of heterocyclic bases containing a nitro function, if the pK of the starting material or the product formed on reduction was greater than 6, it was necessary to add 1 equiv of AcOH or HCl to obtain complete reduction. If, for solubility reasons, it was necessary to reduce in dilute solution, difficulty was sometimes experienced in starting the reaction. Addition of a few drops of nitrobenzene successfully initiated reduction.

Unsuccessful attempts to prepare hydrogen methyl terephthalate on a large scale led us to use the monobenzyl ester for preparation of monoamides of terephthalic acid.[5] Further investigation has shown that it is possible to prepare methyl potassium terephthalate on a large scale. Dimethyl terephthalate (37.2 g) was suspended in boiling MeOH (500 ml), and a solution of KOH (11.8 g) in MeOH (150 ml) was added dropwise to the vigorously boiling suspension. When approximately half of the alkali was added, a clear solution resulted. When the addition of all the alkali was complete, refluxing was continued for a further hour. After several hours at 0°, the crystals were collected and dried. The solid was suspended in 200 ml of H_2O at 60°, the resulting solution was filtered, and a solution of 150 g of KCl in H_2O (300 ml) at 60° was added. After thorough cooling, the crystalline salt was collected, 27.5 g, mp >360°. Samples of the free acid could be obtained by acidifying a cold, aqueous solution of the salt with HOAc and crystallizing *small* quantities from boiling H_2O. It was essential to heat and cool as rapidly as possible and avoid prolonged contact with hot H_2O. Attempts to crystallize large samples in this fashion invariably gave a product highly contaminated with terephthalic acid. The mono-K salt could be used directly in phosphorazo couplings (*vide infra*) and this was the most convenient method of obtaining monoamide esters of terephthalic acid. The monoamide monomethyl esters were hydrolyzed to the free acid by warming gently with 1 N KOH in 85% aqueous MeOH until solution was complete, then leaving for 1 hr at room temperature. After addition of an equal volume of H_2O, the solution was filtered. The acid was precipitated by the addition of the required amount of acid. Construction of the amide link was conveniently performed by using the phosphorazo method.[6]

(6) "Newer Methods of Preparative Organic Chemistry," Vol. II, W. Foerst, Ed., Academic Press Inc., New York, N. Y., 1963, Chapter 2.

Using the above methods, the intermediates listed in Tables I–III, not described in the literature, were prepared.

The general conditions used for the quaternizations, paper chromatography, and other experimental methods have been described adequately.[2]

Biological Testing.—The standard test consisted of intraperitoneal inoculation of 10^5 L1210 cells into 18.5–22.5-g C_3H/DBA_2 F, hybrids on day 1; drug treatment was initiated 24 hr later and was continued for 5 days. Average survivals were 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. Lower doses at 0.2 log intervals have then been tested until a nontoxic or nonactive dose level has been reached.

Table IV shows the data obtained and is virtually self-explanatory. All dosage has been intraperitoneal in 0.2-ml volume, H_2O being used as medium. Groups of six animals per dose level have been used (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 as or longer than controls are listed under survivors. Doses have been rounded off to 2 significant figures.

Compounds that have been tested under these conditions and have given no increase in life span have been classed as negative, and this is noted with the analytical data. Full details of testing of negative compounds has not been given.

No effort has been made to determine optimum dosage schedule, routes of administration, etc. Orders of activity are ganged on percentage T/C and breadth of dose range from maximum increase in life span (ILS) to that giving only 40% ILS, figures being taken from a plot of log dose/ILS.

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Potential Antitumor Agents. VII. Bisquaternary Salts

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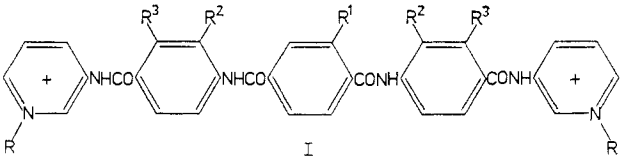
The effects of a series of substituents on the biological activity of a bisquaternary ammonium heterocycle have been determined against the L1210 leukemia system.

The last communications from this laboratory[2,3] disclosed the high experimental antileukemic activity of compound I with $R^1 = R^2 = R^3 = H$; $R = C_2H_5$.



I

While no claim is made that this compound is the most active in this series of quaternary salts, the relative ease with which the substitution pattern could be altered at will prompted a closer examination in this area. It was hoped that the results might give an insight into what would be the most rewarding area for future modifications.

Introduction of a substituent alters the lipophilic–hydrophilic balance of a species. The quaternary salts then offer a novel opportunity for examination of true structure-activity relationships since the balance of physical properties can be restored by compensatory adjustment of the quaternary function.

