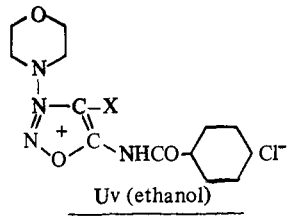
Table II



	X	Ir (KBr), cm^{-1}	Uv (ethanol) λ_{max}, mμ	ε	Nmr (DMSO), τ
PR-G-138	H	1725, 1625	325 243	11,400 9,100	3N-CH_2, 3.83 4C-H, 9.22
12	Cl	1727, 1625	348 254	10,500 10,000	3N-CH_2, 3.97
13	Br	1720, 1620	348 250	9,500 9,000	3N-CH_2, 3.88
14	I	1720, 1600	350 253	6,000 7,800	3N-CH_2, 4.01

material PR-G-138 (10 mg/kg po, –40 mm, SHR). The iodo compound **14** appeared slightly less active, as did the iodo compound of the series **15-17**. The pharmacological profile‡ and a favorable therapeutic index make the 3-amino-4-halosydnone imines promising candidates for further evaluation.

Experimental Section§

The nmr spectra were determined on a Varian A-60; uv spectra were recorded on a Bausch and Lomb 505 and the ir spectra on a Perkin-Elmer Infracord 237B.

Method A. Halosuccinimide. The acylated 3-aminosydnone imine hydrochloride was dissolved in H_2O, basified with excess Na_2CO_3 solution, and extracted into $CHCl_3$. The solution was dried and evaporated. To 0.05 mol of free base dissolved in 150 ml of CCl_4, 0.15 mol of *N*-halosuccinimide was added.# The mixture was heated to 75° for 3 hr,** cooled and mixed with 100 ml of H_2O, and worked up as usual. The residue was dissolved in MeOH and acidified with HCl-Et_2O and crystallized.

Method B. Br_2-NaOAc. To 0.05 mol of 3-aminosydnone imine hydrochloride suspended in 350 ml of Et_2O, 16 g of NaOAc was added. At reflux temperature 16 g (0.1 mol) of Br_2 in 25 ml of $CHCl_3$ was dropped in and refluxed 5 hr. The mixture was cooled and filtered and filtrate evaporated to dryness. The residue was dissolved in EtOH, acidifled with HCl, and crystallized.

Method C. Br_2-$NaHCO_3$. 3-Aminosydnone imine hydrochloride (0.05 mol) suspended in 20 ml of $CHCl_3$ and 300 ml of Et_2O were refluxed with 25 g of $NaHCO_3$ under stirring. Br_2 (12 g, 0.75 mol) was dropped in. The mixture was refluxed for 3 hr, cooled, and filtered and the filtrate evaporated. The residue was crystallized.

Hydrolysis of Acylated 3-Amino-4-halosydrone Imines (III → IV). III (0.01 mol) dissolved in 50 ml of H_2O was left at room temperature for 1 week. The clear solution was repeatedly extracted with Et_2O to remove the organic acid. The aqueous layer was evaporated to dryness and crystallized. Compounds 8, 12, 15, and 16 were obtained by this method.

Bromo(morpholinoimino)acetonitrile from 3-Morpholinosydnone Imine Hydrochloride (VI from V). To 0.1 mol of V and 32 g of NaOAc suspended in 600 ml of Et_2O, 32 g (0.2 mol) of Br_2 in 50 ml of $CHCl_3$ was added under reflux. The mixture was heated for 4 hr and filtered and filtrate concentrated to dryness. The residue was crystallized from Et_2O-petroleum ether: yield 5.0 g (23%); mp 54–56°; ir (KBr) 2200, 1575 cm^{-1}; uv (EtOH) λ_{max} 282, 230 nm (ε 8700, 3500).

Bromo(morpholinoimino)acetonitrile from (Morpholinoimino)-acetonitrile (VI from VII). To a solution of 0.1 mol of VII in 200 ml of Et_2O, 23 g of NaOAc was added. The mixture was refluxed and 24 g (0.15 mol) of Br_2 in 50 ml of $CHCl_3$ was dropped in, refluxed 2 hr, cooled, and filtered and filtrate evaporated: yield 6.0 g (25%); mp 54–55° (Et_2O-petroleum ether); ir, uv, and tlc identical with VI from V.

‡The detailed pharmacology will be published separately: J. T. Oliver, unpublished results.

§Where analyses are indicated only by symbols of the elements, analytical results were obtained within ±0.4% of the theoretical values.

#For chlorinations a trace of benzoyl peroxide was added.

**Occasionally the reaction proceeded satisfactorily also at room temperature.

References

(1) G. Wehlmann, K. Zeile, M. Götz, and K. Freter, German Offen. 1,942,854 (Sept 1970) (Appl. 22 Aug 1969); *Chem. Abstr.*, 73, 131005 (1970).
(2) M. Götz and K. Grozinger, *J. Heterocycl. Chem.*, 7, 123 (1970).
(3) M. Götz and J. T. Oliver, *Chem. Can.*, 24 (9), 20 (1972).
(4) K. Maranda, Y. Imashiro, and T. Kaneko, *Chem. Pharm. Bull.*, 18, 128 (1970).
(5) K. Kirkuchi, M. Hirata, A. Nagaoka, and Y. Aramaki, *Jap. J. Pharmacol.*, 20, 23 (1970).
(6) R. E. McMahon in "Medicinal Chemistry," A. Burger, Ed., Wiley-Interscience, New York, N. Y., 1970, p 51.
(7) H. Kato, M. Hashimoto, and M. Ohta, *Nippon Kagaku Zasshi*, 78, 707 (1957).
(8) M. Ohta and H. Kato, "Nonbenzenoid Aromatics," Academic Press, New York and London, 1969, p 178.
(9) Y. Asaki, K. Shinozaki, and M. Nagaoka, *Chem. Pharm. Bull.*, 19, 1079 (1971).
(10) R. F. Skelton, *Proc. Soc. Exp. Biol. Med.*, 90, 342 (1955).

