20 units/mL solution) was employed to give an easily measurable rate which was followed by removing 65-μL aliquots at 30-s intervals for the first 5 min and immediately quenching each aliquot in 10.0 mL of 0.1 M phosphate buffer at pH 7.0 containing 0.15 M semicarbazide hydrochloride. After complete conversion of methylglyoxal to the bis(semicarbazone) (ca. 10 h), the initial rate of the enzymatic reaction could be determined by measuring the absorption of the bis(semicarbazone) in these solutions at 286 nm (ϵ 3.2×10^4 M^{-1} cm^{-1}). Methylglyoxal (16.36 mM), GSH (1.42–7.09 mM), inhibitor (none or 5.6–16.7 mM) in 400 μL of DMF, and buffer (10.3–9.9 mL) were allowed to equilibrate at 30 °C for 5 min to allow time for the formation of the substrate before addition of the enzyme. The initial substrate concentration $[S]_0$ was varied by increasing the GSH concentration from 1.42 to 7.09 mM and calculated at equilibrium from a quadratic equation using the dissociation constant of 3.0 mM.[20] A computer program was used to determine the line of best fit by the method of least squares. The dissociation constants of the enzyme inhibitor complex (K_i) were obtained from double reciprocal plots[18] of $1/V_0$ vs. $1/[S]_0$ and $1/V_i$ vs. $1/[S]_0$. V_0 = initial velocity of the uninhibited enzymatic reaction and V_i = initial velocity of the inhibited reaction at a suitable inhibitor concentration (5.6–16.7 mM). The concentration of inhibitor required to inhibit the enzymatic reaction 50% was calculated using the expression $[I]_{50} = K_i(K_m + [S]_0)/K_m$ for the substrate component concentrations of 16.36 mM methylglyoxal and 2.84 mM glutathione.

Biological Testing. 10^5 L1210 lymphoid leukemia were implanted intraperitoneally into BDF_1 mice on day 0. Ip drug treatment was administered on day 1; day 1, 5, and 9; or day 2 and 6; and evaluated by the mean survival time in days of the treated animals over that of the control. Multiple dose assay levels included 400, 200, and 100 mg/kg and used a three- or six-mouse assay procedure.

Acknowledgment. This investigation was supported primarily by PHS Research Grant No. CA 12984 from the National Cancer Institute and in part by grants from the Research Council (Rutgers University), the National Institutes of Health (Biomedical Sciences Support Grant), and Mrs. Lauretta Horstmann, Bagley, Wis. In addition, the authors thank the Drug Research and Development Program, Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Bethesda, Md., for screening our compounds; and Drs. F. Scheidl and W. Benz, Hoffmann-La Roche Inc., Nutley, N.J., for the microanalyses and mass spectra.

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Potential Antitumor Agents. 25. Azalogues of the 4′-(9-Acridinylamino)methanesulfonanilides

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Tumor inhibition produced by isomeric, nitro-substituted 4′-(9-acridinylamino)methanesulfonanilide analogues of low base strength (pK_a = 4.79–5.72) might result from in vivo reduction to the corresponding, higher pK_a (7.15–9.80), tumor active amines. The aza analogues, –N= in place of –C(NO_2)=, have been prepared as nonclassical bioisosteres and screened in the L1210 system. Significant L1210 inhibition produced by isomeric 3- and 4-azalogues, of similar base strength to the corresponding nitro-substituted derivatives, demonstrates that weakly basic analogues can provide biologic activity when there is no prospect of in vivo reduction to more strongly basic products. Obligatory reduction of nitro function, for biologic activity, need not be postulated in this drug series.

Certain nitro group substituted 4′-(9-acridinylamino)-methanesulfonanilide (AMSA, 1) congeners are significantly tumor inhibitory in L1210 screening assays[1,2] with the 3-nitro isomer (**13**, Table I) proving particularly effective. The powerfully electron-withdrawing nitro function markedly lowers acridine base strength and from the pK_a values of the L1210 active isomers (**12**, **13**) it can be calculated that less than 2% of these compounds would be ionized at physiologic pH (7.4). Additionally, the 3,6-dinitro-substituted AMSA agent (pK_a = 3.68), which would be less than 0.03% ionized at pH 7.4, is also convincingly L1210 active.[3] It has usually been considered that the cation of chemotherapeutic acridines provides observed biologic activity.[4] More highly ionized acridines normally prove either more efficacious or more dose po-

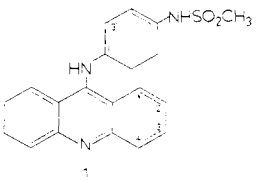


Table I. Structural Details, Physicochemical Properties, and Antitumor Screening Data for the Aza-AMSA Congeners

