soln and crystn of the residue from EtOAc–petr ether (bp 60–80°) gave **55**, mp 142–143° (3.3 g). *Anal.* ($C_{18}H_{20}O_7$) C, H.

6-(5-Carboxy-3-methylpent-2-enyl)-5-ethoxy-7-hydroxy-4-methylphthalan-1-one (56) and 6-(5-Carboxy-3-methylpent-2-enyl)-7-hydroxy-4-methyl-5-*n*-propyloxyphthalan-1-one (7).—A soln of **53** (5 g) and EtI (20 ml) in Me_2CO (125 ml) was heated under reflux in the presence of anhyd K_2CO_3 (20 g) until tlc showed that a significant amount of the desired product had been formed (36–48 hr). The reaction mixt was filtered and the solid residue acidified with 2 *N* HCl and extd with EtOAc. The washed (H_2O) and dried ($MgSO_4$) EtOAc soln was evapd and the residual solid subjected to prep tlc. Product **56** (500 mg) had mp 143–145° [from EtOAc–petr ether (bp 60–80°)]; τ ($CDCl_3$) 6.18 (q, 2 H) and 8.62 (t, 3 H) (CH_2CH_2O). *Anal.* ($C_{18}H_{22}O_6$) C, H.

Using a similar procedure **53** (3 g) was converted into **57**: mp 125–127° (from EtOAc–petr ether (bp 60–80°) (240 mg); τ ($CDCl_3$) 6.25 (t, 2 H), 8.1 (m, 2 H), and 8.91 (t, 3 H) ($CH_3CH_2CH_2O$). *Anal.* ($C_{19}H_{24}O_6$) C, H.

6-(5-Carboxy-3-methylpent-2-enyl)-5-hydroxy-7-methoxy-4-methylphthalan-1-one (60).—A soln of **55** (500 mg) in MeOH (70 ml) was allowed to stand with excess of CH_2N_2 in Et_2O for 1 hr at 5°. The Et_2O was evapd, 3 *N* NaOH (10 ml) was added, and the soln was kept at room temp for 20 min. The reaction mixt was acidified with 3 *N* HCl and extd with EtOAc. Evapn of the washed (H_2O) and dried ($MgSO_4$) EtOAc soln and crystn of the residue from EtOAc–petr ether (bp 60–80°) gave **60**, mp 156–158° (310 mg). *Anal.* ($C_{17}H_{20}O_6$) C, H.

3-(5-Carboxy-3-methylpent-2-enyl)-2-hydroxy-6-hydroxymethyl-4-methoxytoluene (62).—A soln of **60** (100 mg) in 3 *N* NaOH (30 ml) was heated under reflux under N_2 for 24 hr. After acidification of the reaction mixt with 3 *N* HCl the product was isolated in EtOAc and crystd from EtOAc–petr ether (bp 60–80°) to give **62**: mp 128–132° (55 mg); τ (Me_2CO-d_6) 3.42 (s, 1 H), 4.7 (t, 1 H), 5.5 (s, 2 H), 6.3 (s, 3 H), 6.7 (d, 2 H), 7.76 (m, 4 H), 7.92 (s, 3 H), and 8.28 (sb, 3 H). *Anal.* ($C_{16}H_{22}O_5$) C, H.

3-(5-Carboxy-3-methylpent-2-enyl)-4-hydroxy-6-hydroxymethyl-2-methoxytoluene (63).—A soln of **1** (2 g) in 3 *N* NaOH (100 ml) was heated under reflux for 18 hr during which time a fast stream of N_2 caused a reduction in vol to 20 ml and the production of a ppt. The reaction mixt was acidified with 3 *N* HCl and extd with EtOAc. The EtOAc soln was washed (H_2O), dried ($MgSO_4$), and evapd and the residue was subjected to prep tlc. Isolation of the component giving an immediate response to CrO_3–H_2SO_4 spray and crystn from EtOAc–petr ether (bp 60–80°) gave **63**: mp 126–128° (150 mg); τ 3.28 (s, 1 H, ArH) and 5.54 (s, 2 H, $ArCH_2OH$). *Anal.* ($C_{16}H_{22}O_5$) C, H.

3-(5-Carboxy-3-methylpent-2-enyl)-2,4-dihydroxy-6-hydroxymethyltoluene (64).—A soln of **53** (1 g) in 3 *N* NaOH (80 ml) was heated under reflux under N_2 for 18 hr. It was acidified with 3 *N* HCl and extd with EtOAc. Concn of the washed (H_2O) and dried ($MgSO_4$) EtOAc soln gave **64**; mp 152–154° (800 mg); τ (Me_2CO-d_6) 3.5 (s, 1 H, ArH) and 5.52 (s, 2 H, $ArCH_2OH$). *Anal.* ($C_{15}H_{20}O_5$) C, H.

