

144°, was obtained; λ_{\max} (10% EtOH), 242, 268–293 (plateau); pH 1, 299; pH 13, 277 (plateau) m μ . *Anal.* (C₁₉H₁₉N₃O₂) C, 11, 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 NaNO₂ in 5 ml H₂O. 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 H₂O; 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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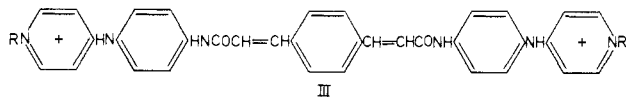
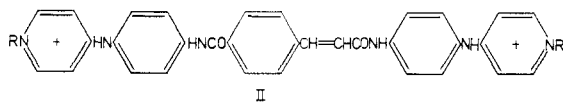
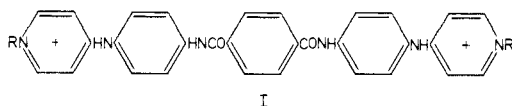
An investigation of types of quaternary ammonium heterocycles acceptable as basic functions in experimental antileukemic bisquaternary salts is described. Some aspects of the dependence for activity on charge separation and on certain steric features are discussed.

Since the initial observation of experimental antileukemic activity in quaternary salts of N,N¹-(6-quinolyl)terephthalamide² 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,N¹-(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

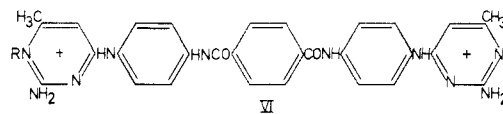
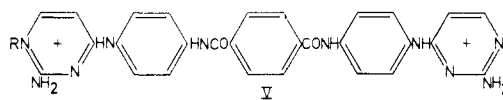
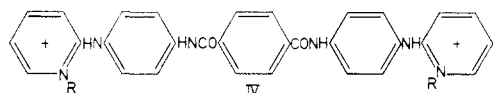
A further example of the permissibility of variable charge separation is provided by the three series I–III which all show convincing experimental antileukemic effectiveness in mice.



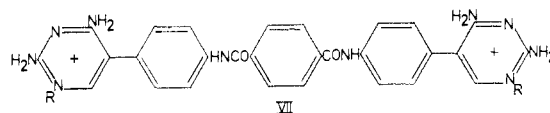
These three series utilize the new basic function 4-anilinopyridine; this function is somewhat more

lipophilic than those previously used; for example, antileukemic effectiveness drops from I (R = CH₃) 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 antrycide⁵ and prothidium⁶ 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.



Consideration of the above results coupled with the activity of the 3-phenylpyridine series described earlier² 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 = C₂H₅) in early treatment groups giving a proportion of 100-day survivors.



(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).

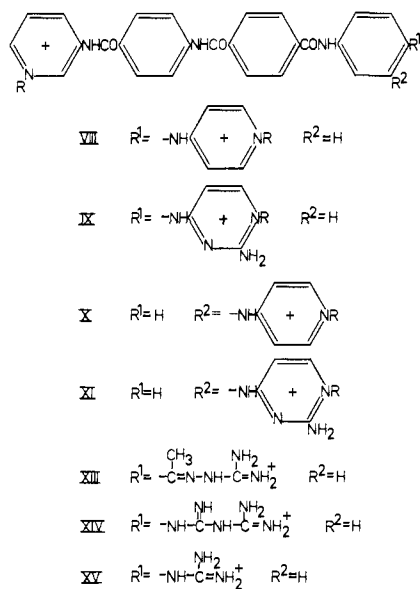
(4) Part VII: G. J. Atwell, B. F. Cain, and R. N. Seelye, *ibid.*, **11**, 300 (1968).

(5) A. D. Ainley, F. H. S. Cued, W. Hepworth, A. G. Murray, and C. H. Vasey, *J. Chem. Soc.*, 59 (1953).

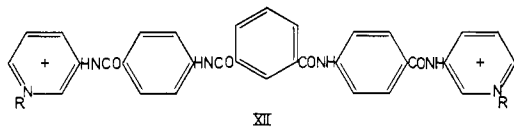
(6) T. I. Watkins and G. Woolfe, *Nature*, **178**, 368, 727 (1956).

As the majority of the compounds so far prepared had identical 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



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 rapid² 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.



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' -(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 interchange 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-anilino-pyridine.