No.	Substituents in 1	Mp, °C	Formula	Analyses[a]	pK_a[b]	R_m[c]	O.D.[d]	L1210[e] ILS %
1	Parent for comparison purposes[f]				7.19	0.00	45	107
7	1,-N=[g]	285 dec	$C_{19}H_{16}N_4O_2S \cdot HCl$	C, H, N; Cl[h]	6.90	-0.56	>500	
8	2,-N=	298 dec	$C_{19}H_{16}N_4O_2S$	C, H, N, S	5.73	-0.39	>500	
9	3,-N=	249-251	$C_{19}H_{16}N_4O_2S \cdot HCl \cdot 0.5H_2O$	C, H, N	5.32	-0.33	>500	
10	4,-N=	295 dec	$C_{19}H_{16}N_4O_2S \cdot HCl \cdot 0.5H_2O$	C, H, N, Cl	5.93	-0.27	>500	
11	1-NO_2[i]				4.79	-0.09	75	
12	2-NO_2[i]				5.42	-0.21	250	27
13	3-NO_2[i]				5.52	-0.17	25	123 (2)
14	4-NO_2[i]				4.88	-0.17	>500	
15	2-NH_2[i]				7.15	-0.31	25	78
16	3-NH_2[i]				9.80	-0.17	2.5	81 (1)
17	1,-N=,3'-OCH_3	192-194	$C_{20}H_{18}N_4O_3S \cdot HCl$	C, H, N, Cl	7.19	-0.39	220	
18	2,-N=,3'-OCH_3	255-256	$C_{20}H_{18}N_4O_3S$	C, H, N, S	5.84	-0.25	500	
19	3,-N=,3'-OCH_3	201-203	$C_{20}H_{18}N_4O_3S \cdot HCl \cdot H_2O$	C, H, N, Cl	5.53	-0.13	90	88
20	4,-N=,3'-OCH_3	209-211	$C_{20}H_{18}N_4O_3S \cdot HCl$	C, H, N, Cl	6.09	-0.07	62	89
21	1-NO_2,3'-OCH_3	206-209	$C_{21}H_{18}N_4O_5S \cdot HCl$	C, H, N, Cl	4.98	-0.01	180	44
22	2-NO_2,3'-OCH_3	288-289	$C_{21}H_{18}N_4O_5S \cdot HCl$	C, H, N, Cl	5.63	-0.06	>500	
23	3-NO_2,3'-OCH_3[i]				5.72	0.11	4.5	84
24	4-NO_2,3'-OCH_3	248 dec	$C_{21}H_{18}N_4O_5S \cdot HCl$	C, H, N, Cl	5.05	0.09	75	38

[a] Analyses for the indicated elements were within ±0.4% of the theoretical figures for the formula quoted. [b] Ionization constants measured as detailed in ref 33. [c] Relative measure of agent lipophilic-hydrophilic balance from reversed-phase partition chromatography; ref 31. [d] O.D. = optimum dose. That dose in mg/kg/day providing maximum increase in life span in leukemic animals or, for inactive drugs, the maximum tolerated dose. [e] ILS = increase in life span in L1210 assays. ILS % = (T/C % - 100). Numbers of animals surviving 50 days after dosing, from a group of six, are provided in parentheses. A negative sign denotes that significant life extension (>25%) was not obtained at employed dose levels. [f] Reference 31. [g] Azalogue; ring nitrogen (-N=) replacing the designated ring carbon atom. [h] Cl: calcd, 8.8; found, 9.3. [i] Reference 1. [j] Reference 3.

tent.[4] The above examples then appear exceptional, as do certain other recorded 3-nitro-9-aminoacridine derivatives (pK_a = 7.1-7.7)[4] which demonstrate high antibacterial activity in vitro[5] and in vivo[6-8] as well as antitrypanosomal[8] and antiviral activity.[9,10] A vinylogous 1-nitro-9-aminoacridine derivative, presumably of comparable low base strength (cf. **11**, **21**, Table I), has also received clinical trial as an antitumor agent.[11,12]

In the present drug series, whenever a nitro-substituted variant has been found L1210 active, the corresponding more strongly basic amine, produced by nitro group reduction, has also proved active.[1-3] These amines (cf. **15**, **16**, Table I)[3] inevitably prove more dose potent than the corresponding nitro compounds (cf. **12**, **13**). Enzymatic production of trace amounts of the more dose potent amines might then explain the in vivo chemotherapeutic activity of the low basicity nitroacridines. Besides the corresponding amines, nitro group reduction could furnish a series of intermediates, at varying oxidation levels, some of which might also display antitumor activity. The observed chemotherapeutic activity of the nitro-substituted acridines might then result from the applied nitro compound alone, the combined effects of the nitro compound plus reduced products, or from the reduction products alone.