Potential Antitumor Agents. 13. Bisquaternary Salts

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Forty-two variants of bisquaternary salts of 4-[*p*-(*p*-(4-pyridylamino)phenylcarbamoyl)anilino]-quinoline have been synthesized for evaluation in the L1210 system. The N^1-alkyl-4-pyridylamino cationic function could be replaced by N^1-alkyl-4-pyridyl-, N^1-alkyl-5-(2,4-diaminopyrimidinyl)-, or amidinohydrazone and L1210 activity retained. Congeners substituted in the quinoline ring (Cl, CH_3, OCH_3, NO_2, NH_2) were screened and when the log *P* contribution of the quinoline substituent to antileukemic activity was compensated for, it appeared that electron-donor substituents provided the most L1210 active compounds. Reversal of the central –CONH– bond or replacement by –NHCONH– or –CH=CH– provided L1210 active molecules. 6- or 7-aminoquinoline variants were highly active and provided numbers of indefinite survivors in early ip L1210 tests. The 7-NO_2 quinoline congeners showed anomalously high activity while all 6-NO_2 variants proved inactive.

Series of bisquaternary ammonium heterocycles prepared earlier showed very high apparent activity in L1210 tests, some examples producing *T/C* values of greater than 300%.[1-8] In leukemic test groups treated for five consecutive days with certain of these agents (*e.g.*, I, R′ = H; R = C_2H_5), it was found that the animals dying later than 30 days after the start of dosing were free of leukemia. Such findings could have been predicted by extrapolating the

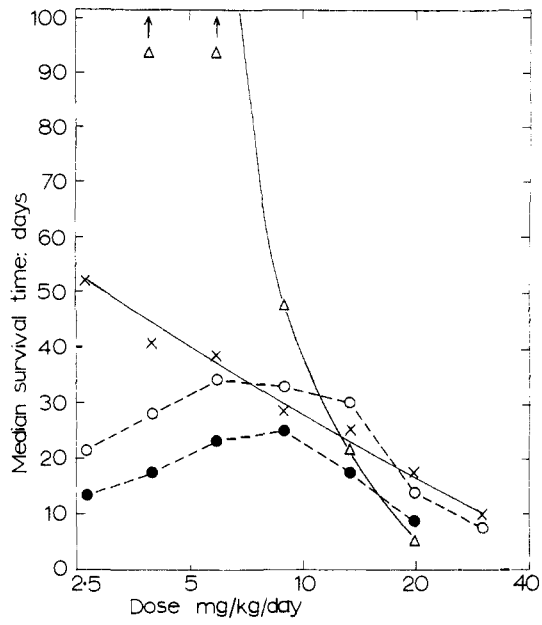
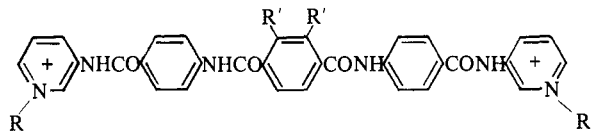


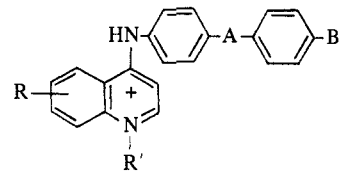
Figure 1. Drugs administered once daily for 5 days ip: ○—○, I (R′ = H; R = C_2H_5) administered to L1210 (10^5 ip) bearing animals; ×—×, dosed to tumor-free mice; ●—●, IIa (R = H; R′ = R″ = CH_3) dosed to L1210 bearing animals; Δ—Δ, dosed to tumor-free animals. Ten animals per point; arrows denote mice survived >100 days.

kinetic data obtained at the Southern Research Institute;[9] one viable leukemic cell remaining at the end of a 5-day dosing period could be expected to multiply to lethal tumor cell numbers in a further 14-19 days. Animals in our test groups should then either die leukemic deaths within 23 days from the start of dosing or survive free of the disease. Administration of examples of our bisquaternary salts to tumor-free animals showed that late occurring deaths were due to chronic drug toxicity (Figure 1). It appeared that animals dying after 30 days in leukemic tests had been cleared of the leukemia but later died from drug toxicity. It is noteworthy that any dose of I (R′ = H; R = C_2H_5) which will provide life extension in L1210 tests will ultimately prove lethal to tumor-free animals.

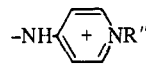
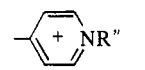
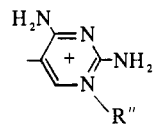
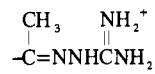
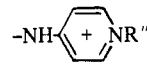
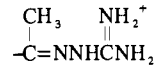
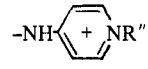
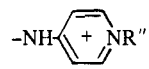
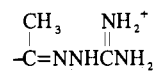
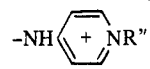
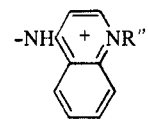


I

When two closely related variants, I (R′ = Me; R = Me) and I (R′ = H; R = C_2H_5), were dosed to tumor-free animals, very similar patterns of delayed deaths were observed. However, these two congeners differed widely in their ability to extend life in L1210 tests.[1] It therefore appeared possible that chronic toxicity and antileukemic effectiveness might be separable. In addition, by amino group substitution of analogs of I, it was possible to produce agents which could provide numbers of 100-day survivors in L1210 tests.[2] It could be demonstrated that chronic toxicity was attenuated in such amino-substituted variants. A systematic study of structure-chronic toxicity relationships, using the wide range of structural types available,[1-3] demonstrated that the variant IIa (R = H; R′ = R″ = CH_3) had little tendency to produce delayed animal deaths at tumor active doses (Figure 1). It then became necessary to examine structure-antileukemic activity relationships of congeners of this latter agent; this publication reports preliminary results obtained in this area.



