, Br	0.90	+
IV	*a*	327–328	$C_{30}H_{24}N_6O_2$	C, H, N		
IV	CH_3	328–329	$C_{46}H_{44}N_6O_8S_2 \cdot 2H_2O$	C, H, S	0.91	−
IV	C_2H_5	256–258[g]	$C_{34}H_{34}N_6O_2I_2 \cdot H_2O$	C, H, I	0.96	−
IV	$CH_3(CH_2)_2$	149–152	$C_{50}H_{52}N_6O_8S_2 \cdot 4H_2O$	C, H, S	1.16	−
V	*a*	337–338	$C_{28}H_{24}N_{10}O_2$	C, H, N		
V	CH_3	352–353	$C_{44}H_{44}N_{10}O_8S_2 \cdot 2H_2O$	C, H, S	0.75	++
V	C_2H_5	295–300	$C_{46}H_{48}N_{10}O_8S_2 \cdot 0.5H_2O$	C, H, S	0.88	++
VI	*a*	>360	$C_{30}H_{28}N_{10}O_2$	C, H, N		
VI	CH_3	>360	$C_{46}H_{48}N_{10}O_8S_2 \cdot 1.5H_2O$	C, H, S	0.79	++
VI	C_2H_5	336–342	$C_{48}H_{52}N_{10}O_8S_2 \cdot 2H_2O$	C, H, S	0.89	++
VII	*a*	>360	$C_{28}H_{24}N_{10}O_2 \cdot H_2O$	C, H, N		
VII	CH_3	>360	$C_{44}H_{44}N_{10}O_8S_2 \cdot 2H_2O$	C, H, N, S	0.75	++
VII	C_2H_5	315–317	$C_{46}H_{48}N_{10}O_8S_2 \cdot 2H_2O$	C, H, S	0.80	++
VII	$CH_3(CH_2)_2$	320–322	$C_{48}H_{52}N_{10}O_8S_2 \cdot 2H_2O$	C, H, S	0.92	++
VIII	*a*	>360	$C_{31}H_{24}N_6O_3$	C, H, N		
VIII	CH_3	344–345[g]	$C_{33}H_{30}N_6O_3I_2$	C, H, I	0.81	++
IX	CH_3	306–308[g]	$C_{33}H_{32}N_5O_3I_2$	C, H, I	0.73	+
X	*a*	345–347	$C_{31}H_{24}N_6O_3$	C, H, N		
X	CH_3	213–214[g]	$C_{33}H_{30}N_6O_3I_2 \cdot H_2O$	C, H, I	0.97	+
XI	CH_3	225–227[g]	$C_{33}H_{32}N_5O_3I_2$	C, H, I	0.85	−
XIII	CH_3	151–153	$C_{44}H_{44}N_8O_3S_2$	C, H, S	0.84	+
XIV	CH_3	272–273	$C_{29}H_{29}N_5O_3I_2$	C, H; I[h]	0.82	++
XV	CH_3	318–319[g]	$C_{28}H_{27}N_7O_3I_2$	C, H, I	0.87	++
XII	*a*	>360	$C_{32}H_{24}N_6O_3$	C, H, N		
XII	CH_3	287–289	$C_{48}H_{44}N_6O_{10}S_2$	C, H, S	0.89	+
XII	C_2H_5	159–161	$C_{50}H_{48}N_6O_{10}S_2$	C, H, S	0.97	±

[a] Free base. [b] R_f relative to dimidium; see ref 2. [c] L1210 results according to our experimental procedure. Increase in life span 25–50%, ±; 50–100%, +; >100%, ++. [d] Anion used throughout this paper, unless otherwise stated, is *p*-toluenesulfonate. [e] Prior shrinkage from 178°. [f] Anion bromide. [g] Anion iodide. [h] I: calcd, 31.5; found, 30.9.

2-Amino-4-(*p*-aminoanilino)pyrimidine was prepared from the above nitro compound by reduction with iron in aqueous EtOH solution.[3] The amine crystallized from small volumes of H_2O containing a little NH_3 as colorless plates, mp 155.5–156°. *Anal.* ($C_{10}H_{11}N_5$) C, H, N.

