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Potential Antitumor Agents. 15. Bisquaternary Salts

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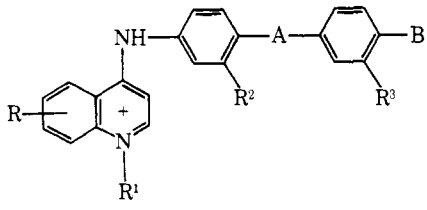
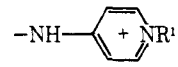
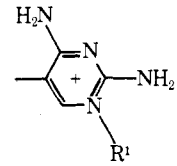
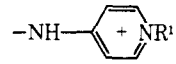
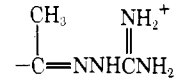
A series of 37 amine-substituted variants of bisquaternary salts of 4-[*p*-[*p*-(4-pyridylamino)phenylcarbamoyl]anilino]quinoline was prepared and evaluated against the L1210 system. Lipophilic–hydrophilic balance of the agents was adjusted with the *N*-alkyl quaternary function. The variants differed in (a) the terminal basic function, the N^1-alkyl-4-pyridylamino component being interchanged with either N^1-alkyl-5-(2,4-diaminopyrimidinyl) or amidinohydrazone; (b) the number and (c) the positioning of the primary amine substituents; (d) reversal of the central amide bond; and (e) the *N*-alkyl quaternary function. Six of the variants prepared provided 100-day survivors when administered intraperitoneally to mice bearing either intraperitoneally or subcutaneously implanted L1210 cells (10^5).

We have demonstrated that our earlier prepared symmetrical bisquaternary salts [*e.g.*, 3,3′-[terephthaloylbis(imino-*p*-phenylenecarbonylimino)] bis[1-ethylpyridinium *p*-toluenesulfonate]] showed marked chronic toxicity, any L1210 active dose proving lethal.[1] However, certain primary amine substituted variants of these agents could produce numbers of indefinite survivors in early intraperitoneal L1210 tests.[2] The role of the amine substituents in these latter variants could be to either attenuate chronic toxicity or increase drug selectivity toward the leukemic population. Our later demonstration that asymmetric bisquaternary salts of generic formula I were much less chronically toxic[1] then raised the possibility that amine substitution might further increase the antileukemic activity of this generic type. We have already shown that such agents bearing a 6- or 7-amino group on the quinoline ring will furnish a percentage of 100-day survivors in early intraperitoneal L1210 tests.[1] The present communication details the preparation and antileukemic (L1210) activity of isomeric mono- and diamine substituted variants of I.

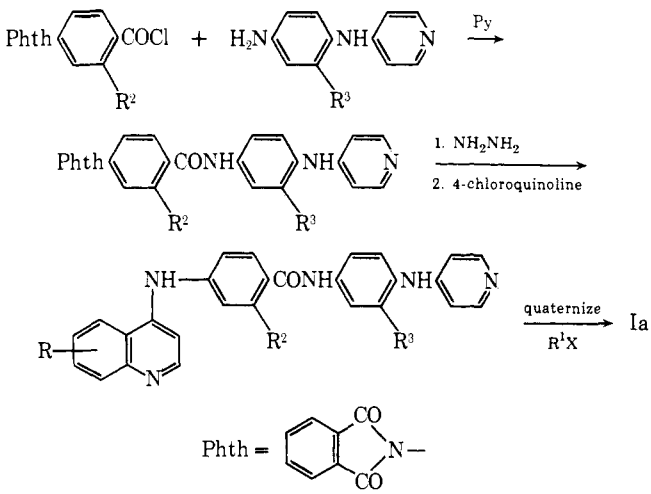
Chemistry. The general synthetic method is detailed in Scheme I. Nitro groups were used as precursors to the required amine substituents, the nitro functions being ulti-



	A	B
Ia	–CONH–	
Ib	–CONH–	
Ic	–NHCO–	
Id	–CONH–	$-COCH_3$
Ie	–CONH–	

mately reduced with Fe–H^+.[3] The same general route (Scheme I) with minor modifications served to prepare generic types Ia–e; the modifications necessary are clear from the intermediates quoted in the Experimental Section. 4-Anilinopyridines were prepared by a modification of the method of Jerchel and Jakob,[4] the *p*-toluenesulfonate salt of the requisite aniline being treated with *N*-pyridyl-4-pyridinium chloride hydrochloride.[5]

Scheme I



Biological Activity. A selection of the nitro-substituted bisquaternary salts, prepared as intermediates to the desired amino compounds, was screened (**3, 10–12, 17, 24, 31,** Table I)† but these were either inactive or much less active than the corresponding amines.