II

	A	B
IIa	-CONH-	
IIb	-CONH-	
IIc	-CONH-	
IId	-CONH-	-$COCH_3$
IIe	-CONH-	
IIf	-NHCO-	
IIg	-NHCO-	-$COCH_3$
IIh	-NHCO-	
IIi	-CH=CH-	
IIj	-NHCONH-	
IIk	-NHCONH-	-$COCH_3$
III	-NHCONH-	
IIm	-CH=CHCONH-	
IIn	-CONH-	

We have repeatedly stressed[1-8] the crucial importance of lipophilic-hydrophilic balance of agent for anti-L1210 activity in the bisquaternary salt series. In order to be able to compensate for changing lipophilic character in congeners, a standard reference curve was constructed by plotting log maximum life extension obtained in L1210 tests for the members of the homologous series IIa (R = H; R′ = R″ = CH_3, C_2H_5, n-C_3H_7) *vs.* $\Sigma\pi$ for the *N*-alkyl quaternary function. The contributions made to antileukemic activity by the electronic and steric efforts of a further substituent could then be determined by examining whether, at the π value for the substituent added, the life extension measured lay significantly above or below the reference curve. For example, a chlorine substituent on the quinoline benzene ring (**4**, **6**, **8**; Table I) produced a greater decrease in activity than that expected from the increased lipophilic character due to the Cl atom alone. Presumably, electronic and/or steric effects due to the added chlorine substituent were unfavorable. When the increased lipophilic character due to an additional methyl group was compensated for by these methods, it appeared that the 6- and 8-Me congeners (**12**, **14**) had activity comparable to the parent. However,

a 3-Me substituent **10** abolished activity; in this variant steric interactions will rotate the side chain to a greater than normal angle and destroy the close overall approach to planarity thought necessary for activity.[1,3]

Methoxyl groups placed at positions 6 or 8 on the quinoline ring (**16**, **18**) appeared to slightly enhance activity. Amine substituents on the quinoline ring definitely enhanced activity, amine-substituted variants providing a proportion of 100-day survivors in early L1210 tests (**26**, **27**, **48**, **59**, **63**, **69**, **88**). Since the amino group is strongly hydrophilic it was deemed necessary to synthesize the homologous series of quaternary salts **26–28** to ensure that the Me congener did not have a suboptimal log *P*. Methyl and ethyl quaternary salts **26** and **27** displayed very similar activities; alternative testing against subcutaneously implanted L1210 also failed to distinguish between the activities of these homologs. If, in this agent series, there is the usually observed parabolic dependence of biologic activity on log *P*, then the equivalent activity of these two homologs requires that they have log *P* values which lie to each side of the optimum log *P* for this biologic test system.

The inability to quaternize 8-nitroquinolines (*e.g.*, **25**) made the 8-aminoquinolines unavailable with the synthetic methods detailed. When the effect of the log *P* contribution of the substituents examined (Cl, Me, OMe, NH_2) had been compensated for, it appeared that L1210 activity increased with increasing electron-donor character of the substituent. However, the powerfully electron-withdrawing nitro group placed at the 7 position of the quinoline ring provided compounds with high activity (**24**, **29**, **51**, **52**, **62**, **63**). In contrast, all 6-NO_2 congeners examined proved inactive (**20–22**, **57**, **58**, **75**, **78**). This apparently anomalous effect of a 7-NO_2 group finds a parallel in a related series of acridines[5] where 3-nitro isomers are found to be active while the 2-nitro compounds are inactive.† No satisfactory explanation for the apparently anomalous activity of these nitro derivatives is as yet available (Table II).

Earlier research had disclosed a range of positively charged functions which could act as the cationic center in related drug series.[6] Examination of certain of these alternatives in the present series provided many examples with excellent activity (**38–52**) but no clearly superior variant emerged.

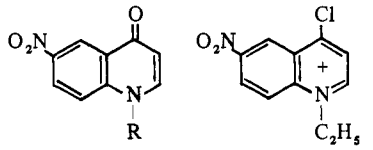
The intermediary unquaternized bis bases (*e.g.*, **3**, **5**, **11**, **17**) are strong bases and exist predominantly as dications in aqueous solution at physiological pH's. In contrast to the corresponding bisquaternary salts (**4**, **6**, **12**, **18**), these compounds failed to influence the L1210 leukemia. A monoquaternary salt should act as a better source of dication than the corresponding bis base but a synthesized example (**30**) showed no L1210 activity. It appears that quaternization accomplishes more than just ensuring an adequate supply of dicationic species.

Replacement of the quinoline moiety of the parent (IIa) by the quinazoline azalog abolished activity (**33**). In contrast, the pyridine terminus of such molecules could be acceptably replaced by pyrimidine (**39–41**).