In the preparation of terephthaloyl derivatives of the anilinopyrimidines and aminophenylpyrimidines, to minimize acylation of the heterocyclic amino groups, the temperature was kept below 0° and excess amine was used. For example, V (R = H) was prepared by adding a solution of terephthaloyl chloride (2.03 g) in dioxane (25 ml) dropwise with vigorous stirring to a solution of 2-amino-4-(*p*-aminoanilino)pyrimidine (5.04 g, 2.5 equiv) in pyridine (25 ml), the temperature being maintained below 0°. After 1 hr of stirring the solution was heated on a water bath for 15 min and then evaporated dry *in vacuo*. After addition of MeOH (25 ml) and NH_3 (15 ml), the crude product was collected and repeatedly crystallized from DMF–MeOH until paper chromatograms using *n*-BuOH–AcOH–H_2O (4:1:5) showed the material to be homogeneous.

Quaternizations of the substituted pyrimidines were carried out at water-bath temperatures for 1 hr with excess quaternizing agent followed by 15 min at 140°. There is adequate evidence in the literature that substituted 2,4-diaminopyrimidines quaternize predominantly at N-1.[9] Quaternary salts were repeatedly crystallized until only a single spot was observed on paper chromatograms using an *n*-BuOH–aqueous sodium *p*-toluenesulfonate system.[2]

(9) D. J. Brown and T. Teitei, *J. Chem. Soc.*, 755 (1965), and references quoted therein.

2-Amino-4-(*p*-nitroanilino)-6-methylpyrimidine.—2-Amino-4-chloro-6-methylpyrimidine (1.14 g) and *p*-nitroaniline (1.13 g) were dissolved in 2-ethoxyethanol (40 ml) by warming. Concentrated HCl (3 ml) was added to the solution and the whole was heated under reflux for 0.5 hr. On cooling, solid started to separate; saturated aqueous NaCl (10 ml) was then added and product was collected when thoroughly cold. After trituration with aqueous NH_3 the free base was crystallized from H_2O–EtOH separating as yellow needles, mp 264°. *Anal.* ($C_{11}H_{11}N_5O_2$) C, H, N.

2-Amino-4-(*p*-aminoanilino)-6-methylpyrimidine was prepared by iron reduction[3,4] of the foregoing nitro compound. The product separated from hot H_2O containing a little NH_3 as colorless plates, mp 201–202°. *Anal.* ($C_{11}H_{13}N_5$) C, H, N.

3-[*p*-(*p*-Methoxycarbonylbenzamido)benzamido]pyridine. A suspension of 3-(*p*-aminobenzamido) pyridine[3] (4.0 g) and terephthalic acid monomethyl ester (3.35 g) in dry pyridine (30 ml) was stirred vigorously at 0° while PCl_3 (0.83 ml) was added dropwise. After stirring 1 hr at 0° the mixture was heated on a steam bath for 1 hr and cooled well, and then a large excess of 5% aqueous $NaHCO_3$ was added. The solid was collected, washed well with water, and crystallized from DMF–H_2O as colorless prisms, mp 266–265.5°. *Anal.* ($C_{21}H_{17}N_3O_4$) C, H, N.

3-[*p*-(*p*-Carboxybenzamido)benzamido]pyridine (XVI).—The foregoing methyl ester (4.0 g) was suspended in DMF (30 ml) at room temperature and a solution of NaOH (2.0 g) in 50 ml of 80% H_2O–MeOH was added to the stirred suspension. After a few minutes a clear solution resulted. It was allowed to stand at room temperature for 1 hr, boiling H_2O (800 ml) was then added, and the hot solution was quickly filtered. Addition of AcOH (4.0 ml) precipitated the acid, which, after washing

TABLE II[a]