For preliminary investigations the substituents chlorine, methyl, methoxyl, and amino were selected, since they are relatively similar in size but cover a range of electron-donor properties. The nitro compounds required as intermediates for preparation of the amino compounds were also screened (see Table I).

Introduction of a chlorine substituent into the terephthaloyl unit gave a more lipophilic series of quaternary salts (I, $R^2 = R^3 = H$; $R^1 = Cl$) than the parent, maximum antileukemic activity being ob-

(1) Author to whom inquiries should be addressed.
(2) G. J. Atwell and B. F. Cain, *J. Med. Chem.*, **11**, 295 (1968).
(3) G. J. Atwell and B. F. Cain, *ibid.*, **10**, 706 (1967).

TABLE I

Drug	R	R¹	R²	R³	Mp, °C	Formula	Analyses	L1210[c]	R_D[d]
I	...[a]	Cl	H	H	>360	$C_{32}H_{23}ClN_6O_4$	C, H, Cl, N		
I	CH_3[b]	Cl	H	H	328–330	$C_{48}H_{43}ClN_6O_{10}S_2 \cdot 4H_2O$	C, H, S	++	0.89
I	C_2H_5	Cl	H	H	281–283	$C_{50}H_{47}ClN_6O_{10}S_2$	C, H, Cl, S	±	0.94
I	$CH_3(CH_2)_2$	Cl	H	H	266–267	$C_{52}H_{51}ClN_6O_{10}S_2$	C, H, Cl, S	−	0.99
I	...[a]	CH_3	H	H	354–356	$C_{33}H_{26}N_6O_4$	C, H, N		
I	CH_3	CH_3	H	H	327–328	$C_{49}H_{46}N_6O_{10}S_2$	C, H, S	++	0.88
I	C_2H_5	CH_3	H	H	301–302	$C_{51}H_{50}N_6O_{10}S_2$	C, H, S	+	0.93
I	$CH_3(CH_2)_2$	CH_3	H	H	283–285	$C_{53}H_{54}N_6O_{10}S_2 \cdot H_2O$	C, H, S	−	0.98
I	...[a]	CH_3O	H	H	351–353	$C_{33}H_{26}N_6O_5$	C, H, N		
I	CH_3	CH_3O	H	H	325–327	$C_{49}H_{46}N_6O_{11}S_2 \cdot H_2O$	C, H, S	±	0.74
I	C_2H_5	CH_3O	H	H	303–304	$C_{51}H_{50}N_6O_{11}S_2$	C, H, S	+	0.87
I	$CH_3(CH_2)_2$	CH_3O	H	H	293–295	$C_{53}H_{54}N_6O_{11}S_2 \cdot 3H_2O$	C, H, S	±	0.93
I	...[a]	NO_2	H	H	>360	$C_{32}H_{23}N_7O_6$	C, H, N		
I	CH_3	NO_2	H	H	294–296	$C_{48}H_{43}N_7O_{12}S_2$	C, H, S	±	0.89
I	C_2H_5	NO_2	H	H	288–291	$C_{50}H_{47}N_7O_{12}S_2$	C, H, S	−	0.95
I	$CH_3(CH_2)_2$	NO_2	H	H	328–330	$C_{52}H_{51}N_7O_{12}S_2 \cdot 2H_2O$	C, H, S	−	1.00
I	CH_3	NH_2	H	H	301–303	$C_{48}H_{45}N_7O_{10}S_2 \cdot H_2O$	C, H, S	++	0.72
I	C_2H_5	NH_2	H	H	298–300	$C_{50}H_{49}N_7O_{10}S_2 \cdot H_2O$	C, H, S	++	0.86
I	$CH_3(CH_2)_2$	NH_2	H	H	298–300	$C_{52}H_{53}N_7O_{10}S_2 \cdot H_2O$	C, H, S	±	0.99
I	...[a]	H	Cl	H	>360	$C_{32}H_{23}Cl_2N_6O_4$	C, H, Cl, N		
I	CH_3	H	Cl	H	318–320	$C_{48}H_{42}Cl_2N_6O_{10}S_2 \cdot 0.5H_2O$	C, H, S	±	0.91
I	...[a]	H	CH_3	H	>360	$C_{34}H_{28}N_4O_4$	C, H, N		
I	CH_3	H	CH_3	H	305–307	$C_{50}H_{48}N_6O_{10}S_2 \cdot 1.5H_2O$	C, H, S	±	0.89