Variants of type Ia with a primary amino group ortho to the 4-pyridylamino function ($R^3 = NH_2$ in Ia) were less active than the corresponding unsubstituted compounds (**13–15, 27–29,** Table I).† In contrast, a congener with an amine function in the adjacent aromatic ring ($R^2 = NH_2$ in Ia) appeared to have slightly enhanced activity (**6**). Since the powerfully hydrophilic amino group could have been expected to depress log *P*, possibly below the optimum figure, the homologous series of quaternary salts **6–8** was prepared and screened. As in the unsubstituted parent series[6] the dimethyl quaternary salt **6** ($R^1 = CH_3$ in Ia) proved the most active. We have shown, in the antileukemic bisquaternary salts, that dramatic changes in biological activity result from changes in log *P* and have used partition chromatography as a convenient measure of this factor.[6,7] The addition of an amino group ortho to the central amide bond ($R^2 = NH_2$ in Ia) produces minor changes in R_f values suggesting that an NH_2 in such a position does not have a large negative π value. Anomalous π values have been noted when interaction between adjacent polar substituents is possible.[8]

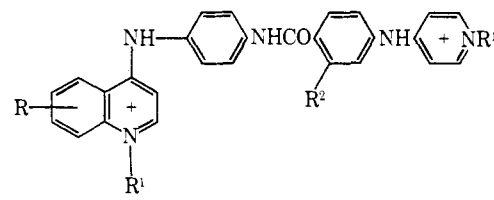
We have shown that two separate amine substituents independently augment antileukemic activity, one as a 6-aminobenzamide component ($R^2 = NH_2$ in Ia) and the other as a 6-aminoquinoline unit.[1] Combination of these two augmenting substituents in one molecule (**20, 21**) furnishes remarkable active experimental antileukemic agents which can provide high percentages of indefinite survivors in early dosed intraperitoneal L1210 tests.†

It has become our practice further to discriminate congeners by a more rigorous test utilizing intraperitoneal inoculation of drug to animals bearing remote, subcutaneously implanted L1210. Such testing often discloses differences in activity which are not readily apparent from the results of screening when drugs are administered intraperitoneally to mice bearing intraperitoneally implanted tumor. Even in the considerably more difficult subcutaneous L1210 test system the diamino variants **20** and **21** are still capable of producing a percentage of 100-day survivors.†

In earlier work[1] 7-aminoquinoline congeners appeared to have the same order of activity as the 6-amino isomers. There was no significant change in biological activity when the 6-aminoquinoline moiety of the highly active diaminoquaternary salts **20** and **21** was replaced by a 7-aminoquinoline component (**45, 46**).†

As shown earlier, the pyridine function in L1210 active bisquaternary salts could be acceptably replaced by alternative cationic functions.[1-3,5-7] However, replacement of the pyridine function, in the diamino variant **20**, by amidinohydrazone (type Ie, **62–64**) or 2,4-diaminopyrimidine (type Ib, **50, 51**) provided less active agents; the distinctions in activity were most readily made by testing against subcutaneous L1210.†

We have shown that the amide function in the parent system can be reversed (*cf.* types Ia and Ic) and full biological activity retained. As demonstrated, addition of an amino group to the benzamide component of the parent structure as in **6** augments activity; in the reversed amide series placement of this substituent in an equivalent position in relation to the pyridine and quinoline rings causes a marked drop in activity (**54,** Table I).† If in the reversed amide series an amino group is appended to the benzamide ring, ortho to the amide function, a highly active material results (**67,** type II). This latter variant (**67**) corresponds to the highly active diamino bisquaternary salt **20** with the bridge unit linking the two cationic functions completely reversed. There were no significant differences between life extensions recorded when the isomers **20** and



II

†See paragraph at end of paper regarding supplementary material.