From an examination of molecular models of these agents and our projected site,[1] it was mooted that reversal of the amide function in IIa should not reduce site binding contact. The amide reversed isomers **53–73** did indeed appear to have equivalent activity. This retention of activity following amide reversal should be compared with earlier series in which this modification produced marked decreases in activity.[7] The amide function could also be replaced by urea, moderately active agents resulting (**77–85**). Such results suggest that the linking function in these agents (A in II) acts predominantly as a bridge to maintain the correct orientation and separation of the quinoline and pyridine rings. The excellent activity of the stilbene (**76**) lends further support to this view. The activity of this variant (**76**) is higher than would be expected from its lipophilic character; possibly this reflects the closer approach to planarity possible in the two rings of a stilbene in comparison to those in a benzanilide.[1,3]

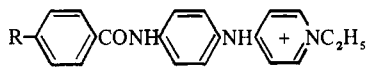
Previously investigated methods of increasing intercharge separation[4,7] proved acceptable in the present instance (**84–88**) but there was no clear enhancement in activity. A bisquinoline variant **90** proved inactive contrasting with the active bisquinoline quaternary salts detailed earlier.[1,3,7,8]

Gram quantities of the agents described can be readily prepared by the synthetic methods detailed. On attempted scale up (kilogram quantities), difficulties were encountered at the quaternization step, a mixture of the required product and partially quaternized material resulting. Rigorous purification of such mixtures on a large scale proved a formidable proposition. An alternate route, quaternizing the basic termini separately at an earlier stage in the synthesis, circumvented this difficulty. For example, **27** was prepared by the following route: 6-nitroquinoline (IIIa) was alkylated to provide the *N*-ethylquinolone (IIIb) and this on treatment with $SOCl_2$ and trace quantities of DMF yielded the quinolinium salt IV. Acid-catalyzed coupling of IV and the pyridinium salt V (R = NH_2) produced the bisquaternary salt **20** in excellent yield and purity. A following reduction produced **27** in high yield and of excellent purity. The variant **27** as NSC113,089 is listed by DRD of NCI as a Decision Network 2 compound.[10]



IIIa, R = H
b, R = C_2H_5

IV



V

Experimental Section

Melting points were determined with an Electrothermal melting point apparatus with the makers supplied stem-corrected thermometer. Open capillaries were used for melting point determinations; a heating rate of 2°/min from 20° below the melting point was used; melting points are as read. Microanalyses were performed by Dr. A. D. Campbell, Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Where analyses are indicated by symbols of the elements, analytical results obtained were within ±0.4% of the theoretical values.

The synthetic routes employed are obvious from the intermediates quoted. Methods used for preparation of amides,[2] pyridylation of amines,[6] quaternization,[4] chromatographic methods,[4] etc., have been adequately described. Formerly, paper chromatography was used as an index of purity for bisquaternary salts; subsequent work has shown tlc (Merck SiO_2 F254; top phase *n*-BuOH–HAc–H_2O, 5:1:4) to give superior resolutions and provides a better index of purity.

Necessary intermediates not listed in Table I are as follows. 2,4-Diamino-5-[*p*-(*p*-nitrobenzamido)phenyl]pyrimidine: yellow prisms (DMF–EtOH), mp 328–329°. *Anal.* ($C_{17}H_{14}N_6O_3$) C, H, N. 2,4-Diamino-5-[*p*-(*p*-aminobenzamido)phenyl]pyrimidine: colorless needles (EtOH–H_2O), mp 286–287°. *Anal.* ($C_{17}H_{16}N_6O$) C, H, N. *p*-(*p*-Nitrophenylcarbamoyl)acetophenone: yellow prisms (EtOH–H_2O), mp 231–232°. *Anal.* ($C_{15}H_{12}N_2O_4$) C, H, N. *p*-(*p*-Aminophenyl-

†Unpublished results.