I	C_2H_5	H	CH_3	H	290–292	$C_{52}H_{52}N_6O_{10}S_2 \cdot 1.5H_2O$	C, H, S	−	0.94
I	...[a]	H	CH_3O	H	307–309	$C_{39}H_{28}N_6O_6$	C, H, N		
I	CH_3	H	CH_3O	H	308–309	$C_{50}H_{48}N_6O_{12}S_2 \cdot 2H_2O$	C, H, S	+	0.75
I	C_2H_5	H	CH_3O	H	249–253	$C_{52}H_{52}N_6O_{12}S_2 \cdot H_2O$	C, H, S	+	0.86
I	$CH_3(CH_2)_2$	H	CH_3O	H	291–293	$C_{54}H_{56}N_6O_{12}S_2$	C, H, S	±	0.97
I	...[a]	H	H	Cl	>360	$C_{32}H_{22}Cl_2N_6O_4$	C, H, N, Cl		
I	CH_3	H	H	Cl	294–296	$C_{48}H_{42}Cl_2N_6O_{10}S_2 \cdot H_2O$	C, H, S	−	0.93
I	...[a]	H	H	CH_3	>360	$C_{34}H_{28}N_6O_4$	C, H, N		
I	CH_3	H	H	CH_3	309–311	$C_{50}H_{48}N_6O_{10}S_2 \cdot H_2O$	C, H, S	±	0.89
I	...[a]	H	H	CH_3O	313–314	$C_{34}H_{28}N_6O_6$	C, H, N		
I	CH_3	H	H	CH_3O	327–328	$C_{50}H_{48}N_6O_{12}S_2 \cdot H_2O$	C, H, S	±	0.61
I	C_2H_5	H	H	CH_3O	289–291	$C_{52}H_{52}N_6O_{12}S_2 \cdot 0.5H_2O$	C, H, S	±	0.76
I	$CH_3(CH_2)_2$	H	H	CH_3O	301–302	$C_{54}H_{56}N_6O_{12}S_2 \cdot H_2O$	C, H, S	±	0.91
I	...[a]	H	H	NO_2	>360	$C_{32}H_{22}N_8O_8$	C, H, N		
I	CH_3	H	H	NO_2	332 dec	$C_{48}H_{42}N_6O_{14}S_2 \cdot 0.5H_2O$	C, H, S	−	0.94
I	C_2H_5	H	H	NO_2	285–288	$C_{50}H_{46}O_{14}S_2 \cdot 0.5H_2O$	C, H, S	−	1.01
I	$CH_3(CH_2)_2$	H	H	NO_2	308–310	$C_{52}H_{50}N_8O_{14}S_2$	C, H, S	−	1.13
I	CH_3	H	H	NH_2	296 dec	$C_{48}H_{46}N_8O_{10}S_2 \cdot 1.5H_2O$	C, H, S	++	0.65
I	C_2H_5	H	H	NH_2	298 dec	$C_{50}H_{50}N_8O_{10}S_2 \cdot 0.5H_2O$	C, H, S	++	0.76
I	$CH_3(CH_2)_2$	H	H	NH_2	282–283	$C_{52}H_{54}N_8O_{10}S_2$	C, H, S	+	0.88
II	...[a]	NO_2			349 dec	$C_{32}H_{22}N_8O_8$	C, H, N		
II	C_2H_5	NO_2			225–228	$C_{36}H_{32}N_8O_8I_2$[e]	C, H, I	−	0.76
II	C_2H_5	NH_2			345 dec	$C_{36}H_{36}N_8O_4I_2 \cdot 1.5H_2O$[e]	C, H, I	++	0.75
I	...[a]	NO_2	H	NO_2	311–312	$C_{32}H_{21}N_9O_{10}$	C, H, N		
I	C_2H_5	NO_2	H	NO_2	184–186	$C_{50}H_{45}N_9O_{16}S_2 \cdot H_2O$	C, H, S	−	0.98
I	$CH_3(CH_2)_2$	NO_2	H	NO_2	171–173	$C_{52}H_{49}N_9O_{16}S_2 \cdot 1.5H_2O$	C, H, S	−	1.13
I	C_2H_5	NH_2	H	NH_2	294–295	$C_{50}H_{51}N_9O_{10}S_2 \cdot 0.5H_2O$	C, H, S	++	0.69
I	$CH_3(CH_2)_2$	NH_2	H	NH_2	163–165	$C_{52}H_{55}N_9O_{10}S_2$	C, H, S	++	0.78

[a] Free base. [b] Anion throughout this paper is *p*-toluenesulfonate unless otherwise indicated. [c] Activity against the L1210 system according to our experimental procedure: ±, increase in life span 25–50%; +, 50–100%; ++, >100%. [d] R_f value relative to internal standard; see ref 2. [e] Anion iodide.