Table I

No.	Type	Quinoline substituents	R^1	R^2	R^3	Mp, °C	Formula	Analyses[b]	R_d[c]	L1210[d]
2	Ia		*a*	NO_2	H	181–182	$C_{27}H_{20}N_6O_3$	C, H, N		
3	Ia		CH_3	NO_2	H	233–235	$C_{29}H_{26}N_6O_3Br_2$	C, H, Br	1.00	−
4	Ia		C_2H_5	NO_2	H	222–224	$C_{31}H_{30}N_6O_3Br_2$	C, H, Br	1.04	
5	Ia		$CH_3(CH_2)_2$	NO_2	H	216–218	$C_{33}H_{34}N_6O_3Br_2 \cdot 0.5H_2O$	C, H, Br	1.07	
6	Ia		CH_3	NH_2	H	221–222	$C_{29}H_{28}N_6OI_2$	C, H, I	0.98	++
7	Ia		C_2H_5	NH_2	H	220–221	$C_{31}H_{22}N_6OI_2$	C, H, I	1.01	+
8	Ia		$CH_3(CH_2)_2$	NH_2	H	215–216	$C_{33}H_{36}N_6OI_2$	C, H, I	1.03	±
9	Ia			H	NO_2	300–301	$C_{27}H_{20}N_6O_3$	C, H, N		
10	Ia		CH_3	H	NO_2	236–238	$C_{29}H_{26}N_6O_3Br_2 \cdot 0.5H_2O$	C, H, Br	0.99	±
11	Ia		C_2H_5	H	NO_2	231–233	$C_{31}H_{30}N_6O_3Br_2$	C, H, Br	1.03	−
12	Ia		$(CH_3(CH_2)_2$	H	NO_2	212–214	$C_{33}H_{34}N_6O_3Br_2$	C, H, Br	1.06	−
13	Ia		CH_3	H	NH_2	233–235	$C_{29}H_{28}N_6OI_2 \cdot 0.5H_2O$	C, H, I	0.92	+
14	Ia		C_2H_5	H	NH_2	211–213	$C_{31}H_{32}N_6OI_2$	C, H, I	0.97	±
15	Ia		$CH_3(CH_2)_2$	H	NH_2	203–205	$C_{33}H_{36}N_6OI_2$	C, H, I	0.99	−
16	Ia	6-NO_2		NO_2	H	261–262	$C_{27}H_{19}N_7O_5$	C, H, N		
17	Ia	6-NO_2	CH_3	NO_2	H	301–302	$C_{29}H_{25}N_7O_5I_2$	C, H, I	1.04	−
18	Ia	6-NO_2	C_2H_5	NO_2	H	234–235	$C_{31}H_{29}N_7O_5I_2$	C, H; I[f]	1.07	
19	Ia	6-NO_2	$CH_3(CH_2)_2$	NO_2	H	240–242	$C_{33}H_{33}N_7O_5Br_2 \cdot H_2O$	C, H, Br	1.11	
20	Ia	6-NH_2	CH_3	NH_2	H	229–230	$C_{29}H_{29}N_7OI_2$	C, H, I	0.90	++
21	Ia	6-NH_2	C_2H_5	NH_2	H	220–222	$C_{31}H_{33}N_7OI_2 \cdot 0.5H_2O$	C, H, I	0.96	++
22	Ia	6-NH_2	$CH_3(CH_2)_2$	NH_2	H	207–209	$C_{33}H_{37}N_7OI_2 \cdot 0.5H_2O$	C, H, I	0.99	+
23	Ia	6-NO_2		H	NO_2	264–265	$C_{27}H_{19}N_7O_5$	C, H, N		
24	Ia	6-NO_2	CH_3	H	NO_2	260–261	$C_{29}H_{25}N_7O_5Br_2$	C, H, Br	1.06	−
25	Ia	6-NO_2	C_2H_5	H	NO_2	217–218	$C_{31}H_{29}N_7O_5I_2$	C, H, I	1.09	
26	Ia	6-NO_2	$CH_3(CH_2)_2$	H	NO_2	200–201	$C_{33}H_{33}N_7O_5I_2$	C, H, I	1.12	
27	Ia	6-NH_2	CH_3	H	NH_2	233–236	$C_{29}H_{29}N_7OI_2$	C, H, I	0.90	++
28	Ia	6-NH_2	C_2H_5	H	NH_2	221–223	$C_{31}H_{33}N_7OI_2$	C, H, I	0.94	+
29	Ia	6-NH_2	$CH_3(CH_2)_2$	H	NH_2	215–217	$C_{33}H_{37}N_7OI_2$	C, H, I	0.99	+
30	Ia			NO_2	NO_2	260–261	$C_{27}H_{19}N_7O_5$	C, H, N		
31	Ia		CH_3	NO_2	NO_2	188–190	$C_{29}H_{25}N_7O_5I_2$	C, H, I	0.09	−
32	Ia		C_2H_5	NO_2	NO_2	165–166	$C_{31}H_{29}N_7O_5I_2$	C, H, I	1.03	
33	Ia		$CH_3(CH_2)_2$	NO_2	NO_2	159–161	$C_{33}H_{33}N_7O_5I_2$	C, H, I	1.06	
34	Ia		CH_3	NH_2	NH_2	219–221	$C_{29}H_{29}N_7OI_2$	C, H, I	0.93	±
35	Ia		C_2H_5	NH_2	NH_2	262–263	$C_{31}H_{33}N_7OI_2$	C, H, I	0.96	−
36	Ia		$CH_3(CH_2)_2$	NH_2	NH_2	233–234	$C_{33}H_{37}N_7OI_2$	C, H, I	1.01	−
37	Ia	2-CH_3, 6-NO_2		NO_2	H	288–289	$C_{28}H_{21}N_7O_5$	C, H, N		
38	Ia	2-CH_3, 6-NO_2	CH_3	NO_2	H	247–249	$C_{30}H_{27}N_7O_5Br_2$	C, H, Br	1.05	
39	Ia	2-CH_3, 6-NO_2	C_2H_5	NO_2	H	266–269	$C_{32}H_{31}N_7O_5Br_2$	C, H, Br	1.08	
40	Ia	2-CH_3, 6-NH_2	CH_3	NH_2	H	237–239	$C_{30}H_{31}N_7OI_2 \cdot H_2O$	C, H, I	0.93	++
41	Ia	2-CH_3, 6-NH_2	C_2H_5	NH_2	H	236–237	$C_{32}H_{35}N_7OI_2$	C, H, I	0.97	+
42	Ia	7-NO_2		NO_2	H	298–299	$C_{27}H_{19}N_7O_5$	C, H, N		
43	Ia	7-NO_2	CH_3	NO_2	H	267–268	$C_{29}H_{25}N_7O_5Br_2$	C, H, N, Br	1.06	±
44	Ia	7-NO_2	C_2H_5	NO_2	H	215–217	$C_{31}H_{29}N_7O_5Br_2$	C, H, N	1.09	−
45	Ia	7-NH_2	CH_3	NH_2	H	255–257	$C_{29}H_{29}N_7OBr_2$	C, H, N, Br	0.90	++
46	Ia	7-NH_2	C_2H_5	NH_2	H	248–250	$C_{31}H_{33}N_7OBr_2$	C, H, N, Br	0.96	++
47	Ib	6-NO_2		NO_2	H	345–346	$C_{26}H_{19}N_9O_5$	C, H, N		
48	Ib	6-NO_2	CH_3	NO_2	H	202–204	$C_{42}H_{39}N_9O_{11}S_2$[e]	C, H, S	1.05	
49	Ib	6-NO_2	C_2H_5	NO_2	H	203–204	$C_{44}H_{43}N_9H_{11}S_2$[e]	C, H, S	1.09	