Table I

No.	Type	Quinoline substituents	R′ = R″ =	Mp, °C	Formula	Analyses[d]	R_d[e]	L1210[f]
3	IIa	6-Cl	*a*	312-313	$C_{27}H_{20}N_5OCl$	C, H, N, Cl		--
4	IIa	6-Cl	CH_3	167-169	$C_{43}H_{40}ClN_5O_7S_2$[b]	C, H, N, S	0.95	+
5	IIa	7-Cl		325-326	$C_{27}H_{20}ClN_5O$	C, H, N, Cl		--
6	IIa	7-Cl	CH_3	249-251	$C_{29}H_{26}ClN_5OBr_2$	C, H, N, Cl, Br	0.94	±
7	IIa	8-Cl		302-303	$C_{27}H_{20}ClN_5O$	C, H, N, Cl		
8	IIa	8-Cl	CH_3	176-178	$C_{43}H_{40}ClN_5O_7S_2$[b]	C, H, N, S	0.96	+
9	IIa	3-CH_3		240-241	$C_{28}H_{25}N_5O_9Cl_2$[c]	C, H, N, Cl		
10	IIa	3-CH_3	CH_3	226-228	$C_{30}H_{29}N_5OBr_2$	C, H, N, Br	0.91	--
11	IIa	6-CH_3		301-302	$C_{28}H_{23}N_5O$	C, H, N		--
12	IIa	6-CH_3	CH_3	168-170	$C_{44}H_{43}N_5O_7S_2$[b]	C, H, N, S	0.92	++
13	IIa	8-CH_3		268-269	$C_{28}H_{23}N_5O$	C, H, N		
14	IIa	8-CH_3	CH_3	169-170	$C_{44}H_{43}N_5O_7S_2$[b]	C, H, N, S	0.94	++
15	IIa	6-OCH_3		203-204	$C_{28}H_{23}N_5O_2$	C, H, N		
16	IIa	6-OCH_3	CH_3	167-169	$C_{44}H_{43}N_5O_8S_2$[b]·0.5H_2O	C, H, S	0.94	++
17	IIa	8-OCH_3		178-179	$C_{28}H_{23}N_5O_2$	C, H, N		–
18	IIa	8-OCH_3	CH_3	266-268	$C_{30}H_{29}N_5O_2Br_2$	C, H, N, Br	0.93	++
19	IIa	6-NO_2		280-281	$C_{27}H_{26}N_6O_3$	C, H, N		
20	IIa	6-NO_2	CH_3	273-274	$C_{29}H_{26}N_6O_3Br_2$	C, H, Br	0.88	–
21	IIa	6-NO_2	C_2H_5	256 dec	$C_{31}H_{30}N_6O_3Br_2$	C, H, Br	0.97	–
22	IIa	6-NO_2	$CH_3(CH_2)_2$	204-205	$C_{33}H_{34}N_6O_3I_2$	C, H, I	1.05	--
23	IIa	7-NO_2		327-328	$C_{27}H_{20}N_6O_3$	C, H, N		
24	IIa	7-NO_2	CH_3	176-178	$C_{43}H_{40}N_6O_9S_2$[b]	C, H, N, S	0.88	++
25	IIa	8-NO_2		274-275	$C_{27}H_{20}N_6O_3$	C, H, N		
26	IIa	6-NH_2	CH_3	240-242	$C_{29}H_{28}N_6OBr_2$	C, H, Br	0.85	++
27	IIa	6-NH_2	C_2H_5	332-333	$C_{31}H_{32}N_6OBr_2$·0.5H_2O	C, H, Br	0.97	++
28	IIa	6-NH_2	$CH_3(CH_2)_2$	200-202	$C_{33}H_{36}N_6OI_2$	C, H, I	1.03	+
29	IIa	7-NH_2	CH_3	242-243	$C_{29}H_{28}N_6OBr_2$	C, H, N, Br	0.83	++
30	IIa		R′ = H; R″ = CH_3	161-164	$C_{42}H_{39}N_5S_2O_7$[b]	C, H, N, S	0.94	–
31	IIa	6-NO_2, 3-N=[g]		343-344	$C_{26}H_{19}N_7O_3$	C, H, N		
32	IIa	6-NO_2, 3-N=	CH_3	341 dec	$C_{42}H_{39}N_7S_2O_9$[b]	C, H, N, S	0.82	–
33	IIa	6-NH_2, 3-N=	CH_3	181-183	$C_{42}H_{41}N_7S_2O_7$[b]	C, H, N, S	0.80	–
34	IIb			282-283	$C_{27}H_{20}N_4O$	C, H, N		
35	IIb		CH_3	182-183	$C_{43}H_{40}N_4S_2O_7$[b]	C, H, S	0.92	++
36	IIb		C_2H_5	227-228	$C_{31}H_{30}N_4OBr_2$·H_2O	C, H, Br	0.97	+
37	IIb		$CH_3(CH_2)_2$	305-306	$C_{33}H_{34}N_4OBr_2$	C, H, Br	1.02	–
38	IIc			314-315	$C_{26}H_{21}N_7O$	C, H, N		
39	IIc		CH_3	191-193	$C_{42}H_{41}N_7O_7S_2$[b]	C, H, S	0.94	++
40	IIc		C_2H_5	214-216	$C_{30}H_{31}N_7OI_2$	C, H, I	0.97	++
41	IIc		$CH_3(CH_2)_2$	209-210	$C_{32}H_{35}N_7OI_2$	C, H, I	1.01	+
42	IId			298-299	$C_{24}H_{19}N_3O_2$	C, H, N		
43	IId		CH_3	289-290	$C_{25}H_{22}N_3O_2Br$	C, H, N, Br		
44	IIe		CH_3	354-355	$C_{26}H_{27}N_7OBr_2$	C, H, N, Br	0.89	+
45	IId	6-NO_2		243-244	$C_{24}H_{18}N_4O_4$	C, H, N		
46	IId	6-NO_2	CH_3	175-177	$C_{32}H_{28}N_4O_7S$[b]	C, H, N, S		
47	IId	6-NH_2	CH_3	205-207	$C_{25}H_{23}N_4O_2Br$	C, H, N, Br		
48	IIe	6-NH_2	CH_3	245-247	$C_{26}H_{28}N_8OBr_2$	C, H, N; Br[h]	0.94	++
49	IId	7-NO_2		302-303	$C_{24}H_{18}N_4O_4$	C, H, N		
50	IId	7-NO_2	CH_3	225-226	$C_{32}H_{27}N_4SO_7$[b]·H_2O	C, H, N, S		
51	IIe	7-NO_2	CH_3	284 dec	$C_{26}H_{26}N_8O_3Cl_2$·H_2O	C, H, N, Cl	0.84	++
52	IIe	7-NH_2	CH_3	236-238	$C_{40}H_{42}N_8S_2O_7$[b]·H_2O	C, H, N, S	0.81	++
53	IIf			319-320	$C_{27}H_{21}N_5O$	C, H, N		
54	IIf		CH_3	196-197	$C_{43}H_{41}N_5O_7S_2$[b]	C, H, S	0.95	++
55	IIf		C_2H_5	223-225	$C_{31}H_{31}N_5OI_2$	C, H, I	0.99	++
56	IIf	6-NO_2		308-310	$C_{27}H_{20}N_6O_3$	C, H, N		
57	IIf	6-NO_2	CH_3	194-196	$C_{43}H_{40}N_6O_9S_2$[b]	C, H, S	0.88	–
58	IIf	6-NO_2	C_2H_5	240-242	$C_{31}H_{30}N_6O_3Br_2$	C, H, Br	0.97	–
59	IIf	6-NH_2	CH_3	180-182	$C_{43}H_{42}N_6O_7S_2$[b]	C, H, S	0.84	++
60	IIf	6-NH_2	C_2H_5	220-222	$C_{31}H_{32}N_6OI_2$	C, H, I	0.98	++
61	IIf	7-NO_2		>360	$C_{27}H_{20}N_6O_3$	C, H, N		
62	IIf	7-NO_2	CH_3	183-185	$C_{43}H_{40}N_6O_9S_2$[b]	C, H, N, S	0.86	++
63	IIf	7-NH_2	CH_3	182-184	$C_{43}H_{42}N_6O_7S_2$[b]	C, H, N, S	0.82	++
64	IIf	7-$NHCOCH_3$		202-204	$C_{43}H_{40}N_6O_8S_2$[b]	C, H, N		
65	IIf	7-$NHCOCH_3$	CH_3	186-188	$C_{45}H_{44}N_6O_8S_2$[b]	C, H, N, S	0.85	++
66	IIg	7-NO_2		302-303	$C_{24}H_{18}N_4O_4$	C, H, N		
67	IIg	7-NO_2	CH_3	299 dec	$C_{32}H_{28}N_4O_7S$[b]	C, H, N, S		
68	IIg	7-NH_2	CH_3	315-316	$C_{25}H_{23}N_4O_2Br$	C, H, N, Br		
69	IIh	7-NH_2	CH_3	234-237	$C_{40}H_{42}N_8S_2O_7$·H_2O	C, H, N, S	0.80	++
70	IIg	6-NO_2		250-251	$C_{24}H_{18}N_4O_4$	C, H, N		
71	IIg	6-NO_2	CH_3	309-310	$C_{32}H_{28}N_4O_7S$[b]	C, H, N, S		
72	IIg	6-NH_2	CH_3	285-286	$C_{32}H_{30}N_4O_5S$[b]	C, H, N, S		
73	IIh	6-NH_2	CH_3	250-252	$C_{40}H_{42}N_8O_7S_2$[b]·0.5H_2O	C, H, S	0.89	++
74	IIi	6-NO_2		340-341	$C_{28}H_{21}N_5O_2$	C, H, N		
75	IIi	6-NO_2	CH_3	165-166	$C_{44}H_{41}N_5O_8S_2$[b]·H_2O	C, H, S	1.01	–
76	IIi	6-NH_2	CH_3	239-241	$C_{30}H_{29}N_5Br_2$	C, H, Br	0.99	++
77	IIj	6-NO_2		284-286	$C_{27}H_{21}N_7O_3$	C, H, N		
78	IIj	6-NO_2	CH_3	187-189	$C_{43}H_{41}N_7O_9S_2$[b]	C, H, N, S	0.93	–