TABLE II

DERIVATIVES OF PYRIDINE

Substituent	Mp, °C	Formula	Analyses
3-(*m*-Chloro-*p*-nitrobenzamido)	159–160	$C_{12}H_8ClN_3O_3$	C, H, N, Cl
3-(*p*-Amino-*m*-chlorobenzamido)	177–177.5	$C_{12}H_{10}ClN_3O$	C, H, N, Cl
3-(*m*-Methyl-*p*-nitrobenzamido)	104–106	$C_{13}H_{11}N_3O_3$	C, H, N
3-(*p*-Amino-*m*-methylbenzamido)	81.5–82	$C_{13}H_{13}N_3O$	C, H, N
3-(*m*-Methoxy-*p*-nitrobenzamido)	116–117	$C_{13}H_{11}N_3O_4$	C, H, N
3-(*p*-Amino-*m*-methoxybenzamido)	177–178	$C_{13}H_{13}N_3O_3$	C, H, N
3-(*o*-Chloro-*p*-nitrobenzamido)	168–169	$C_{12}H_8ClN_3O_3$	C, H, N, Cl
3-(*p*-Amino-*o*-chlorobenzamido)	206–207	$C_{12}H_{10}ClNO_3$	C, H, N, Cl
3-(*o*-Methyl-*p*-nitrobenzamido)	170–171	$C_{13}H_{11}N_3O_3$	C, H, N
3-(*p*-Amino-*o*-methylbenzamido)	165–167	$C_{13}H_{13}N_3O$	C, H, N
3-(*o*-Methoxy-*p*-nitrobenzamido)	192–193	$C_{13}H_{11}N_3O_4$	C, H, N
3-(*p*-Amino-*o*-methoxybenzamido)	162.5–163	$C_{13}H_{13}N_3O_2 \cdot H_2O$	C, H, N

Table III

Drug	R	R^1	R^2	R^3	Dose, mg/kg/day	Wt change	Survivors	Av survival days, Treated	Av survival days, Control	T-C, '
I	CH_3	Cl	H	H	34	−1.8	6	16.0	9.9	162
					23	−0.5	6	20.8	9.9	212
					15	+1.5	6	15.2	9.9	154
					10	+1.0	6	12.3	9.9	124
					6.7	+2.0	6	12.4	9.9	126
I	C_2H_5	Cl	H	H	50	−4.4	3	7.3	9.3	
					33	−1.9	6	13.2	9.3	142
					22	−1.4	6	13.6	9.3	146
					15	+0.8	6	12.2	9.3	131
					10	+1.8	6	9.8	9.3	
I	CH_3	CH_3	H	H	25	−4.5	6	8.6	9.2	
					17	−1.9	6	16.0	9.2	174
					11.3	−0.2	6	20.7	9.2	224
					7.5	+0.9	6	15.5	9.2	168
					5.0	+2.1	6	12.0	9.6	125
I	C_2H_5	CH_3	H	H	25	−2.5	6	15.2	10.6	143
					17	−0.4	6	17.2	10.6	162
					11.0	−0.1	6	14.4	10.6	136
					7.3	+1.7	6	13.2	10.6	124
I	CH_3	CH_3O			22		3			
					15	−0.5	6	15.0	10.2	147
					10	−0.1	6	14.3	10.2	140
					6.7	0.0	6	13.6	10.2	133
					4.4	+2.5	6	12.1	10.2	
I	C_2H_5	CH_3O	H	H	25	−2.5	6	14.3	10.2	141
					17	−0.4	6	19.8	10.2	194
					11	−0.1	6	17.4	10.2	171
					7.5	+1.8	6	14.2	10.2	139
					5.0	+2.3	6	12.8	10.2	126
I	$CH_3(CH_2)_2$	CH_3O	H	H	25		2			
					17	+0.8	6	12.1	9.8	124
					11	+1.3	6	13.0	9.8	132
					7.5	+0.9	6	12.0	9.8	123
I	CH_3	NO_2	H	H	25		2			
					17	−1.2	6	14.5	10.2	142
					11	+0.8	6	13.1	10.2	128
					7.5	+1.1	6	11.6	10.2	
I	CH_3	NH_2	H	H	20	−3.1	6	22.6	9.5	237
					13	−0.7	6	23.2	9.5	244
					9	0.0	6	20.6	9.5	217
					6	+0.8	6	15.2	9.5	161
					4	+2.7	6	12.4	9.5	130
					2.7	+2.2	6	11.3	9.5	
I	C_2H_5	NH_2	H	H	17	−3.2	6	17.1	9.7	176
					11	−0.8	6	22.9	9.7	236
					7	+0.9	6	17.8	9.7	184
					4.7	+0.5	6	16.9	9.7	174
					3.1	+1.5	6	15.0	9.7	154
					2.0	+2.8	6	12.2	9.7	126
I	$CH_3(CH_2)_2$	NH_2	H	H	80		0			
					53	−1.8	6	11.6	9.5	122
					35	+0.2	6	12.3	9.5	129
					23	+2.4	6	10.9	9.5	
I	CH_3	H	Cl	H	50	−2.7	5	12.8	9.9	129
					33	+2.0	6	12.4	9.9	126
					22	+4.8	6	9.8	9.9	
I	CH_3	H	CH_3	H	75		4			
					50	−0.7	6	14.3	10.2	140
					33	+0.2	6	14.9	10.2	146
					22	+1.8	6	11.8	10.2	
I	CH_3	H	CH_3O	H	37		3			
					25	−4.8	6	16.0	9.6	167
					17	−0.4	6	15.2	9.6	158
					11	+0.7	6	12.5	9.6	130
I	C_2H_5	H	CH_3O	H	34		0			
					23	−2.6	6	17.6	10.4	169
					15	−0.6	6	15.6	10.4	150
					10	+0.8	6	12.9	10.4	124
					6.7	+1.6	6	9.6	9.8	