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 $\pm 0.4\%$ of the calculated figure.

Melting 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-Anilino-pyridines.—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 heterogeneous 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 acetyl-amino 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.* ($\text{C}_{11}\text{H}_{11}\text{N}_3$) 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.* ($\text{C}_{10}\text{H}_9\text{N}_5\text{O}_2$) 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	LT1210 ^c
I	a	>360	C ₃₉ H ₂₄ N ₆ O ₂	C, H, N		
I	CH ₃ ^d	310-311	C ₄₆ H ₄₄ N ₆ O ₂ S ₂	C, H, S	0.92	++
I	C ₂ H ₅	295-296	C ₄₈ H ₄₈ N ₆ O ₂ S ₂ ·0.5H ₂ O	C, H, S	0.96	++
I	CH ₃ (CH ₂) ₂	303-304	C ₅₀ H ₅₂ N ₆ O ₂ S ₂ ·0.5H ₂ O	C, H, S	1.00	++
II	a	313-315	C ₃₂ H ₂₆ N ₆ O ₂	C, H, N		
II	CH ₃	248 ^e	C ₄₈ H ₄₆ N ₆ O ₂ S ₂ ·1.5H ₂ O	C, H, S	0.91	++
II	C ₂ H ₅	160-161	C ₅₀ H ₅₀ N ₆ O ₂ S ₂	C, H, S	0.95	++
III	a	>360	C ₃₄ H ₂₈ N ₆ O ₂	C, H, N		
III	CH ₃	323-325	C ₅₀ H ₄₈ N ₆ O ₂ S ₂	C, H, S	0.82	+
III	C ₂ H ₅	188-191	C ₅₂ H ₅₂ N ₆ O ₂ S ₂	C, H, S	0.85	+
III	CH ₃ (CH ₂) ₂	340-341 ^f	C ₄₆ H ₄₂ N ₆ O ₂ Br ₂ ·H ₂ O	C, H, Br	0.90	+
IV	a	327-328	C ₃₆ H ₂₄ N ₆ O ₂	C, H, N		
IV	CH ₃	328-329	C ₄₆ H ₄₄ N ₆ O ₂ S ₂ ·2H ₂ O	C, H, S	0.91	--
IV	C ₂ H ₅	256-258 ^g	C ₄₈ H ₄₈ N ₆ O ₂ I ₂ ·H ₂ O	C, H, I	0.96	--
IV	CH ₃ (CH ₂) ₂	149-152	C ₅₀ H ₅₂ N ₆ O ₂ S ₂ ·4H ₂ O	C, H, S	1.16	--
V	a	337-338	C ₂₈ H ₂₂ N ₆ O ₂	C, H, N		
V	CH ₃	352-353	C ₄₄ H ₄₄ N ₆ O ₂ S ₂ ·2H ₂ O	C, H, S	0.75	++
V	C ₂ H ₅	295-300	C ₄₆ H ₄₈ N ₆ O ₂ S ₂ ·0.5H ₂ O	C, H, S	0.88	++
VI	a	>360	C ₃₀ H ₂₈ N ₆ O ₂	C, H, N		
VI	CH ₃	>360	C ₄₆ H ₄₈ N ₆ O ₂ S ₂ ·1.5H ₂ O	C, H, S	0.79	++
VI	C ₂ H ₅	336-342	C ₄₈ H ₅₂ N ₆ O ₂ S ₂ ·2H ₂ O	C, H, S	0.89	++
VII	a	>360	C ₂₈ H ₂₄ N ₆ O ₂ ·H ₂ O	C, H, N		
VII	CH ₃	>360	C ₄₄ H ₄₄ N ₆ O ₂ S ₂ ·2H ₂ O	C, H, N, S	0.75	++
VII	C ₂ H ₅	315-317	C ₄₆ H ₄₈ N ₆ O ₂ S ₂ ·2H ₂ O	C, H, S	0.80	++
VII	CH ₃ (CH ₂) ₂	320-322	C ₄₈ H ₅₂ N ₆ O ₂ S ₂ ·2H ₂ O	C, H, S	0.92	++
VIII	a	>360	C ₃₀ H ₂₄ N ₆ O ₂	C, H, N		
VIII	CH ₃	344-345 ^h	C ₃₃ H ₃₆ N ₆ O ₃ I ₂	C, H, I	0.81	++
IX	CH ₃	306-308 ^h	C ₃₃ H ₃₂ N ₆ O ₃ I ₂	C, H, I	0.73	+
X	a	345-347	C ₃₀ H ₂₄ N ₆ O ₂	C, H, N		
X	CH ₃	213-214 ^h	C ₃₃ H ₃₆ N ₆ O ₃ I ₂ ·H ₂ O	C, H, I	0.97	+
XI	CH ₃	225-227 ^h	C ₃₃ H ₃₂ N ₆ O ₃ I ₂	C, H, I	0.85	-
XIII	CH ₃	151-153	C ₄₄ H ₄₄ N ₆ O ₂ S ₂	C, H, S	0.84	+
XIV	CH ₃	272-273	C ₂₈ H ₂₈ N ₆ O ₃ I ₂	C, H, I ^h	0.82	++
XV	CH ₃	318-319 ^h	C ₂₈ H ₂₇ N ₆ O ₃ I ₂	C, H, I	0.87	++
XII	a	>360	C ₃₂ H ₂₄ N ₆ O ₂	C, H, N		
XII	CH ₃	287-289	C ₄₈ H ₄₈ N ₆ O ₂ S ₂	C, H, S	0.89	+
XII	C ₂ H ₅	159-161	C ₅₀ H ₅₂ N ₆ O ₂ S ₂	C, H, S	0.97	±