Table I (Continued)

No.	Type	Quinoline substituents	R′ = R″ =	Mp, °C	Formula	Analyses[d]	R_f[e]	L1210[f]
79	IIj	6-NH_2	CH_3	194–196	$C_{43}H_{43}N_7O_7S_2$[b]	C, H, N, S	0.91	+
80	IIk	6-NO_2		255–256	$C_{24}H_{19}N_5O_4$	C, H, N		
81	IIk	6-NO_2	CH_3	257–258	$C_{32}H_{29}N_5O_7S$[b]	C, H, S		
82	IIk	6-NH_2	CH_3	253–255	$C_{32}H_{31}N_5O_5S$[b]	C, H, S		
83	III	6-NH_2	CH_3	257–259	$C_{26}H_{29}N_9OBr_2 \cdot H_2O$	C, H, N, Br	0.94	++
84	IIm			348–349	$C_{29}H_{23}N_5O$	C, H, N		
85	IIm		CH_3	233–235	$C_{31}H_{29}N_5OI_2$	C, H, N, I	0.94	++
86	IIm	6-NO_2		325–326	$C_{29}H_{22}N_6O_3$	C, H, N		
87	IIm	6-NO_2	CH_3	232–233	$C_{45}H_{42}N_6O_9S_2$[b] $\cdot 0.5H_2O$	C, H, N, S		
88	IIm	6-NH_2	CH_3	180–182	$C_{45}H_{44}N_6O_7S_2$[b]	C, H, N, S	0.91	++
89	IIn			>360	$C_{31}H_{23}N_5O$	C, H, N		
90	IIn		CH_3	310–312	$C_{47}H_{43}N_5S_2O_7$[b] $\cdot H_2O$	C, H, N, S	1.01	–

[a]Free base. [b]Anion *p*-toluenesulfonate. [c]Perchlorate salt. [d]Analyses for the indicated elements were within ±0.4% of the calculated figures. [e]R_f relative to dimidium; see ref 4. [f]L1210 results in our standard test. Increase in life span 25–50%, ±; 50–100%, +; >100%, ++. [g]4-Quinazolyl in place of 4-quinolyl. [h]Br: calcd, 26.0; found, 25.4.

Table II. L1210 Screening Results

Drug	Optimum dose,[a] mg/kg/day	Wt change[b]	*T/C*, %	Dose, ILS 40%[c]	C.I.[d]	100-Day survivors[e]
4	40	−0.3	159	18	2.2	
6	40	−0.7	183	14	2.8	
8	50	−1.2	129			
12	10	−0.4	242	3.4	2.9	
14	60	−1.8	197	19	3.2	
16	50	−0.7	275	12	4.8	
18	5	−2.2	241	1.3	4.7	
24	27	−2.3	204	9.0	3.0	
26	3.3	+1.4	273	0.48	6.9	2
27	6.7	+0.3	268	0.67	6.7	2
28	8.9	−1.3	218	2.6	3.4	
29	10	−2.1	228	1.9	5.3	
35	10	−0.3	206	2.7	3.7	
36	6.7	+1.3	174	2.5	2.7	
39	15	+2.8	216	4.4	3.4	
40	20	+1.0	204	9.0	2.2	
41	27	−0.7	161	15	1.8	
44	33	−0.4	182	13	2.5	
48	22	+0.9	286	3.0	7.3	4
51	40	−1.7	232	12	3.3	
52	17	−0.8	208	4.2	4.1	
54	7.5	+0.5	227	2.2	3.4	-
55	6.7	−1.2	217	2.0	3.4	
59	8.9	+0.6	280	1.6	5.6	2
60	8.0	−1.0	272	1.7	4.7	
62	24	−0.3	218	8.0	3.0	
63	27	−1.2	298	4.0	6.7	2
65	100	−2.4	272	18	5.5	
69	180	−1.4	282	27	6.7	4
73	120	−2.1	247	28	4.3	2
76	11	−1.9	243	2.2	5.0	
79	5.0	−2.4	142			
83	180	−2.1	252	53	3.4	
85	22	−1.9	265	6.7	3.3	
88	20	−1.8	228	2.9	6.9	2