TABLE III (*Continued*)

Drug	R	R¹	R²	R³	Dose, mg/kg/day	Wt change	Survivors	Av survival, days Treated	Av survival, days Control	T/C, %
I	$CH_3(CH_2)_2$	H	CH_3O	H	150	−2.8	6	12.1	9.6	126
					100	−1.2	6	11.9	9.6	124
					67	+0.8	6	10.0	9.6	
I	CH_3	H	H	CH_3	15		1			
					10	−1.2	6	12.4	9.7	128
					6.7	+0.2	6	11.6	9.7	120
I	CH_3	H	H	CH_3O	50	−2.0	5	10.8	9.6	
					37.5	+0.7	6	12.1	9.4	129
					25	−2.1	6	12.6	9.4	134
					17	+0.7	6	13.9	9.4	148
					11	+2.3	6	10.8	10.2	
I	C_2H_5	H	H	CH_3O	37.5	−4.2	6	14.5	10.2	142
					25	−1.3	6	14.0	9.6	146
					17	−1.1	6	13.6	9.6	142
					11	+1.5	6	12.9	9.8	132
					7.3	+2.5	6	12.0	9.8	122
I	$CH_3(CH_2)_2$	H	H	CH_3O	40	−0.7	5	13.3	9.4	142
					26	+1.2	6	13.9	9.4	148
					18	+2.4	6	12.2	9.4	130
					12	+3.2	6	10.4	10.2	
I	CH_3	H	H	NH_2	100	−5.8	4	10.4	9.2	
					67	−2.3	6	27.5	9.7	283
					44	−0.7	6	30.4	9.7	314
					30	+0.5	6	31.4	9.7	324
					20	0.0	6	29.6	9.7	305
					13	+1.0	6	27.6	10.4	265
					8.9	+0.9	6	24.7	10.4	238
					5.9	+0.5	6	21.6	10.4	207
					3.9	+1.2	6	17.3	10.4	166
					2.6	+1.4	6	14.6	10.4	140
					1.7	+2.1	6	10.8	10.4	
I	C_2H_5	H	H	NH_2	25	−7.2	6	23.1	10.4	222
					17	+0.3	6	28.2	10.4	272
					11	+1.4	6	33.1	10.4	318
					7	+1.7	6	>100	10.4	>1000[a]
					4.7	+0.4	6	22.8	10.4	219
					3.1	+1.9	6	15.6	10.4	160
					2.1	+2.0	6	14.4	10.4	138
I	$CH_3(CH_2)_2$	H	H	NH_2	34	−0.9	5	15.8	10.2	155
					23	+0.1	6	14.9	10.1	147
					15	−0.5	6	18.1	10.1	179
					10	+0.4	6	16.8	10.1	166
					6.7	+1.9	6	10.4	10.1	
II	C_2H_5	NH_2			10	−2.6	6	12.6	10.2	124
					6.7	+0.2	6	28.8	10.2	283
					4.4	+0.5	6		10.2	*b*
					3.0	+0.8	6	23.6	10.2	232
					2.0	+1.2	6	16.6	10.2	162
					1.3	+1.6	6	12.4	10.2	122
I	C_2H_5	NH_2	H	NH_2	20	−2.9	6	10.7	9.5	
					13	−2.0	6	21.9	9.7	226
					9	+1.1	6	29.1	9.7	301
					6	−0.2	6	25.6	9.7	263
					4	+2.1	6	20.4	9.7	210
					2.7	+1.8	6	13.4	10.0	134
I	$CH_2(CH_2)_2$	NH_2	H	NH_2	15	−3.1	6	12.0	10.0	120
					10	−0.4	6	22.6	10.0	226
					6.7	+0.2	6	21.2	10.0	212
					4.5	+0.7	6	17.4	10.0	174
					3.0	+2.5	6	18.8	10.0	188
					2.0	+2.9	6	13.5	10.0	135