50	Ib	6-NH_2	CH_3	NH_2	H	202–204	$C_{42}H_{43}N_9O_7S_2$[e]	C, H, S	0.89	++
51	Ib	6-NH_2	C_2H_5	NH_2	H	194–195	$C_{44}H_{47}N_9O_7S_2 \cdot 0.5H_2O$[e]	C, H, S	0.93	++
52	Ic			NO_2	H	>360	$C_{27}H_{30}N_6O_3$	C, H, N		
53	Ic		CH_3	NO_2	H	151–152	$C_{43}H_{40}N_6O_9S_2$[e]	C, H, N, S	1.00	+
54	Ic		CH_3	NH_2	H	241–242	$C_{29}H_{28}N_6OBr_2$	C, H, N, Br	0.93	+
55	Id	6-NO_2		NO_2	H	261–263	$C_{24}H_{17}N_5O_6$	C, H, N		
56	Id	6-NO_2	CH_3	NO_2	H	320–321	$C_{32}H_{21}N_5O_9S$[e]	C, H, N, S		
57	Id	6-NO_2	C_2H_5	NO_2	H	180–183	$C_{33}H_{29}N_5O_9S$[e]	C, H, N, S		
58	Id	6-NO_2	$CH_3(CH_2)_2$	NO_2	H	179–181	$C_{34}H_{31}N_5SO_9$	C, H, N, S		
59	Id	6-NH_2	CH_3	NH_2	H	171–173	$C_{32}H_{31}N_5O_5S$[e]	C, H, N, S		
60	Id	6-NH_2	C_2H_5	NH_2	H	187–189	$C_{33}H_{33}N_5O_5S$[e]	C, H, N, S		
61	Id	6-NH_2	$CH_3(CH_2)_2$	NH_2	H	165–167	$C_{34}H_{35}N_5O_5S$[e]	C, H, N, S		
62	Ie	6-NH_2	CH_3	NH_2	H	141–144	$C_{26}H_{29}N_9OBr_2 \cdot 0.5H_2O$	C, H, N, Br	0.92	+++
63	Ie	6-NH_2	C_2H_5	NH_2	H	121–124	$C_{27}H_{31}N_9OBr_2 \cdot H_2O$	C, H, N; Br[g]	0.97	+++
64	Ie	6-NH_2	$CH_3(CH_2)_2$	NH_2	H	102–107	$C_{42}H_{47}N_9O_7S_2 \cdot 1.5H_2O$[e]	C, H, N, S	1.02	++
65	II	6-NO_2		NO_2		334–335	$C_{27}H_{19}N_7O_5$	C, H, N		
66	II	6-NO_2	CH_3	NO_2		184–185	$C_{43}H_{39}N_7O_9S_2$[e]	C, H, N, S	1.03	
67	II	6-NH_2	CH_3	NH_2		253–254	$C_{29}H_{29}N_7OBr_2$	C, H, N, Br	0.91	++
68	II	7-NO_2		NO_2		321–322	$C_{27}H_{19}N_7O_5$	C, H, N		
69	II	7-NO_2	CH_3	NO_2		259–260	$C_{29}H_{25}N_7O_5Br_2$	C, H, N	1.04	++
70	II	7-NH_2	CH_3	NH_2		324–325	$C_{43}H_{43}N_7O_7S_2$[e]	C, H, N, S	0.93	++