2-(5-Carboxy-3-methylpent-2-enyl)-5-hydroxymethylresorcinol (65).—A soln of **61** (100 mg) in 3 *N* NaOH was heated under reflux under N_2 for 18 hr. Acidification of the reaction mixt with 3 *N* HCl, isolation of the product in EtOAc, and crystn from EtOAc–petr ether (bp 60–80°) gave **65**: mp 135–138° (46 mg); τ (Me_2CO-d_6) 3.67 (s, 2 H, ArH) and 5.64 (s, 2 H, $ArCH_2OH$). *Anal.* ($C_{14}H_{18}O_5$) C, H.

2-(5-Carboxy-3-methylpent-2-enyl)-5-hydroxymethyl-3-methoxyphenol (66).—A soln of **49**[12] (400 mg) in 3 *N* NaOH was heated under reflux under N_2 for 18 hr. After acidification with 3 *N* HCl the reaction mixt was extd with EtOAc and the ext washed (H_2O), dried ($MgSO_4$), and evapd. Prep tlc of the residue gave **59** (24 mg) and a solid which was crystd from EtOAc–petr ether (bp 60–80°) to give **66**: mp 115–118° (52 mg); τ (Me_2CO-d_6) 3.57 (s, 1 H), 3.58 (s, 1 H) (ArH), and 5.6 (s, 2 H, $ArCH_2OH$). *Anal.* ($C_{15}H_{20}O_5$) C, H.

Acknowledgments.—We thank Dr. S. B. Carter, Mrs. G. M. Evans, and Mr. H. H. Jones for permission to include biological test results. We thank Mr. D. M. Carr, Mr. J. A. Platt, and Mr. W. J. Priest for technical assistance and the Physical Methods Section for elementary analyses and spectroscopic measurements.

Potential Antitumour Agents. 11. 9-Anilinoacridines

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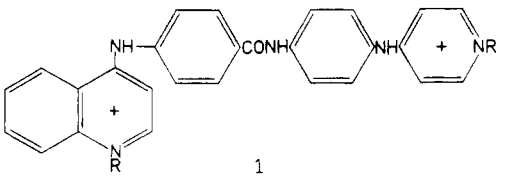
Received August 19, 1970

It is suggested that the group of coplanar, fully ionized, cationic agents which demonstrate high activity against the L1210 leukemia may lodge temporarily in the minor groove of a polynucleotide helix as a site of residence. A later intercalated mode of drug binding leads to cell death. From a consideration of binding to these sequential sites and to modes of cellular entry certain nonquaternary bis bases active against the L1210 have been prepared.

In the last paper of this series[1] we developed a view of the structure–activity relationships of a large series of cationic agents which are essentially fully ionized at physiological pH values. It was then suggested that the structural requirements for activity with these agents [for example, **1** (R = CH_3) and isometamidium **2**] could indicate a site equivalent to the minor groove in a polynucleotide duohelix. Further, such molecules might reside in this site until unwinding of the helix caused separation of the purine–pyrimidine pairs. The agents could then intercalate between adjacent base pairs with the planar aromatic nuclei contacting the purine–pyrimidine nuclei and the cationic charges matching to negative charges on the sugar–phosphate chains.

Intercalation has been well documented as regards homidium (**3**).[1] It also appears reasonable to expect derived agents such as isometamidium (**2**) to intercalate. Extension of such views to the coplanar biscationic agents grouped earlier[1] (*e.g.*, **1**) leads to the speculation that intercalation may be an important event with this group also. Combining this concept with structure–activity relationships concerned with matching of drug structure to the minor groove of a polynucleotide helix, a novel viewpoint emerges that two sets of structure–activity relationships may operate for these agents: (a) structural characteristics allowing binding in the minor groove of a polynucleotide helix as a site of residence; (b) features consonant with binding in a later available intercalation site–the actual site of action. Such views could conveniently explain the requirement for an approximate charge separation of 20 Å in molecules such as **1**[1] for high antileukemic activity. Such dimensions would allow the agent to extend through a

(1) B. F. Cain, G. J. Atwell, and R. N. Seelye, *J. Med. Chem.*, **12**, 199 (1969).



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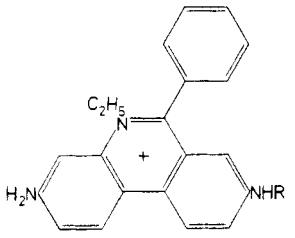
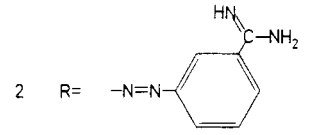




2 R= –N=N–

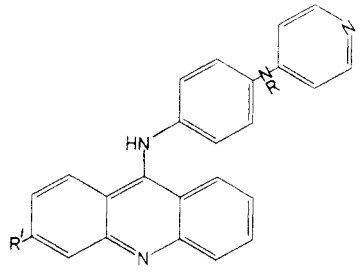
3 R=H

polynucleotide stack, the cationic charges matching to phosphates on chains A and B resulting in effective cross linking.