[a] Repeat experiments with larger numbers of animals have given between 30 and 100% 100-day survivors over the dose range from 6 to 9 mg/kg/day against early treatment on a once daily for 5 days intraperitoneal dosage schedule. [b] One animal died on day 29, one at 33, and one at 42 days; the remainder survived 100 days. Essentially similar results were obtained on repeated experiments.

served with the methyl quaternary salt in contrast to the ethyl salt in the unsubstituted parent series. There was a rapid drop off in activity on homologation; the ethyl homolog (I, $R^2 = R^3 = H$; $R^1 = Cl$; $R = C_2H_5$) was weakly active, and the propyl derivative inactive.

Methyl substitution of the terephthaloyl unit also added to the lipophilic character of the resulting series although to a lesser extent than chlorine sub-

stitution as judged by paper chromatographic data. Once again the methyl quaternary salt (I, $R^2 = R^3 = H$; $R^1 = R = CH_3$) proved to be the most active of the homologous series.

Peak activity returned to the ethyl quaternary salt in the relatively more hydrophilic methoxyterephthaloyl analogs (I, $R^2 = R^3 = H$; $R^1 = OCH_3$).

The nitroterephthaloyl derivatives were also more lipophilic and only the methyl quaternary salt (I, $R^2 = R^3 = H$; $R^1 = NO_2$; $R = CH_3$) showed any antileukemic efficacy and this was of a very low order.

Higher activity, comparable to the parent series, was found in the amino derivatives (I, $R^2 = R^3 = H$; $R^1 = NH_2$). The methyl and ethyl salts of this series were of almost equivalent activity, the *n*-propyl derivative being markedly less active.

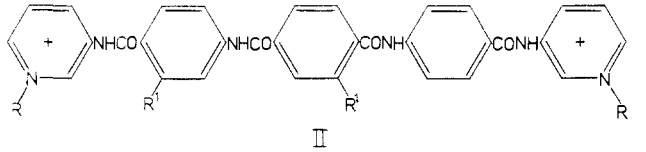
The more readily prepared symmetrically disubstituted modifications involving the *p*-aminobenzoate units were first examined. The increase in lipophilicity due to introduction of two chlorines *ortho* to the amino group (I, $R^1 = R^3 = H$; $R^2 = Cl$) was marked and the methyl salt of this series showed only borderline inhibition. A similar effect was shown with the dimethyl-substituted series (I, $R^1 = R^3 = H$; $R^2 = CH_3$). Less change in physical properties was noted with methoxyl substitution (I, $R^1 = R^3 = H$; $R^2 = CH_3O$); the methyl and ethyl quaternary salts were moderately active, and the *n*-propyl homolog showed only slight inhibition of the leukemia.

Unfortunately the dinitro analogs (I, $R^1 = R^3 = H$; $R^2 = NO_2$) could not be obtained. While it proved possible to benzoylate amides of 3-nitro-4-aminobenzoic acid under forcing conditions with an excess of acid chloride, attempts to obtain symmetrical bisamides, with necessarily limited amounts of terephthaloyl chloride, failed. The combination of steric hindrance and deactivation make it difficult to obtain even simple derivatives of this aminonitro acid.

Reference to the tables of biological data shows that shift of the substituents to the adjacent position (from I, $R^1 = R^3 = H$; $R^2 = Cl, CH_3, OCH_3$, to I, $R^1 = H$; $R^3 = Cl, CH_3, OCH_3$) produced no significant change in order or magnitude of biological activity. However, the amino-substituted derivatives investigated in this area were extremely active. Maximum activity appeared to be associated with the N-ethyl salt (I, $R^1 = R^2 = H$; $R^3 = NH_2$; $R = C_2H_5$). This, at doses between 6 and 9 mg/kg/day and with early treatment schedules gave a proportion of 100-day survivors (30–100% of animals in tests at different times over this dose range[4]).

The exemplary level of effectiveness of these compounds promoted an examination of other variously substituted amino derivatives.

The asymmetric derivative II ($R = C_2H_5$; $R^1 = NH_2$) also gave a proportion of 100-day survivors but appeared to offer no real advantage over the more readily prepared symmetrical derivative.