[a] Free base. [b] Analyses for the indicated elements were within ±0.4% of the theoretical figures. [c] R_f value relative to dimidium; see ref 5. [d] L1210 results according to our test system: increase in life span 25–50%, ±; 50–100%, +; >100%, ++. [e] Anion *p*-toluenesulfonate. [f] Calcd, 30.5; found, 29.9. [g] Br: calcd, 23.7; found, 23.1.

67 were screened against either intraperitoneally or subcutaneously implanted L1210.† Variant **20** as NSC114347 is listed by Department of Research and Development of the National Cancer Institute as a Decision Network 2 compound.

Experimental Section

Melting points were determined in open capillaries on an Electro-thermal melting point apparatus with the makers stem corrected thermometer and are as read. A 2°/min heating rate from 20° below the melting point was used. Analyses were by Dr. A. D. Campbell, Microchemical Laboratory, University of Otago, Dunedin, New Zealand. The symbol for the requisite element has been used to signify that analytical results were within ±0.4% of the calculated values.

Experience showed that the quaternary salts listed in the tables were best prepared from the six-membered heterocyclic terminus. From the requisite heterocycle a further ring was added by a variety of devices; thus, *p*-aminobenzamido compounds were prepared by Fe reduction[4] of the corresponding nitrobenzamides which were in turn produced by acylation of the amine with *p*-nitrobenzoyl chloride in pyridine or from the corresponding acid *via* the phosphorazo method.[3] If a complicating nitro function was present use of a *p*-phthalimidobenzoyl chloride followed by hydrazinolysis in pyridine solution preserved the nitro function intact. This method served to generate 2-nitro-4-aminobenzamides *via* the phthalimido derivative previously described.[3] The coupled phthalimido derivatives were invariably extremely insoluble, high-melting materials and could only be recrystallized from large volumes of high boiling solvents [*N*-methyl-2-pyrrolidone (NMePy), $C_6H_6NO_2$].