[a]Taken from a plot of *T/C vs.* log dose, the dose which provides maximum life extension. Tests as detailed in the Experimental Section. [b]Difference between average animal weights day 8 − day 1 at optimum dose, grams. [c]That dose providing increase in life span of 40% taken from the plot of *T/C vs.* log dose. [d]Chemotherapeutic index; ratio of optimum dose to dose providing ILS 40%. [e]At optimum dose for a group of six animals.

carbamoyl)acetophenone: colorless prisms (EtOH–H_2O), mp >360°. *Anal.* ($C_{15}H_{14}N_2O_2$) C, H, N. 4-[*p*-(*p*-Nitrophenylcarbamoyl)anilino]-pyridine: colorless prisms (DMF–H_2O), mp 294–295°. *Anal.* ($C_{18}H_{14}N_4O_3$) C, H, N. 4-[*p*-(*p*-Aminophenylcarbamoyl)anilino]-pyridine: colorless needles (EtOH–H_2O), mp 257–258°. *Anal.* ($C_{18}H_{16}N_4O$) C, H, N. *p*-(*p*-Nitrophenylcarbamido)acetophenone: yellow prisms (DMF–H_2O), mp 264–266°. *Anal.* ($C_{15}H_{13}N_3O_4$) C, H, N. *p*-(*p*-Aminophenylcarbamido)acetophenone: colorless needles (DMF–H_2O), mp >360°. *Anal.* ($C_{15}H_{15}N_3O_2$) C, H, N. 4-(4-Pyridylamino)-4′-nitrostilbene: red prisms (DMF–H_2O), mp 304–305°. *Anal.* ($C_{19}H_{15}N_3O_2$) C, H, N. 4-(4-Pyridylamino)-4′-aminostilbene: yellow needles (EtOH), mp 255–257°. *Anal.* ($C_{19}H_{17}N_3$) C, H, N. 4-[*p*-(*p*-Nitrocinnamido)anilino]pyridine: yellow needles (DMF–H_2O), mp 285–286°. *Anal.* ($C_{20}H_{16}N_4O_3$) C, H, N. 4-[*p*-(*p*-Aminocinnamido)anilino]pyridine: colorless needles (EtOH–H_2O), mp 267–268°. *Anal.* ($C_{20}H_{18}N_4O$) C, H, N.

1-Ethyl-1,4-dihydro-6-nitroquinol-4-one (IIIb). A mixture of 6-nitro-4-hydroxyquinoline (0.53 *M*), CaO (powder, 0.53 *M*), and KI (2 g) was suspended in DMF (400 ml) and heated on the steam bath for 20 min. After cooling to 10° Et_2SO_4 (0.53 *M*) was added portionwise to the red solution. The mixture was heated until the red color disappeared, further CaO (0.53 *M*) and Et_2SO_4 (0.53 *M*) were then added as before, and heating was continued for 2 hr with occasional shaking. Water (250 ml) and concentrated HCl (150 ml) were added and the suspension was warmed until all Ca salts dissolved; crude product was precipitated with water (1 l.). Unreacted starting material was removed by digestion with 2 *N* NaOH; the remaining solid was dissolved in boiling 5 *N* HCl (400 ml), clarified,

and reprecipitated with NH_4OH. Recrystallization from AcOH–H_2O and then DMF–H_2O gave product homogenous to tlc: mp 253–254°; yield 78 g (68%). *Anal.* ($C_{11}H_{10}N_2O_3$) C, H, N.

1-Ethyl-4-chloro-6-nitroquinolinium Chloride (IV). *N*-Ethylquinolone IIb (0.15 *M*) was suspended in $SOCl_2$ (2 *M*) and DMF (0.2 ml) was added; the suspension was heated under reflux for 0.5 hr, a solution resulting. Further DMF (0.2 ml) was added and refluxing continued for 0.5 hr more. The solution was concentrated *in vacuo;* on cooling product crystallized. Sample acid-catalyzed couplings with simple aromatic amines gave high yields of the 6-nitro-4-anilinoquinoline etho quaternary salts; accordingly further processing of this product was not attempted.

1-Ethyl-4-[*p*-(*p*-nitrobenzamido)anilino]pyridinium *p*-Toluenesulfonate (V, R = NO_2). A suspension of 4-[*p*-(*p*-nitrobenzamido)-anilino]pyridine (60 g, 0.18 *M*) in nitrobenzene (120 ml) and ethyl *p*-toluenesulfonate (0.36 *M*) was heated at 160° in an oil bath, until a homogeneous solution resulted, and then for 10 min longer. After cooling C_6H_6 (600 ml) and H_2O (600 ml) were added and the mixture was stirred until crystalline. NaOTs (30 g) was added and stirring continued until this salt had dissolved. After cooling to 5° the quaternary salt was collected, washed with C_6H_6, 5% NaOTs–H_2O, and C_6H_6, and dried *in vacuo*. The salt was dissolved in H_2O (12 l.) by boiling for 15 min and filtered through a preheated sinter covered with a Celite pad. NaOTs (145 g) was dissolved in the boiling filtrate and the whole solution cooled until just turbid. Seeding and slow cooling then provided highly crystalline quaternary salt. A further crystallization (EtOH–NaOTs–H_2O) gave product homogeneous to tlc: mp 256–258°; yield 81 g (87%). *Anal.* ($C_{27}H_{26}N_4O_6S$) C, H, N, S.