Drug	R	Dose, mg/kg/day	Survivors	Wt change, g	Av survival, days Treated	Control	T/C, %
I	CH_3	5.0	6	−0.5	11.5	10.1	
		3.3	6	−1.8	32.3	10.1	321
		2.2	6	−1.9	37.7	10.1	373
		1.5	6	+0.3	32.7	10.1	323
		1.0	6	+1.8	27.0	10.1	267
		0.67	6	+3.6	21.4	10.1	212
		0.44	6	+3.0	14.5	10.1	143
I	C_2H_5	5.0	0				
		3.3	6	−2.8	23.5	9.8	240
		2.2	6	−0.8	33.5	9.8	342
		1.5	6	+0.2	25.4	9.8	258
		1.0	6	+0.8	21.3	9.8	217
		0.67	6	+1.7	15.8	9.8	161
		0.44	6	+2.1	10.7	9.8	
I	$CH_3(CH_2)_2$	5.0	6	−2.0	16.8	9.6	175
		3.3	6	+0.2	19.4	9.6	202
		2.2	6	+0.6	19.6	9.6	204
		1.5	6	+1.3	17.9	9.6	187
		1.0	6	+1.3	16.1	9.6	178
		0.67	6	+2.2	13.6	9.6	142
II	CH_3	15	6	−0.7	10.9	9.6	
		10	6	−0.6	36.6	9.6	381
		6.7	6	−0.1	20.5	9.6	213
		4.4	6	+1.7	16.1	9.6	168
		3.0	6	+2.0	14.2	9.6	148
		2.0	6	+3.1	10.8	9.6	
II	C_2H_5	15	6	−2.2	19.4	10.1	192
		10	6	−1.9	22.0	10.1	218
		6.7	6	−0.2	21.4	10.1	212
		4.4	6	+0.5	16.2	10.1	160
		3.0	6	+1.3	12.3	10.1	122
III	CH_3	15	6	−1.8	14.2	10.0	142
		10	6	+0.2	16.4	10.0	164
		6.7	6	+1.1	15.8	10.0	158
		4.4	6	+1.6	13.8	10.0	138
		3.0	6	+2.5	11.4	10.0	
III	C_2H_5	75	2				
		50	6	−1.9	15.2	10.0	152
		33	6	−1.7	17.2	10.0	172
		22	6	−0.8	17.0	10.0	170
		15	6	+0.9	16.2	10.0	162
		10	6	+1.3	15.6	10.0	156
		6.7	6	+1.5	13.2	10.0	132
III	$CH_3(CH_2)_2$	50	6	−1.8	11.4	10.0	
		33	6	−1.1	13.6	10.0	136
		22	6	−0.7	15.2	10.0	152
		15	6	−0.2	14.2	10.0	142
		10	6	+1.3	12.6	10.0	126
V	CH_3	75	6	−1.0	22.8	9.9	231
		50	6	+0.1	30.7	9.9	310
		33	6	+0.8	22.9	9.9	232
		22	6	+1.1	15.4	9.9	156
		15	6	+1.7	12.2	9.9	123
V	C_2H_5	250	5	−1.2	23.6	10.2	232
		170	6	−0.2	26.4	10.2	259
		110	6	−0.1	22.8	10.2	224
		75	6	+1.7	18.9	10.2	185
		50	6	+1.0	14.6	10.2	143
VI	CH_3	500	4	−2.7	13.7	9.6	143
		330	6	−1.2	22.8	9.6	237
		220	6	+0.2	22.4	9.6	233
		150	6	+3.7	13.6	9.6	142
VII	CH_3	15	5	−2.1	13.0	10.4	125
		10	6	−1.8	22.2	10.4	213
		6.7[b]	6	−1.2	44.8	10.4	432
		4.4	6	+1.0	29.3	10.4	282
		3.0	6	+1.3	20.9	10.4	202
		2.0	6	+1.4	13.8	10.4	133
VII	C_2H_5	30	4	−1.7			
		20	6	−1.0	25.3	10.6	257
		13	6	−0.3	43.5[c]	10.6	410
		9	6	+1.1	36.2	10.6	342
		6	6	+2.1	22.2	10.6	208
		4	6	+2.8	14.6	10.6	138
VII	$CH_2(CH_2)_2$	150	4	−2.5			
		100	6	−0.6	22.9	9.9	231
		67	6	−0.2	25.6	9.9	258
		44	6	+0.3	20.8	9.9	211
		30	6	+0 9	17.6	9.9	178
		20	6	+0.7	13.4	9.9	138
VIII	CH_3	5	6	−2.8	14.8	10.4	143
		3.3	6	−0.3	23.2	10.4	223
		2.2	6	+1.1	19.4	10 4	187
		1.5	6	+1.8	14.6	10.4	141
IX	CH_3	22	6	−1.4	13.2	10.8	122
		15	6	−0.7	16.8	10.8	156
		10	6	+1.2	14.9	10.8	138
		6.7	6	+2.5	12.1	10.8	112
X	CH_3	15	2				
		10	6	−3.1	13.9	10.1	137
		6.7	6	−2.3	18.0	10.1	178
		4.4	6	+0.4	17.8	10.1	176
		3.0	6	+1.8	16.1	10.1	159
		2.0	6	+1.5	12.3	10.1	122
XIII	CH_3	25	6	−2.4	14.4	9.8	147
		17	6	−1.2	17.4	9.8	178
		11	6	+1.9	17.6	9.8	179
		7.5	6	+2.3	15.7	9.8	160
		5.0	6	+3.1	12.5	9.8	127
XIV	CH_3	15	4				
		10	6	−3.1	20.8	9.9	211
		6.7	6	−0.2	25.2	9.9	254
		4.4	6	+0.7	20.2	9.9	200
		3.0	6	+2.8	16.3	9.9	161
		2.0	6	+5.1	13.2	9.9	131
XV	CH_3	10	6	−2.3	12.4	9.8	127
		6.7	6	+0.1	33.2	9.8	338
		4.4	6	+0.9	37.6	9.8	383
		3.0	6	+1.8	28.6	9.8	292
		2.0	6	+2.9	19.4	9.8	198
		1.3	6	+2.5	12.2	9.8	124
XII	CH_3	50	3				
		33	6	−3.2	17.4	9.9	176
		22	6	+0.2	14.3	9.9	145
		15	6	+2.3	11.4	9.9	115
XII	C_2H_5	15	6	−2.8	12.2	9 9	123
		10	6	−1.2	13.2	9.9	134
		6.7	6	+0.2	12.1	9.9	122