The preparation of 4-anilinopyridines has been adequately described;[3] when an acetamido aniline is used in this reaction less cleavage of the acetylamino function takes place if NMePy is used as solvent in place of phenol.[3]

The quinolyl function was attached by the usual acid-catalyzed reaction of the substituted 4-chloroquinoline with the requisite aromatic amine. The coupling was usually carried out in EtOH–H_2O (2:1), sufficient HCl being added to account for the basic centers present and to provide 1 equiv excess; reaction time of 30 min at reflux was ample in all cases examined.

The extended heterocyclic bis bases listed in Table I were extremely insoluble and were best crystallized from Py, DMF, or NMePy with or without addition of MeOH.

Details of quaternization methods, paper chromatography, tlc, etc., have been described.[1,5] Many bis bases quoted in this paper are difficult to completely quaternize. In general, preparation of Me quaternary salts presents no great difficulties, MeOTs–$C_6H_6NO_2$ at 160° (internal) *ca.* 10 min being adequate for complete reaction. Higher homologs (Et, *n*-Pr) invariably present difficulties; less troubles are encountered if small batches (<2 g) are quaternized. The internal temperature should not exceed 165°, otherwise the quaternary salt produced tends to eliminate olefin. Completion of reaction with the higher alkyl homologs is best judged by sampling and following the reaction using paper chromatography or tlc.

With the 6- and 7-nitroquinoline quaternary salts prepared it was found that purification could be considerably simplified if the crude product obtained from the quaternization was extracted to completion with large volumes of boiling water; precipitation from the clarified aqueous solutions with a salt of the appropriate anion provided more easily purifiable quaternary salt.

It cannot be too strongly emphasized that with these normally extensively hydrated quaternary salts, which are extremely hygroscopic when thoroughly dried, melting points do not serve as a reliable guide to purity. Paper chromatography and tlc provide a much superior gauge of homogeneity.[1,5]

In coupling aminoguanidine bicarbonate with the substituted acetophenones listed in Table I it was found that the best yields of guanylhydrazones were obtained if the concentration of mineral acid in the reaction was adjusted to 2 *N*.

Protecting acetylamino functions were removed by heating a solution in 2 *N* HCl–EtOH under reflux conditions for 45 min.

Nitroquaternary salts were reduced to the corresponding amines by Fe reduction as previously described.[4]

Many of the final products (Table I) tend to separate from solution as gels. Crystallization from small volumes of EtOH–H_2O keeping EtOH concentration high and depressing solubility by the addition of Na or the NH_4 salt of the anion required will combat gel formation. Considerable experimentation with various an-

ions and conditions is sometimes necessary to obtain satisfactory crystalline end products.

Necessary intermediates not listed in Table I or described in the literature are listed below; methods of synthesis are obvious from the preamble.

4-[p-(p-Amino-o-nitrobenzamido)anilino]pyridine: bronze plates; EtOH; mp 259–260°. *Anal.* ($C_{18}H_{15}N_5O_3$) C, H, N.

4-(p-Acetamido-o-nitroanilino)pyridine: red needles; *n*-BuOH; mp 265.5–266.5°. *Anal.* ($C_{13}H_{12}N_4O_3$) C, H, N.

4-(p-Amino-o-nitroanilino)pyridine: deep red, almost black needles; EtOH; mp 167.5–168°. *Anal.* ($C_{11}H_{10}N_4O_2$) C, H, N.

4-[p-(p-Aminobenzamido)-o-nitroanilino]pyridine: scarlet plates; DMF–H_2O; mp 244–245°. *Anal.* ($C_{18}H_{15}N_5O_3$) C, H, N.

4-[p-(p-Amino-o-nitrobenzamido)-o-nitroanilino]pyridine: orange plates; DMF–H_2O; mp 265.5–277°. *Anal.* ($C_{18}H_{14}N_6O_5$) C, H, N.

2,4-Diamino-5-[p-(p-amino-o-nitrobenzamido)phenyl]pyrimidine: yellow needles; DMF–H_2O; mp 247–249°. *Anal.* ($C_{17}H_{15}N_7O_3$) C, H, N.

p-(p-Acetamido-o-nitrophenylcarbamoyl)aniline: orange-red prisms; DMF–EtOH; mp 283.5–284°. *Anal.* ($C_{15}H_{14}N_4O_4$) C, H, N.