1-Ethyl-4-[*p*-(*p*-aminobenzamido)anilino]pyridinium Bromide (V, R = NH_2). The above nitro compound (70 g, 0.135 *M*) was dissolved in 65% EtOH–H_2O (1 l.) by boiling, the flask was removed from the heat, and, while stirring vigorously, fine Fe powder (200 g) was added portionwise, a vigorous reaction resulting. When the reaction abated 15% $FeCl_3$–EtOH (5 ml) was added and the suspension boiled and stirred for 30 min. $CaCO_3$ powder (10 g) was then added and boiling and stirring continued for a further 10 min. Iron oxides were removed and the filtrate was concentrated *in vacuo* until product started to separate as an oil. The mixture was heated until a clear solution resulted. To this solution $CaCO_3$ powder (0.5 g) was added followed by solid NaBr portionwise until product started to crystallize. The crystal cake from the cold solution was suspended in H_2O (3 l.), EtOH (500 ml), and NH_4OH (10 ml), and the whole mixture was boiled until solution resulted. After clarifying NaBr (350 g) was dissolved at the boil and the solution cooled slowly. The crystals obtained were dissolved in boiling H_2O (3 l.) and a trace of insoluble material removed. EtOH (500 ml) was added to the filtrate which was then boiled and NH_4Br (100 g) and NaBr (250 g) dissolved in the solution. Slow cooling provided highly crystalline product which proved homogenous to tlc: mp 316–317°; yield 48 g (86%). *Anal.* ($C_{20}H_{21}N_4OBr$) C, H, N, Br.

20. A solution of the above quaternary bromide (51.6 g, 0.125 *M*) in 65% EtOH–H_2O (2.5 l.) plus HAc (18 ml) was prepared and maintained at 50° while stirring. To the 4-chloroquinolinium salt IV (excess of 0.15 *M* IIIb) was added 65% EtOH–H_2O (1 l.) which had been chilled to −15°. This suspension was stirred vigorously while allowing to warm to room temperature; as soon as all the chloro compound had dissolved the solution was added to the solution of the amine component. The mixture was heated to reflux for 30 min; then NaBr (150 g) was added and ethanol distilled off until crystallization started. The crystals were washed with ice-cold 10% NaBr–H_2O. After dissolution in boiling water (1.5 l.) the solution was clarified, AcOH (to 1 *N*) added, followed by NaBr (120 g). On cooling the quaternary salt separated as orange-red needles. One further crystallization (EtOH–H_2O–NaBr) gave product homogenous to tlc: mp 256° dec; yield 72 g (83%). *Anal.* ($C_{31}H_{30}N_6O_3Br_2$) C, H, N, Br.

27. A sample of the nitro quaternary salt 20 (50 g, 0.072 *M*) was reduced with Fe in 65% EtOH–H_2O (1 l.) as described above. The final clarified solution of product was concentrated as far as possible *in vacuo*. The resulting crude yellow gel was dissolved in boiling 60% EtOH (0.5 l.), then NH_4Br (60 g) was dissolved in the hot solution; boiling absolute EtOH (750 ml) was added and the solution cooled and seeded. Product separated slowly as a mixture of orange anhydrous and yellow hydrated forms. The crystals were dissolved in 90% MeOH (600 ml) by boiling; to the hot solution was added boiling EtOH (600 ml) and the clarified solution distilled until crystallization commenced (*ca.* 300 ml removed). Product separated as orange needles of the anhydrous form. A further crystallization gave 38 g (78%) of product homogenous to tlc, mp 333° dec. *Anal.* ($C_{31}H_{32}N_6OBr_2$) C, H, N, Br.

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_1 hybrids on day 1; drug treatment was initiated 24 hr later and was continued for 5 days. Doses were arranged at 0.18 log dose intervals. 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). Average survivals were calculated in the usual way. Doses have been rounded off to two significant figures.

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References

(1) B. F. Cain, G. J. Atwell, and R. N. Seelye, *J. Med. Chem.*, 12, 199 (1969).
(2) G. J. Atwell, B. F. Cain, and R. N. Seelye, *ibid.*, 11, 300 (1968).
(3) B. F. Cain, G. J. Atwell, and R. N. Seelye, *ibid.*, 11, 963 (1968).
(4) G. J. Atwell and B. F. Cain, *ibid.*, 10, 706 (1967).
(5) G. J. Atwell, B. F. Cain, and R. N. Seelye, *ibid.*, 15, 611 (1972).
(6) G. J. Atwell, B. F. Cain, and R. N. Seelye, *ibid.*, 11, 690 (1968).
(7) G. J. Atwell and B. F. Cain, *ibid.*, 11, 295 (1968).
(8) G. J. Atwell and B. F. Cain, *ibid.*, 10, 706 (1967).
(9) H. E. Skipper, F. M. Schabel, Jr., and W. S. Wilcox, *Cancer Chemother. Rep.*, 35, 54 (1964).
(10) "Some Chemical Structures of Current Interest to the Chemotherapy Program," Drug Development Branch, Chemotherapy, NCI, Sept 1971.