In essence, our current view is that the frequency of occurrence of a deranging intercalation step will be markedly increased by measures which concentrate agents in the immediate neighborhood of the potential intercalation site.

In preceding papers of this series we have described a series of bisquaternary salts which exhibit high activity when tested against the early intraperitoneal L1210 test system. Not surprisingly, none of the quaternary salts so far prepared have shown significant activity against intracerebral leukemia when administered ip. Attempts have been made, by molecular modification, to obtain agents with better distributive capacities while still retaining the high activity of the quaternary salts.

Current concepts in molecular pharmacology[2-6] suggest that rates of cellular penetration by passive diffusion can be increased by modifying agents so that they are: (a) more lipophilic; (b) less ionized at physiological pH values. Taking cognizance of these factors and considering our own present views of the importance of intercalation, a series of bis bases **4** was prepared. These can be considered as equivalent to nonquaternary analogs of isometamidium (**2**). The strongly basic γ-aminoheterocyclic systems in **4** provide cationic charge without the necessity for quaternization. Similarities between **4** and **2** are seen clearly from comparison of molecular models in which the similar intercharge separation and the degree of correspondence of the fused ring areas become apparent. Lipophilic character was controlled by adjustment of the alkyl function R; consideration of p*k* control and lipophilic character prompted a concurrent examination of the 3-chloro isomers. None of these agents (Table I) showed activity against the L1210 system.



4

TABLE I

SUBSTITUENTS IN **4**

R′	R	Mp, °C	Formula	*Anal.*
H	H	>360	$C_{24}H_{18}N_4 \cdot 2HBr$	C, H, N, Br
H	C_2H_5	234–235	$C_{26}H_{22}N_4$	C, H, N
H	$CH_3(CH_2)_3$	243–244	$C_{28}H_{26}N_4$	C, H, N
H	$CH_3(CH_2)_5$	107.5–108	$C_{30}H_{30}N_4$	C, H, N
H	$CH_3(CH_2)_7$	173–175	$C_{32}H_{34}N_4 \cdot 2HBr$	C, H, N, Br
H	$CH_3(CH_2)_9$	167–169	$C_{34}H_{38}N_4 \cdot 2HBr$	C, H, N, Br
Cl	H	336–338	$C_{24}H_{17}ClN_4 \cdot 2HI$	C, H, N, I
Cl	C_2H_5	142–143	$C_{26}H_{21}ClN_4$	C, H, N, Cl
Cl	$CH_3(CH_2)_3$	237–238	$C_{28}H_{25}ClN_4$	C, H, N, Cl
Cl	$CH_3(CH_2)_5$	232–233	$C_{30}H_{29}ClN_4 \cdot 2HBr$	C, H, N, Br
Cl	$CH_3(CH_2)_7$	94–95	$C_{32}H_{33}ClN_4$	C, H, N, Cl
Cl	$CH_3(CH_2)_9$	86–87	$C_{34}H_{37}ClN_4$	C, H, N

Earlier,[1] predictions from our site model hypothesis had led us to prepare bisquaternary salts such as **1** (R = CH_3) which showed high activity against the L1210 leukemia. The base **1** (R = H) showed no inhibitory effects against leukemia L1210. Recognition of the possible importance of intercalation led to the preparation of the acridinium salt **5** (R = CH_3). This compound provided convincing life extension when tested against leukemia L1210 although it was certainly not as active as **1** (R = CH_3). The corresponding free base **5** (R = H) proved inactive. The lower activity of **5** (R = CH_3) in relation to **1** (R = CH_3) had been predicted before synthesis because of our accumulated knowledge of the importance of lipophilic–hydrophilic balance. The biscationic agents discussed previously[1] show a pronounced dependence on lipophilic–hydrophilic balance for activity. The fusion of a benzene ring to **1** (R = CH_3), where the physical properties are in close to optimum balance, yielding **5** (R = CH_3) would certainly produce a more lipophilic and, therefore, less active molecule.[7]

Our previous experience in this area, coupled with model fitting, predicted that a species such as **6** (R = CH_3) would be considerably more active — the fit to the sites of residence and action appearing the same as for **5** (R = CH_3) but the added amide and 2-amino group in **6** would allow a closer approach to the optimum figure required for the lipophilic–hydrophilic balance. This proved to be the case since **6** (R = CH_3) shows very high activity against the L1210 test system.[8] Surprisingly, the unquaternized base **6** (R = H) was unequivocally active against the L1210 system. By synthesis

(2) B. B. Brodie and C. A. M. Hogben, *J. Pharm. Pharmacol.*, **9**, 345 (1957).