[a] See the Experimental Section for the details of the biological testing. [b] Occasional 100-day survivors were obtained at this dose level. [c] Not including three animals which survived 100 days.

with H_2O and MeOH, was recrystallized from DMF–MeOH; colorless prisms, mp 343–344°. *Anal.* ($C_{20}H_{15}N_3O_4$) C, H, N.

VIII (R = H).—A sample of the acid XVI (1.0 g) and 4-(*p*-aminoanilino)pyridine (0.52 g) were dissolved in dry N-methyl-2-pyrrolidone (NMPy) (12 ml) by warming, the solution was cooled to 5°, and pyridine (1 ml) was added followed by PCl_3 (0.125 ml). The reaction mixture was heated on a steam bath for 1 hr and cooled well, and crude product was precipitated with excess 2 *N* NH_3. Crystallization from DMF–MeOH–H_2O mixtures gave yellow prisms, mp >360°.

3-{*p*-[*p*-(*p*-Nitrophenylcarbamoyl)benzamido]benzamido}-pyridine.—A solution of acid XVI (2.0 g) and *p*-nitroaniline (0.81 g) in dry NMPy (25 ml) was cooled to 5°, and pyridine (2 ml) then PCl_3 (0.25 ml) were added in that order to the stirred solution. During 1 hr of steam bath heating, the product separated. The solid collection from the well-cooled reaction mixture was triturated with aqueous NH and then crystallized from DMF–MeOH mixture, pale yellow prisms, mp >360°. *Anal.* ($C_{26}H_{19}N_5O_5$) C, H, N.

Pyridinium 1-Methyl-3-{*p*-[*p*-(*p*-nitrophenylcarbamoyl)-benzamido]benzamido}-*p*-toluenesulfonate.—The preceding nitro compound was heated with 2 molar equiv of methyl *p*-toluenesulfonate in NMPy solution to 150° for 5 min. Crude product was precipitated with Et_2O and crystallized repeatedly from DMF–H_2O containing 2% sodium *p*-toluenesulfonate. Pure material separated in colorless prisms, mp 324–325°. *Anal.* ($C_{34}H_{29}N_5O_8S_2$) C, H, S.

Pyridinium 1-Methyl-3-{*p*-[*p*-(*p*-aminophenylcarbamoyl)benzamido]benzamido}-*p*-toluenesulfonate (XVII).—The preceding nitro quaternary salt (10 g) was suspended in 50% H_2O–DMF (200 ml), and Fe dust (30 g) was then added followed by AcOH (2 ml). The heterogeneous mixture was heated under reflux until reduction was complete.[3] The Fe–FeO mixture was filtered from the boiling solution, the solids were extracted with hot DMF (two 50-ml portions), and the combined filtrates were evaporated to dryness. The residue was crystallized repeatedly from H_2O–EtOH containing sodium *p*-toluenesulfonate until homogeneous by paper chromatography.[2] The resultant pure

amine separated from solution as pale yellow plates (5.9 g), mp 268–269°. *Anal.* ($C_{34}H_{31}N_5O_6S$) C, H, S.

XI (R = CH_3).—A sample of the quaternary salt XVII (1.0 g) was dissolved in 70% H_2O–EtOH (100 ml). To the solution were added concentrated HCl (0.2 ml) and 2-amino-4-chloro-1,6-dimethylpyrimidinium iodide (0.5 g) and the whole was heated under reflux for 0.5 hr. To the hot solution was added a solution of KI (10 g) in H_2O (30 ml); on cooling the bisiodide crystallized. Repeated crystallization from H_2O–MeOH containing KI using paper chromatography as an index of purity gave pure material as yellow prisms, mp 306–308°.