4-[p-(p-Amino-o-nitrophenylcarbamoyl)anilino]pyridine: red prisms; EtOH; mp 269–270°. *Anal.* ($C_{18}H_{15}N_5O_3$) C, H, N.

p-(p-Acetamido-o-nitrobenzamido)acetophenone: cream prisms; EtOH–H_2O; mp 237–238°. *Anal.* ($C_{17}H_{15}N_3O_5$) C, H, N.

p-(p-Amino-o-nitrobenzamido)acetophenone: yellow plates; EtOH–H_2O; mp 239–240°. *Anal.* ($C_{15}H_{13}N_3O_4$) C, H, N.

p-(p-Amino-o-nitrobenzamido)acetanilide: yellow prisms; DMF–EtOH–H_2O; mp 300–302°. *Anal.* ($C_{15}H_{14}N_4O_4$) C, H, N.

4-[p-(p-Acetamidophenylcarbamoyl)-m-nitroanilino]pyridine: yellow plates; EtOH; mp 298–299°. *Anal.* ($C_{20}H_{17}N_5O_4$) C, H, N.

4-[p-(p-Aminophenylcarbamoyl)-m-nitroanilino]pyridine: orange needles; EtOH–H_2O; mp 258–259°. *Anal.* ($C_{18}H_{15}N_5O_3$) C, H, N.

Biological Testing. The routine test consists 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 continued for 5 days. Average survivals were calculated in the usual way. An attempt was made to test all drugs from a level which was frankly toxic, giving either toxic deaths before control deaths or marked weight loss; serial twofold dilutions were then tested until an obviously nontoxic dose was reached; this usually required a total of three tests. Compounds which under these test conditions were not given T/C values greater than 125% have been classified as negative and this is recorded in the requisite column in Table I. On retesting positives doses have been arranged at 0.18 log dose intervals, the levels ensuring tests from toxic levels to those which give less than 40% increase in life span. All dosage has been in 0.2 ml of H_2O. Groups of six animals per dose level were used and one control group for every five tests.

In the subcutaneous tests 10^5 L1210 cells were implanted subcutaneously above the right axilla. Drugs were administered by the intraperitoneal route on days 2–6 as for tests against intraperitoneally implanted tumor.

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Supplementary Material Available. Full details of L1210 screening data will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Journals Department, American Chemical Society, 1155 16th St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JMED-74-930.

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Potential Antitumor Agents. 11. Inhibitors of Alkaline Phosphatase, an Enzyme Involved in the Resistance of Neoplastic Cells to 6-Thiopurines†

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A series of 4′-substituted derivatives of 5-hydroxy-2-formylpyridine thiosemicarbazone (5-HP) has been synthesized and evaluated as inhibitors of alkaline phosphatase partially purified from a murine ascitic cell line of Sarcoma 180 resistant to the antileukemic agents 6-mercaptopurine and 6-thioguanine. These agents were also tested as inhibitors of ribonucleoside diphosphate reductase from rat Novikoff hepatoma and for antineoplastic activity in mice bearing either 6-thiopurine-sensitive or -resistant cells of Sarcoma 180. Structure–activity relationship studies have delineated the bulk requirement for a five-membered ring at the 4′ position for optimum phosphatase-inhibitor interaction. Similar bulk produced loss of activity by α-(N)-heterocyclic carboxaldehyde thiosemicarbazones as inhibitors of ribonucleoside diphosphate reductase. Some of these agents were found to possess potent tumor-inhibitory potential.

Investigation of potential mechanisms by which leukemic cells of man acquire resistance to the antileukemic 6-thiopurines (*i.e.*, 6-mercaptopurine and 6-thioguanine) has been pursued in this laboratory in both an experimental animal model tumor system[1,2] and in leukocytes from patients with leukemia.[3] Initial studies with the animal model system (Sarcoma 180/TG ascites cells) have indicated that the mechanism of acquired insensitivity to the 6-thiopurines exhibited by this variant was not the result of impaired uptake or increased catabolism of the 6-thiopurine *per se*, nor of a decreased capacity to synthesize the inhibitory nucleotide form.[1] Since an increased rate of loss of the active analog nucleotide form occurred in resistant cells as compared with the parent subline, in-

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