(3) L. S. Schanker, D. J. Jocco, B. B. Brodie, and C. A. M. Hogben, *J. Pharmacol. Exp. Ther.*, **123**, 81 (1958).

(4) B. B. Brodie, H. Kurz, and L. S. Schanker, *ibid.*, **130**, 20 (1960).

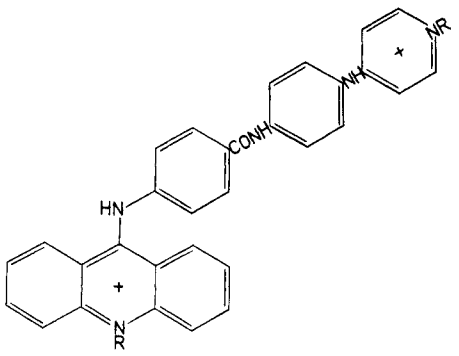
(5) E. J. Ariens, Ed., "Molecular Pharmacology," Vol. 1, Academic Press, New York, N. Y., 1964, pp 8–48.

(6) L. S. Schanker, *J. Med. Pharm. Chem.*, **2**, 343 (1963).

(7) Using partition chromatography to provide a convenient measure of partition coefficients (ref 1) the R_f values of **1** (R = CH_3) and **5** (R = CH_3) relative to dimidium **3** (R = CH_3) as internal standard were 0.87 and 1.22.

(8) R_f for **6** (R = CH_3) relative to dimidium was 0.98.

of the various analogs described below it was shown that the reason for the antileukemic activity of **6** (R = H) in contrast to the nonactivity of **5** (R = H) lay in the lower p*k* of the 3-benzamidopyridine system in **6**. The reversed-amide isomer of **5**, *viz.*, **7** (R = H), proved inactive as did the 3-chloro isomer **7** (R = Cl). However, the more weakly basic diaminopyrimidine[9] **8** proved active. The activity of the 3-nitro compound **7** (R = NO_2) showed that sufficient reduction in the p*k* of either basic terminus would allow anti-L1210 activity to be demonstrated. One conclusion to be drawn here



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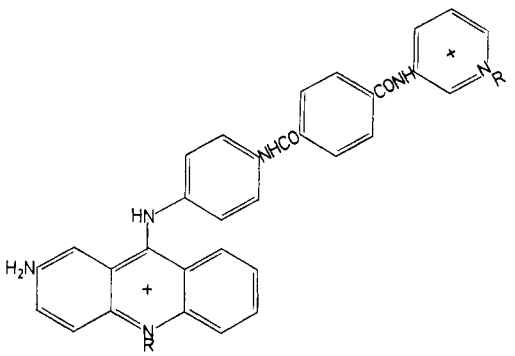

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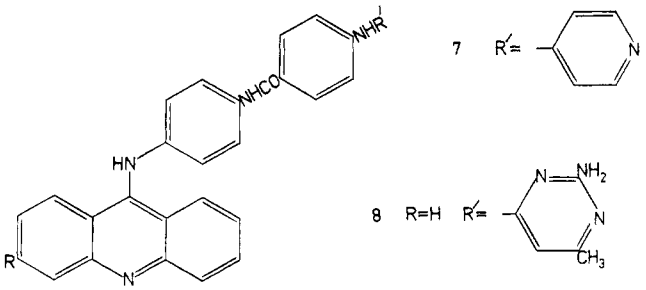

7 R′=

8 R=H R′=

is that cellular penetration is a limiting factor and dependent on the concentration of neutral species (Table II.) This is in accord with Robbins'[10] suggestion that the rate of membrane penetration is proportional to the percentage of neutral form.

It has proved impossible to measure the p*k*'s in H_2O of **5**–**8** listed in Table II by potentiometric or spectrophotometric methods[11] because of the extreme insolubility of the neutral forms. While it is possible to measure the p*k*'s in solvent mixtures the application of solvent correction factors has proved unreliable.[11] Since a set of very similar molecules are considered it is taken that the percentage of neutral forms present at physiological pH values will be related to the p*k*'s of the basic termini when these are examined separately. The figures in Table II have been calculated by such means for a pH of 7.3. It is assumed that the large charge separation (>15 Å) minimizes intercharge interactions.[12] The figures given are certainly not absolute but should be valid in a relative sense over the restricted series presented.