^a Free base. ^b R_f relative to dimidium; see ref. 2. ^c LT1210 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.⁴ The amine crystallized from small volumes of H₂O containing a little NH₃ as colorless plates, mp 155.5-156°. *Anal.* (C₁₀H₁₁N₅) C, H, N.

In the preparation of terephthaloyl derivatives of the anilino-pyrimidines 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 NH₃ (15 ml), the crude product was collected and repeatedly crystallized from DMF-MeOH until paper chromatograms using *n*-BuOH-AcOH-H₂O (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

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 NH₃ the free base was crystallized from H₂O-EtOH separating as yellow needles, mp 264°. *Anal.* (C₁₁H₁₁N₅O₂) 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 H₂O containing a little NH₃ as colorless plates, mp 201-202°. *Anal.* (C₁₁H₁₃N₅) C, H, N.

3-[*p*-(*p*-Methoxycarbonylbenzamido)benzamido]pyridine. A suspension of 3-(*p*-aminobenzamido)pyridine³ (4.0 g) and terephthalic acid monomethyl ester (3.35 g) in dry pyridine (30 ml) was stirred vigorously at 0° while PCl₅ (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 NaHCO₃ was added. The solid was collected, washed well with water, and crystallized from DMF-H₂O as colorless prisms, mp 266-265.5°. *Anal.* (C₂₁H₁₇N₃O₄) 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% H₂O-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 H₂O (800 ml) was then added, and the hot solution was quickly filtered. Addition of AcOH (4.0 ml) precipitated the acid, which, after washing