The *meta* isomers of the above products that are listed in Tables I and II were made by equivalent methods; details of the necessary intermediates are listed below.

3-{*p*-[*p*-(*m*-Nitrophenylcarbamoyl)benzamido]benzamido}-pyridine, colorless prisms from DMF–MeOH, had mp >360°. *Anal.* ($C_{26}H_{19}N_5O_5$) C, H, N.

Pyridinium 1-methyl-3-{*p*-[*p*-(*m*-nitrophenylcarbamoyl)benzamido]benzamido}-*p*-toluenesulfonate, colorless needles from H_2O–DMF, had mp 325–326°. *Anal.* ($C_{34}H_{29}N_5O_8S$) C, H, S.

Pyridinium 1-methyl-3-{*p*-[*p*-(*m*-aminophenylcarbamoyl)benzamido]benzamido}-*p*-toluenesulfonate crystallized from H_2O–EtOH as pale yellow plates, mp 247–250°. *Anal.* ($C_{34}H_{31}N_5O_6S$) C, H, S.

3-{*p*-[*p*-(*p*-Acetophenylcarbamoyl)benzamido]benzamido}-pyridine.—A solution of acid XVI (1.0 g) and *p*-aminoacetophenone (0.4 g) in dry NMPy (10 ml) was cooled to 5° and pyridine (1 ml) and PCl_3 (0.13 ml) were added. After heating for 0.5 hr on a steam bath the solution was cooled and the crude product precipitated with dilute NH_3. Crystallization from NMPy–MeOH gave colorless prisms, mp >360°. *Anal.* ($C_{28}H_{22}N_4O_4$) C, H, N.

Pyridinium 1-Methyl-3-{*p*-[*p*-(*p*-acetophenylcarbamoyl)benzamido]benzamido}-*p*-toluenesulfonate.—Quaternization of the above base in the usual fashion[2] gave the quaternary salt which separated from solutions in H_2O–MeOH as colorless prisms, mp 322–323°. *Anal.* ($C_{36}H_{32}N_4O_7S \cdot 0.5H_2O$) C, H, S.

XIII (R = CH_3).—To a solution of the preceding quaternary salt (0.58 g) in 70% H_2O–DMF (7.5 ml) was added a solution of aminoguanidine bicarbonate (0.175 g) in H_2O (5 ml) plus concentrated HCl (1 ml). The solution was heated under reflux for 20 min then evaporated to dryness *in vacuo*. Crystallization from H_2O–EtOH containing sodium *p*-toluenesulfonate plus a few milligrams of *p*-toluenesulfonic acid gave pure product, mp 151–153°. *Anal.* ($C_{44}H_{44}N_8O_9S_2$) C, H, S.

XIV (R = CH_3).—Normally the preparation of biguanides is carried out in aqueous media; with the difficult examples we have examined in this laboratory we have found that much better results are obtained if completely anhydrous conditions can be maintained. A mixture of anhydrous *p*-toluenesulfonic acid (0.27 g), the quaternary salt XVII (0.90 g, dried *in vacuo* at 100°), and dicyandiamide (0.27 g) was suspended in dry NMPy (8 ml) and the heterogeneous mixture was heated to 140° in an oil bath for 1.5 hr. A homogeneous reaction mixture resulted after approximately 10 min of heating. Excess Et_2O precipitated crude product as an oil. Crystallization from H_2O–MeOH containing NaI gave pure XIV (R = CH_3) as yellow prisms, mp 272–273°.

XV (R = CH_3).—Anhydrous *p*-toluenesulfonic acid (0.52 g), cyanamide (0.17 g), and quaternary salt XVII (1.70 g) in NMPy (8 ml) were treated as for the corresponding biguanide. The bisiodide separated from H_2O–MeOH containing NaI and a trace of HI as colorless needles, mp 318–319°.

Biological Testing.—Our routine test procedure consisted of intraperitoneal inoculation of 10^5 L1210 cells into 18.5–22.5-g C_3H/DBA_2 F_1 hybrids on day 1 with drug treatment initiated 24 hr later and continued for 5 days. All dosage was in 0.2-ml volume, H_2O being used as medium. Groups of six animals per dose level were used with 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.

Compounds that have given no statistically significant increase in life span when tested from a toxic dose level (giving marked weight loss or premature deaths) to a nontoxic level have been classed as negative, and this has been noted in the requisite tables. Full details of the testing of such negative compounds has not been given.

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