TABLE II

Species	Basic functions and their p*k*'s	Percentage of neutral form at pH 7.3 (relative)
5, R = H	9-Aminoacridine, 9.6;[a] 4-aminopyridine, 9.2[b]	0.006
6, R = H	2,9-Diaminoacridine, 10.0;[a] 3-benzamidopyridine, 3.8[c]	0.2
7, R = H	9-Aminoacridine, 9.6; 4-aminopyridine, 9.2	0.006
7, R = Cl	3-Chloro-9-aminoacridine, 8.9;[a] 4-aminopyridine, 9.2	0.03
7, R = NO_2	3-Nitro-9-aminoacridine, 7.5;[a] 4-aminopyridine, 9.2	0.48
8	9-Aminoacridine, 9.6; 2,4-diamino-6-methylpyrimidine, 7.7[d]	0.17
5, R = CH_3	9-Imino-10-methylacridan, 10.7;[a] 1,4-dihydro-4-imino-1-methylpyridine, 12.5[e]	3.6×10^{-6}

[a] A. Albert, Ed., "The Acridines," 2nd ed, E. Arnold, London, 1966, p 437. [b] J. M. Essery and K. Schofield, *J. Chem. Soc.*, 3939 (1961). [c] R. A. Jones and A. R. Katritzky, *ibid.*, 1317 (1959). [d] J. C. Gage, *ibid.*, 469 (1949). [e] S. S. Angyal and C. L. Angyal, *ibid.*, 1461 (1952).

Now the lack of anti-L1210 activity for **4** (Table I) becomes understandable; such strongly basic species would not provide sufficient neutral species for adequate cellular penetration by passive diffusion. Also, the dimethyl quaternary salt of **4** (R = R′ = H) proved much more lipophilic (R_D = 1.34)[7] than is usual,[1] hence its inactivity in the L1210 systems.

The accumulated results draw further attention to the anomalous ease of cellular entry of the quaternary salts already examined.[1] As indicated in Table II, quaternary salts such as **5** (R = CH_3) have virtually no neutral form present at physiological pH values. Partition chromatography has provided an internally consistent scale for measuring the comparative hydrophilic–lipophilic balance of the quaternary salts.[1] However, comparison of this scale with alternate measures of these physical properties, for example, the octanol–H_2O partition system of Hansch,[13] demonstrates the extremely hydrophilic nature of these quaternary salts. Thus, dimidium bromide (**3**, R = H) which has partition figures close to the optimum necessary for antileukemic activity in the quaternary salts and has been used as a convenient internal standard,[1] has a partition coefficient (*P*) of only 0.4 in octanol–H_2O.[13] This low figure contrasts strongly with the optimum suggested[13,14] for passive diffusion to site of action; log $P \sim 2$, *i.e.*, $P \sim$

(9) The earlier demonstration that quaternary pyridinium and diaminopyrimidinium functions could be interchanged as the cationic centres in bisquaternary salts prompted the transposition in this example to gain p*k* control; see G. J. Atwell, B. F. Cain, and R. N. Seelye, *J. Med. Chem.*, **11**, 690 (1968).

(10) R. Robbins, *J. Gen. Physiol.*, **43**, 853 (1960).

(11) A. Albert and E. P. Sergeant, Ed., "Ionization Constants of Acids and Bases," Methuen & Co. Ltd., London, 1962.

(12) J. Clark and D. D. Perim, *Quart. Rev. Chem. Soc.*, **18**, 295 (1964).

(13) C. Hansch, *Account Chem. Res.*, **2**, 232 (1969).

(14) C. Hansch, A. R. Sterrand, S. M. Anderson, and D. Bentley, *J. Med. Chem.*, **11**, 1 (1968).

100. These figures further strengthen our view that a specialized transport mechanism operates with these powerful bases.[1]

Our view would be that a powerful base such as **5** (R = CH_3) with its lipophilic–hydrophilic balance in the range necessary for activity[1] would be transported into cells of the target species. Although considerably less strongly basic, **5** (R = H) distributes itself much less freely than the more fully ionized quaternary salt.

The enhanced lipophilic[15] character of the nonquaternary base **5** (R = H) denies this agent the transport mechanism which operates for **5** (R = CH_3) but, because of its strongly basic functions, cannot provide sufficient neutral species for adequate cellular entry by passive diffusion (Table II). Attenuation of *either* strongly basic terminus to provide the alternates **7** (R = NO_2) and **8** affords molecules where the relative level of neutral species present at physiological pH values (Table II) is sufficient to allow reasonable rates of membrane penetration when biological activity returns.

Experimental Section

Analyses were by D. A. D. Campbell, Microchemical Laboratory, University of Otago, Otago, New Zealand. The symbol for the requisite element has been used to signify that the analytical results were within ±0.4% of the calculated figure. Melting points have been determined on an Electrothermal melting point apparatus with the maker's supplied stem-corrected thermometer. A 2°/min heating rate from 20° below the mp was used.

4-Anilopyridines.—The convenient method of Jerchel and Jakob,[16] treating a salt of an aromatic amine with *N*-pyridyl-4-pyridinium chloride·HCl[17] was used; full experimental details have been published.[18] By this means the nitroanilinopyridines listed in Table III were prepd, these in turn being reduced by Fe[19] to the aminoanilinopyridines (Table IV).