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

TABLE II^a

Drug	R	Dose, mg/kg/day	Surviv- ors	Wt			T/C, %	Drug	R	Dose, mg/kg/day	Surviv- ors	Wt			T/C, %		
				change, g	Av survival, days Treated	Control						change, g	Av survival, days Treated	Control			
I	CH ₃	5.0	6	-0.5	11.5	10.1		VI	CH ₃	500	4	-2.7	13.7	9.6	143		
		3.3	6	-1.8	32.3	10.1	321			330	6	-1.2	22.8	9.6	237		
		2.2	6	-1.9	37.7	10.1	373			220	6	+0.2	22.4	9.6	233		
		1.5	6	+0.3	32.7	10.1	323			150	6	+3.7	13.6	9.6	142		
		1.0	6	+1.8	27.0	10.1	267			15	5	-2.1	13.0	10.4	125		
		0.67	6	+3.6	21.4	10.1	212			10	6	-1.8	22.2	10.4	213		
		0.44	6	+3.0	14.5	10.1	143			6.7 ^b	6	-1.2	44.8	10.4	432		
I	C ₂ H ₅	5.0	0					VII	C ₂ H ₅	4.4	6	+1.0	29.3	10.4	282		
		3.3	6	-2.8	23.5	9.8	240			3.0	6	+1.3	20.9	10.4	202		
		2.2	6	-0.8	33.5	9.8	342			2.0	6	+1.4	13.8	10.4	133		
		1.5	6	+0.2	25.4	9.8	258			30	4	-1.7					
		1.0	6	+0.8	21.3	9.8	217				20	6	-1.0	25.3	10.6	257	
		0.67	6	+1.7	15.8	9.8	161				13	6	-0.3	43.5 ^c	10.6	410	
		0.44	6	+2.1	10.7	9.8				9	6	+1.1	36.2	10.6	342		
I	CH ₃ (CH ₂) ₂	5.0	6	-2.0	16.8	9.6	175	6	6	+2.1	22.2	10.6	208				
		3.3	6	+0.2	19.4	9.6	202	4	6	+2.8	14.6	10.6	138				
		2.2	6	+0.6	19.6	9.6	204	150	4	-2.5							
		1.5	6	+1.3	17.9	9.6	187		100	6	-0.6	22.9	9.9	231			
		1.0	6	+1.3	16.1	9.6	178		67	6	-0.2	25.6	9.9	258			
		0.67	6	+2.2	13.6	9.6	142	44	6	+0.3	20.8	9.9	211				
		II	CH ₃	15	6	-0.7	10.9	9.6		VIII	CH ₃	5	6	-2.8	14.8	10.4	143
10	6			-0.6	36.6	9.6	381	3.3	6			-0.3	23.2	10.4	223		
6.7	6			-0.1	20.5	9.6	213	2.2	6			+1.1	10.4	10.4	187		
4.4	6			+1.7	16.1	9.6	168	1.5	6			+1.8	14.6	10.4	141		
3.0	6			+2.0	14.2	9.6	148	22	6			-1.4	13.2	10.8	122		
2.0	6			+3.1	10.8	9.6		15	6			-0.7	16.8	10.8	156		
II	C ₂ H ₅			15	6	-2.2	19.4	10.1	192			10	6	+1.2	14.9	10.8	138
		10	6	-1.9	22.0	10.1	218	6.7	6	+2.5	12.1	10.8	112				
		6.7	6	-0.2	21.4	10.1	212	15	2								
		4.4	6	+0.5	16.2	10.1	160		10	6	-3.1	13.9	10.1	137			
		3.0	6	+1.3	12.3	10.1	122		6.7	6	-2.3	18.0	10.1	178			
		III	CH ₃	15	6	-1.8	14.2	10.0	142	4.4	6	+0.4	17.8	10.1	176		
				10	6	+0.2	16.4	10.0	164	3.0	6	+1.8	16.1	10.1	159		
6.7	6			+1.1	15.8	10.0	158	2.0	6	+1.5	12.3	10.1	122				
4.4	6			+1.6	13.8	10.0	138	25	6	-2.4	14.4	9.8	147				
3.0	6			+2.5	11.4	10.0		17	6	-1.2	17.4	9.8	178				
III	C ₂ H ₅			75	2					XIII	CH ₃	11	6	+1.9	17.6	9.8	179
				50	6	-1.9	15.2	10.0	152			7.5	6	+2.3	15.7	9.8	160
		33	6	-1.7	17.2	10.0	172	5.0	6			+3.1	12.5	9.8	127		
		22	6	-0.8	17.0	10.0	170	15	4								
		15	6	+0.9	16.2	10.0	162		10			6	-3.1	20.8	9.9	211	
		10	6	+1.3	15.6	10.0	156		6.7			6	-0.2	25.2	9.9	254	
		6.7	6	+1.5	13.2	10.0	132	4.4	6			+0.7	20.2	9.9	200		
III	CH ₃ (CH ₂) ₂	50	6	-1.8	11.4	10.0		3.0	6	+2.8	16.3	9.9	161				
		33	6	-1.1	13.6	10.0	136	2.0	6	+5.1	13.2	9.9	131				
		22	6	-0.7	15.2	10.0	152	10	6	-2.3	12.4	9.8	127				
		15	6	-0.2	14.2	10.0	142		6.7	6	+0.1	33.2	9.8	338			
		10	6	+1.3	12.6	10.0	126		4.4	6	+0.9	37.6	9.8	383			
		V	CH ₃	75	6	-1.0	22.8	9.9	231	3.0	6	+1.8	28.6	9.8	292		
				50	6	+0.1	30.7	9.9	310	2.0	6	+2.9	19.4	9.8	198		
33	6			+0.8	22.9	9.9	232	1.3	6	+2.5	12.2	9.8	124				
22	6			+1.1	15.4	9.9	156	50	3								
15	6			+1.7	12.2	9.9	123		33	6	-3.2	17.4	9.9	176			
V	C ₂ H ₅			250	5	-1.2	23.6		10.2	232	22	6	+0.2	14.3	9.9	145	
				170	6	-0.2	26.4	10.2	259	15	6	+2.3	11.4	9.9	115		
		110	6	-0.1	22.8	10.2	224	15	6	-2.8	12.2	9.9	123				
		75	6	+1.7	18.9	10.2	185	10	6	-1.2	13.2	9.9	134				
50	6	+1.0	14.6	10.2	143	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 H₂O and MeOH, was recrystallized from DMF-MeOH; colorless prisms, mp 343-344°. *Anal.* (C₂₀H₁₃N₃O₄) 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 PCl₃ (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* NH₃. Crystallization from DMF-MeOH-H₂O 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 PCl₃ (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 NH₃ and then crystallized from DMF-MeOH mixture, pale yellow prisms, mp >360°. *Anal.* (C₂₆H₁₉N₃O₅) 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 Et₂O and crystallized repeatedly from DMF-H₂O containing 2% sodium *p*-toluenesulfonate. Pure material separated in colorless prisms, mp 324-325°. *Anal.* (C₃₄H₂₉N₅O₅S₂) 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% H₂O-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 H₂O-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.* (C₃₄H₃₁N₅O₆S) C, H, S.