II

The triamino series (I, $R^2 = H$; $R^1 = R^3 = NH_2$) as the ethyl and propyl quaternary salts gave high levels of life extension but in our hands these have given no 100-day survivors.

It appears from the rather limited data presented in this paper that antileukemic activity in these substituted derivatives roughly correlates with the electron-donor properties of the substituent, amino substitution giving the most active materials.

Experimental Section

Analyses were by Dr. A. D. Campbell, Microchemical Laboratory, University of Otago, New Zealand. Where analyses are indicated only by symbols of the elements analytical results obtained for those elements were within ±0.4% of the theoretical values. 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.

The majority of the required bisbases were symmetrical and were readily prepared by interaction of the substituted terephthaloyl chloride on 2.2 molar proportions of the requisite 3-(*p*-aminobenzamido)pyridine (see Table II) in diethylene glycol dimethyl ether solution. The methods used in isolation, quaternization, paper chromatography, etc., have been adequately described.[2] Intermediates which have not been previously described in the literature are listed below. The bulk of these compounds were prepared by the phosphorazo coupling method.[2]

The nitro quaternary salts were reduced to the amino quaternary compounds by the method used for reduction of nitrophenanthridinium salts.[5]

2-Nitro-4-phthalimidobenzoic Acid.—A mixture of 2-nitro-4-aminobenzoic acid (7.3 g), phthalic anhydride (8.5 g), and pyridine (15 ml) was heated on the steam bath until homogeneous and then for a further 0.5 hr. AcOH (50 ml) was then added, the solution was refluxed for 5 min and cooled to 0°, and Ac_2O (4.5 ml) was added. The resulting clear solution was heated under reflux until the product commenced to crystallize. After several hours of cooling pure product (12.2 g), mp 286°, was collected. *Anal.* ($C_{15}H_8N_2O_4$) C, H, N.

3-(*p*-Amino-*o*-nitrobenzamido)pyridine.—To a suspension of 2-nitro-4-phthalimidobenzoic acid (2 g) in dioxane (10 ml) were added successively pyridine (0.52 ml) and $SOCl_2$ (6 ml). After a few minutes of warming, a clear solution resulted and this was evaporated *in vacuo* as far as possible, a little dioxane was added, and the solution was reevaporated. This crude acid chloride was dissolved in dioxane (7.5 ml) and added dropwise to a well-stirred solution of anhydrous 3-aminopyridine (1 g) in dioxane (5 ml) containing pyridine (1 ml). The mixture was heated on the steam bath for 1 hr, then evaporated as far as possible. The coupled product may be isolated and crystallized from *n*-BuOH but it has a marked tendency to separate from solution as a gel. Higher yields of end product are obtained by not isolating at this stage. The crude phthalimido derivative was dissolved in pyridine (7.5 ml), 100% hydrazine hydrate (0.6 ml) and anhydrous Na_2CO_3 (0.4 g) were added, and the mixture was maintained at 50° for 2 hr. After removal of pyridine *in vacuo* the product was suspended in H_2O (50 ml), HCl was added to a pH of 2.4, and the mixture was shaken well and filtered. Basification with NH_3 precipitated the amino compound which separated from EtOH–H_2O in glistening yellow plates, mp 207–208°, 1.35 g, 77% yield. *Anal.* ($C_{12}H_{10}N_4O_3$) C, H, N.

3-[*p*-(*p*-Methoxycarbonyl-*m*-nitrobenzamido)-*o*-nitrobenzamido]pyridine was prepared by phosphorazo coupling[2] of *p*-methoxycarbonyl-*m*-nitrobenzoic acid and 3-(*p*-amino-*o*-nitrobenzamido)pyridine; mp 215–216°. *Anal.* ($C_{21}H_{15}N_5O_8$) C, H, N. Mild alkaline hydrolysis of this ester by the previously described procedure[2] afforded the corresponding acid, mp 285–286°. *Anal.* ($C_{20}H_{13}N_5O_8$) C, H, N. Coupling of this acid with 3-

(4) The C_3H/DBA_2F_1 hybrid mice used in our screening program are somewhat more robust than the BDF_1 used by the CCNSC or the parent DBA_2 strain judging by the dose levels attainable. Repetition of these experiments in DBA_2 mice has given at various times, 20–70% 100-day survivors over the dose range 5–8 mg/kg/day, under our test conditions.

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

(*p*-aminobenzamido)pyridine by the phosphorazo[2] method gave the free base II ($R^1 = NO_2$).