TABLE III

N-(PYRID-4-YL)-*N*-ALKYL-*p*-NITROANILINES

N-Alkyl substituent	Mp, °C	Formula	*Anal.*
C_2H_5	91–91	$C_{13}H_{13}N_3O_2$	C, H, N
$CH_3(CH_2)_3$	83–84	$C_{15}H_{17}N_3O_2$	C, H, N
$CH_3(CH_2)_5$	245–246	$C_{17}H_{21}N_3O_2 \cdot HBr$	C, H, N, Br
$CH_3(CH_2)_7$	234–235	$C_{19}H_{25}N_3O_2 \cdot HBr$	C, H, N, Br
$CH_3(CH_2)_9$	226–227	$C_{21}H_{29}N_3O_2 \cdot HBr$	C, H, N, Br

TABLE IV

N^1-(PYRID-4-YL)-N^1-ALKYL-*p*-PHENYLENEDIAMINES

N^1-Alkyl substituent	Mp, °C	Formula	*Anal.*
C_2H_5	120–121	$C_{13}H_{15}N_3$	C, H, N
$CH_3(CH_2)_3$	115–116	$C_{15}H_{19}N_3$	C, H, N
$CH_3(CH_2)_5$	108–109	$C_{17}H_{23}N_3$	C, H, N
$CH_3(CH_2)_7$	100–101	$C_{19}H_{27}N_3$	C, H, N
$CH_3(CH_2)_9$	97–98	$C_{21}H_{31}N_3$	C, H, N

5, R = H.—4-[*p*-(*p*-Aminobenzamido)anilino]pyridine[1] (3.04 g) and 9-chloracridine (2.14 g) were dissolved in 90% EtOH (125 ml) by boiling; to the resultant pale yellow soln was added 12 *N* HCl (0.9 ml). The soln was heated under reflux for 0.5 hr becoming progressively more red. NaCl (5 g) and H_2O (40 ml) were added and EtOH was distd off until the product started to sep. Recrystn from H_2O afforded **5** (R = H) dichloride as yellow needles (5.4 g), mp 336–337°. *Anal.* ($C_{31}H_{23}N_5O \cdot 2HCl$) C, H, N, Cl. Soln of this salt in EtOH–H_2O followed by addition to NaOH–H_2O afforded the free base, yellow needles from EtOH–H_2O, mp 176.5–177°. *Anal.* ($C_{31}H_{23}N_5O$) C, H, N.

Quaternization of this base (1.5 g) by soln in MeOTs (2.5 ml) at 160° and maintaining at this temp for 20 min followed by the usual work-up[19] afforded yellow needles of an extensively hydrated bisbromide. Recrystn from EtOH–H_2O–NaBr gave **5** (R = CH_3)·$2Br^-$ as hydrated yellow needles. Thorough drying over H_2SO_4 *in vacuo* removed H_2O of hydration, the anhyd compd being red. A thoroughly dried red sample, dried as for analysis, had mp 262–263°, R_D[1] 1.22. *Anal.* ($C_{33}H_{29}Br_2N_5O$) C, H, N, Br.

3-[*p*-(*p*-Nitrophenylcarbamoyl)benzamido]pyridine.—4-(*p*-Nitrophenylcarbamoyl)benzoic acid (3.02 g) and 3-aminopyridine (1.03 g) were dissolved in Py (35 ml) and the soln was cooled to below 0°. PCl_3 (0.54 ml) was added dropwise to the rapidly stirred soln so that the temp remained below 0°. After 1 hr at this temp the suspension was heated on the water bath for 1 hr, then excess Py was removed *in vacuo*. Dil NH_3–H_2O pptd crude material which on crystn from DMF–H_2O sepd as pale yellow plates (3.9 g), mp 318–319°. *Anal.* ($C_{19}H_{14}N_4O_4$) C, H, N.

The nitro compound was reduced to the corresponding amine with Fe[19] in 65% DMF–H_2O. The amine sepd from EtOH–H_2O as colorless needles, mp 278–280°. *Anal.* ($C_{19}H_{16}N_4O_2$) C, H, N.

3-{*p*-[*p*-(3-Nitroacridin-9-ylamino)phenylcarbamoyl]benzamido}pyridine.—A suspension of the above primary aromatic amine (1.12 g) and 9-chloro-3-nitroacridine (0.86 g) in 240 ml of 90% EtOH was heated under reflux for 0.5 hr. After this time HCl(12 *N*; 0.8 ml) was added in 0.1-ml portions at 10-min intervals. During the acid additions a homogeneous deep red soln resulted, toward the end of the addition the product started to sep. After a further 20-min boiling the suspension was cooled thoroughly and the deep red crystals were collected. After thorough trituration with NH_3–H_2O the resulting free base was crystd from DMF–H_2O–NH_3, separating as orange needles (1.54 g), mp 195–196°. *Anal.* ($C_{32}H_{22}N_6O_4$) C, H, N.