XI (R = CH₃).—A sample of the quaternary salt XVII (1.0 g) was dissolved in 70% H₂O-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 H₂O (30 ml); on cooling the bisiodide crystallized. Repeated crystallization from H₂O-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*-(*m*-Nitrophenylcarbamoyl)benzamido]benzamido]-pyridine, colorless prisms from DMF-MeOH, had mp >360°. *Anal.* (C₂₆H₁₉N₃O₃) C, H, N.

Pyridinium 1-methyl-3-[*p*-(*m*-nitrophenylcarbamoyl)benzamido]benzamido}-*p*-toluenesulfonate, colorless needles from H₂O-DMF, had mp 325-326°. *Anal.* (C₃₄H₂₉N₅O₈S) C, H, S.

Pyridinium 1-methyl-3-[*p*-(*m*-aminophenylcarbamoyl)benzamido]benzamido}-*p*-toluenesulfonate crystallized from H₂O-EtOH as pale yellow plates, mp 247-250°. *Anal.* (C₃₁H₃₁N₅O₈S) C, H, S.

3-[*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 PCl₅ (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 NH₃. Crystallization from NMPy-MeOH gave colorless prisms, mp >360°. *Anal.* (C₂₅H₂₂N₂O₄) C, H, N.

Pyridinium 1-Methyl-3-[*p*-(*p*-acetophenylcarbamoyl)benzamido]benzamido}-*p*-toluenesulfonate.—Quaternization of the above base in the usual fashion² gave the quaternary salt which separated from solutions in H₂O-MeOH as colorless prisms, mp 322-323°. *Anal.* (C₃₆H₃₂N₄O₇S·0.5H₂O) C, H, S.

XIII (R = CH₃).—To a solution of the preceding quaternary salt (0.58 g) in 70% H₂O-DMF (7.5 ml) was added a solution of aminoguanidine bicarbonate (0.175 g) in H₂O (5 ml) plus concentrated HCl (1 ml). The solution was heated under reflux for 20 min then evaporated to dryness *in vacuo*. Crystallization from H₂O-EtOH containing sodium *p*-toluenesulfonate plus

a few milligrams of *p*-toluenesulfonic acid gave pure product, mp 151-153°. *Anal.* (C₄₄H₄₁N₅O₉S₂) C, H, S.

XIV (R = CH₃).—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 Et₂O precipitated crude product as an oil. Crystallization from H₂O-MeOH containing NaI gave pure XIV (R = CH₃) as yellow prisms, mp 272-273°.

XV (R = CH₃).—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 H₂O-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 10⁶ I.1210 cells into 18.5-22.5-g C₅H₇DBA₂F₁ hybrids on day 1 with drug treatment initiated 24 hr later and continued for 5 days. All dosage was in 0.2-ml volume, H₂O 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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