Biological Testing.—Full details of the test procedure have been given previously.[2,3] The legends in Table III correspond to test groups of six animals which have been inoculated with 10^5 L1210 cells intraperitoneally; dosage has been once daily intraperitoneally at the indicated figure for 5 days starting 24 hr after tumor inoculation. Weight change refers to difference in weight at days 1 and 8. Survivors' column lists those animals surviving as long or longer than controls in the group. Compounds which have been tested from a toxic (evidenced by death or marked weight loss) to a nontoxic dose level and have given no increase in life span are classified as negative and so designated in the tables of analytical data. Full details of testing for these negative compounds have not been given.

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Potential Antileukemic and Immunosuppressive Drugs. Preparation and *in Vitro* Pharmacological Activity of Some Benzo-2,1,3-oxadiazoles (Benzofurazans) and Their N-Oxides (Benzofuroxans)

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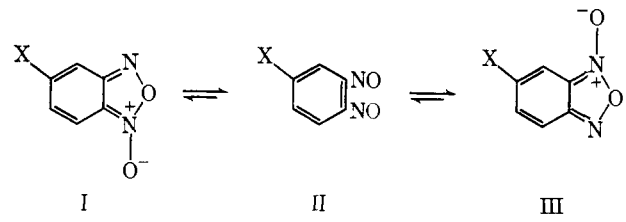
Eight methods for synthesizing various benzo-2,1,3-oxadiazoles (benzofurazans) and their N-oxides (benzofuroxans) are described. The activity of benzofurazans and benzofuroxans as *in vitro* inhibitors of RNA synthesis in sheep lymphocytes is given. Optimal drug activity *in vitro* was exhibited by 4-nitrobenzofurazan and 4-nitrobenzofuroxan and their 7-thio or 7-phenoxy derivatives. These compounds readily formed Meisenheimer complexes with nucleophiles and their drug action was abolished by preincubation with aliphatic thiols, *e.g.*, glutathione. It is concluded that the most active compounds reacted with key intracellular thiol groups. The 4-nitro group and furazan ring were essential for optimal drug activity.

In the course of testing some analogs of the purines and the antiarthritic drug, 2,3-bis(*p*-methoxyphenyl)-indole,[1] as inhibitors of lymphocyte metabolism *in vitro*,[2] we discovered that 4-nitrobenzo-2,1,3-oxadiazole[3] was a powerful inhibitor of nucleic acid and protein biosynthesis in many types of animal cells but with an especially toxic effect upon the metabolism of leukocytes *in vitro*.[4] This report summarizes our exploration of the structure–activity relationship for suppressing the incorporation of tritiated uridine into sheep lymphocyte RNA by benzo-2,1,3-oxadiazoles and their N-oxides. For convenience in distinguishing these two series of compounds, we shall hereafter refer to them by their alternative names, namely, benzofurazans and benzofuroxans (the N-oxide).

Benzofuroxan itself (I, X = H) has been shown by low-temperature nmr studies to be a rapidly equilibrating system, the transformation between the 1- and 3-oxide structure probably proceeding *via* *o*-dinitrosobenzene (II, X = H) as an intermediate.[5a,b] In the case of benzofuroxans substituted in the 5 position, the amount of each tautomer present in solution is dependent on the nature of X. When X is an electron donor, structure I is more abundant, while an electron-accepting group favors structure III.[5c] 4-Nitrobenzofuroxan exists in one form (oxide in the 1 position)



I II III

at all temperatures.[5a,c] For a recent review of benzofuroxans see ref 6.

Preparation of Compounds.—Benzofuroxans were prepared by pyrolysis of the appropriately substituted *o*-nitrophenyl azides (method A) (see Experimental Section for details of each method) or hypohalite oxidation of substituted *o*-nitroanilines (method B). Method A was generally preferred to B because the oxidant used in the latter method may disrupt the furoxan ring[7] or displace a substituent. For example, alkaline hypochlorite reacts with 2,4-dinitroaniline to give 5-chloro-4-methoxybenzofuroxan instead of the expected 5-nitrobenzofuroxan.[8a] *o*-Nitrophenyl azides are readily obtained from *o*-nitroanilines either by diazotization and treatment with aqueous sodium azide, or by nucleophilic displacement of halogeno or nitro groups with sodium azide in dimethyl sulfoxide.[9] Treatment of 2-bromo-3-nitrobenzoic acid with sodium azide in dimethyl sulfoxide gave 2-azido-3-nitrobenzoic acid, previously only obtained from 2-bromo-3-nitrobenzoic acid by conversion to the amino compound

(1) Indoxol. E. M. Glenn, B. J. Bowman, W. Kooyers, T. Koslowske, and M. L. Myers, *J. Pharmacol. Exp. Ther.*, **155**, 157 (1967).
(2) M. W. Whitehouse, *J. Pharm. Pharmacol.*, in press.
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