6, R = CH_3.—The above acridine bis base (1.2 g) was suspended in $PhNO_2$ (4.8 ml) and heated to 160° (internal). Me_2SO_4 (1.5 g) was added initiating an immediate reaction, a deep red soln resulting. After 20 min at 160° the $PhNO_2$ soln was chilled and the red quaternary salt was filtered off and washed well with dry Et_2O. On contact with polar solvents the cryst salt gave gummy solvates. Accordingly the NO_2 compd was immediately reduced by the standard method[19] and the resultant aminoquaternary salt purified as the bis(*p*-toluenesulfonate) salt. Crystn from EtOH–H_2O–NaOTs to the standards of paper chromatography as before[19] gave pure **6** (R = CH_3) as bis(*p*-toluenesulfonate), mp 214° dec. *Anal.* ($C_{48}H_{44}N_6O_8S_2 \cdot 2H_2O$) C, H, N, S.

6, R = H.—The corresponding NO_2 compound described above was reduced with Fe[19] in 65% DMF–H_2O with addition of 3 mole equiv of HCl. When reduction was complete 1 mole of $CaCO_3$ was added and after 10 min stirring on the water bath the hot mixt was filtered through a Celite pad. The filter pad was repeatedly washed with boiling 75% EtOH till no further color was eluted. Removal of solvent *in vacuo* left a red gum which was dissolved in a little 40% EtOH and NaAc (2 g) was added. The mixt was stirred until all NaAc had dissolved then satd NaCl–H_2O was added until all color had pptd from the soln. Recrystn from EtOH–H_2O–NaCl gave scarlet needles of the monohydrochloride, mp > 360°. *Anal.* ($C_{32}H_{24}N_6O_2 \cdot HCl$) C, H, N, Cl.

4-[*p*-(*p*-Nitrophenylcarbamoyl)anilino]pyridine was prepared by interaction of 4-(*p*-nitrophenylcarbamoyl)aniline and *N*-pyridyl-4-pyridinium chloride·HCl as described earlier.[18] The pure base sepd from DMF–H_2O as colorless prisms, mp 294–295°. *Anal.* ($C_{18}H_{14}N_4O_3$) C, H, N.

Reduction of this nitro compound by the usual method in 65% DMF–H_2O afforded **4-[*p*-(*p*-aminophenylcarbamoyl)anilino]pyridine,** colorless needles from EtOH–H_2O–NH_3, mp 257–258°. *Anal.* ($C_{18}H_{16}N_4O$) C, H, N.

Interaction of this primary amine with the requisite 9-chloracridine by the usual acid-catalyzed coupling[20,21] yielded the bis bases **7** (R respectively H, Cl, and NO_2).

7, R = H.—The dihydrochloride sepd from EtOH–H_2O–NaCl as yellow needles, mp 338–339°. *Anal.* ($C_{31}H_{23}N_5O \cdot 2HCl$) C, H, N, Cl.

7, R = Cl.—The orange dihydrobromide sepd from EtOH–

(15) R_f relative to dimidium,[1] R_D 1.43.

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H_2O–NaBr in fine needles, mp 308° dec. *Anal.* ($C_{31}H_{22}Cl$-$N_5O \cdot 2HBr$) C, H, N.

7, R = NO_2.—Scarlet needles were obtained from EtOH–H_2O–HCl, mp > 360°. *Anal.* ($C_{31}H_{22}N_6O_3 \cdot 2HCl$) C, H, N, Cl. Attempted drying of this salt at elevated temp *in vacuo* caused loss of HCl and analyses showing a Cl^- content between 1 and 2 moles of Cl were obtained for samples so dried. For analysis a sample was dried at room temp *in vacuo*.

2-Amino-4-[*p*-(*p*-nitrophenylcarbamoyl)anilino]-6-methylpyrimidine.—4-(*p*-Nitrophenylcarbamoyl)aniline (2.57 g) was dissolved in hot 2-ethoxyethanol (60 ml) and the soln cooled to 60°. 2-Amino-4-chloro-6-methylpyrimidine (1.58 g) was added, the mixt was boiled till homogeneous, concd HCl (0.95 ml) was added, and the clear soln was heated on the water bath for 0.5 hr. After a few minutes a product started to crystallize. After thorough cooling the chunky pale yellow prisms of the hydrochloride were collected, suspended in EtOH (15 ml), and stirred with concd NH_3 (10 ml) for 0.5 hr. The deep yellow base sepd from DMF–H_2O–NH_3 as fine needles, (3.2 g), mp 334–335°. *Anal.* (C_{18}-$H_{16}N_6O_3$) C, H, N.

2-Amino-4-[*p*-(*p*-aminophenylcarbamoyl)anilino]-6-methylpyrimidine was prepd by Fe[19] reduction of the preceding nitro compound in 65% DMF–H_2O. The pure compound sepd from EtOH–H_2O–NH_3 as colorless needles, mp 280–281°. *Anal.* ($C_{18}H_{18}N_6O$) C, H, N.

8.—A sample of the above primary amine (0.98 g) and 9-chloroacridine (0.64 g) were dissolved in 65% EtOH (80 ml) by boiling. HCl (12 *N*, 0.52 ml) was added to the clear soln, yellow crystals of product·2HCl started to sep shortly afterwards. The reaction mixt was thoroughly chilled after a further 0.5-hr heating on the water bath. The sepd crystals were recrystd from DMF–H_2O–HCl–NaCl. The fine yellow needles of dihydrochloride (1.48 g) had mp 358° dec. *Anal.* ($C_{31}H_{25}N_7O \cdot 2HCl$) C, H, N, Cl.

Biological Testing.—The routine screening test consists of ip inoculation of 10^5 L1210 cells into 18.5–22.5 g of C_3H–DBA_2 F_1 hybrids on day 1 with drug treatment initiated 24 hr later and continued for 5 days. All dosage was in 0.2-ml vol in H_2O suspension. Groups of 6 animals/dose level were used with one control group for every 5 tests. The wt change column in Table V records the difference between initial wt 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. Details of testing of inactive compds have not been given.

TABLE V

Drug	Dose, mg/kg per day	Survivors	Wt change	Average survival, days: Treated	Control	*T/C*, %
5, R = CH_3, as dibromide	150	4	−4.2	15.9	10.2	156
	100	6	−1.8	19.2	10.2	192
	67	6	−0.2	18.2	10.2	178
	44	6	+0.6	15.1	10.2	148
	29	6	+1.1	13.7	10.4	132
	20	6	+3.2	12.8	10.4	
6, R = CH_3 as bis(*p*-toluenesulfonate)	75	4	−3.9	14.4	10.1	142[a]
	50	6	−1.8	29.5	10.1	292[b]
	33	6	−2.2	21.7	10.1	215
	22	6	−0.8	18.3	10.1	181
	15	6	+0.3	15.8	10.1	156
	10	6	−0.9	14.0	9.8	143
	6.7	6	+3.0	12.2	9.8	125
6, R = H, as monochloride	225	6	−3.8	14.5	10.2	142
	150	6	+0.8	17.8	10.6	168
	100	6	+2.9	17.2	9.8	172
	67	6	+2.4	13.4	10.1	135
	44	6	+3.5	12.7	10.4	132
7, R = NO_2, as dihydrochloride	60	5	−2.4	14.8	10.4	142
	40	6	−1.2	16.8	10.4	162
	27	6	+0.8	14.0	10.2	143
	18	6	+4.0	13.1	10.1	132
	12	6	+3.8	12.3	10.1	122
8, as dihydrochloride	350	6	−5.2	14.4	9.8	141
	230	6	−2.8	16.5	10.2	168
	155	6	+0.2	14.8	10.4	154
	100	6	+2.4	13.7	10.4	143
	70	6	+4.2	12.3	10.1	124

[a] Not including one 100-day survivor. [b] Not including two 100-day survivors.

Acknowledgments. We are grateful to Miss Lois Armiger and her assistants for technical assistance in performing the many biological tests. This work was supported by the Auckland Division, Cancer Society of New Zealand (Inc.).

Irreversible Enzyme Inhibitors. 181.[1,2] Inhibition of Brain Choline Acetyltransferase by Derivatives of 4-Stilbazole

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Received November 16, 1970

Forty-three analogs of 4-stilbazole were synthesized and evaluated as inhibitors of choline acetyltransferase from rabbit brain. The most active inhibitor found for the acetyltransferase was 3′,4′-dichloro-4-stilbazole (**23**) which complexed to the enzyme 910 times more effectively than the substrate choline and 230 times more effectively than 4-stilbazole. Other highly effective derivatives of choline acetyltransferase were the 3′-Cl (**11**), 3′-CH_3 (**12**), and 3′-CH_3O (**13**) derivatives of 4-stilbazole which complexed 80- to 130-fold more effectively to the enzyme than choline. Compounds **11–13** and **23** were poor inhibitors of the AChE from rabbit brain; for example, **11** was complexed 130-fold more tightly to choline acetyltransferase than AChE.

The major enzyme in nerve endings involved in nerve impulses is acetylcholinesterase (AChE).[3] The order of events appears to be (a) active transport of choline through the nerve membrane,[4] (b) acetylation of choline to ACh with acetyl-CoA mediated by choline acetyltransferase,[5] (c) hydrolysis of ACh to choline and acetate by AChE during the nerve impulse,[3] and (d) reacetylation of CoA.[6] A tremendous amount of

(1) This work was generously supported by Grants CA-08695 and NS-09544 from the U. S. Public Health Service.

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