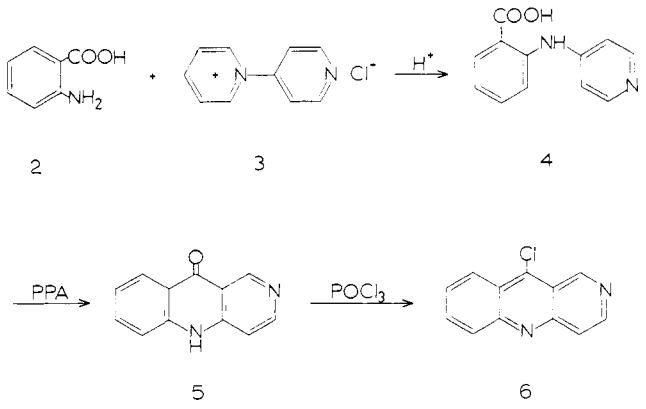
The electronic polarization effects produced, within six-membered heteroaromatic systems, by either a ring nitrogen (-N=) or a nitro-substituted carbon atom [-C(NO_2)=] are often comparable.[13] Classes of chemical reaction[13] and biologic activity, responsive to such polarization, prove common to both members of the pairs of the corresponding aza and nitro heterocycles. Such nonclassical bioisosterism[14] has been demonstrated in series of analeptics:[15] pyridoxal analogues[16] and the antibacterial sulfonamides.[17,18] Additionally, the similar electronic effects of ring nitrogen and a nitro substituent often provide compounds with very similar base strengths and quite reasonable prediction of the pK_a of an aza heterocycle can be made from that of the corresponding nitro heterocycle.[19] The isomeric azaacridine AMSA analogue should then be similar to the nitro congeners in both electron polarization and base strengths. However, the demonstration of convincing antitumor activity for an azaacridine variant, if this had comparable base strength to the corresponding nitro-substituted acridine, would provide supportive evidence for the view that prior reduction was not obligatory in order that the latter display biologic activity. This communication describes the synthesis of, and the L1210 screening data for, the isomeric aza-AMSA analogues.

Chemistry. The isomeric aza-9-chloroacridines have not previously been obtained and it appears generally assumed that they are difficult to produce from the corresponding aza-9(10*H*)-acridones.[26,27] It would be predicted that such conversions, employing compounds containing an additional ring nitrogen atom, would be of comparable ease to those using the corresponding nitro-substituted acridones. The nitro-9(10*H*)-acridones can be readily converted to the corresponding 9-chloro compounds but these, when formed, are quite labile and suffer ready hydrolysis returning the parent acridone. Similar properties have been found for the aza-9-chloroacridines; these compounds are quite readily prepared but extremely easily hydrolyzed to the starting acridone. By careful low-temperature work-up the necessary aza-9-chloroacridines can be obtained. These compounds, as with the nitro-9-chloroacridines, are hydrolyzed at room temperature by atmospheric moisture with the first liberated traces of HCl acting catalytically to accelerate decomposition of remaining chloro compound.

The aza-9-chloroacridines (e.g., **6**, Scheme I) coupled satisfactorily with requisite 4'-aminomethanesulfonanilides, by a modification of the standard method,[1-3] to provide the required agents (**7**-**10**, **17**-**20**, Table I).

Necessary aza-9(10*H*)-acridones were prepared by ring closure of either 2-(*x*-pyridylamino)benzoic acids or (*x* - 1)-phenylaminopyridine-*x*-carboxylic acids, employing variations of the route of Scheme I, as enumerated below. Formerly, extremely vigorous conditions (e.g., molten NaCl-$AlCl_3$)[20] have been employed for such ring closures (**4** → **5**). In all cases it was found that polyphosphoric acid (PPA), under quite moderate conditions, promoted ready

Scheme I



ring closure and furnished higher product yields.

Reaction of 2-chlorobenzoic acid and 3-aminopyridine under modified Jourdan–Ullmann reaction conditions[1] provided 2-(3-pyridylamino)benzoic acid.[23] In agreement with an earlier report[23] ring closure (PPA) of the latter acid provided 1-aza-9(10*H*)-acridone, free from discernable amounts of the isomeric 3-aza compound which might also be produced. 2-(4-Pyridylamino)benzoic acid (**4**, Scheme I) is most conveniently prepared by reaction of anthranilic acid with 1-(4-pyridyl)pyridinium chloride[24,25] (**3**) rather than 4-chloropyridine as used earlier.[20-23,26,27] Under vigorous conditions iodobenzene reacted with 3-aminopyridine-4-carboxylic acid to provide 3-phenylaminopyridine-4-carboxylic acid[23,27] which, on PPA induced ring closure, afforded 3-aza-9(10*H*)-acridone as sole product. 2-Phenylaminopyridine-3-carboxylic acid[28] was most readily available from Jourdan–Ullmann coupling of aniline and 2-chloronicotinic acid. Following PPA ring closure unequivocally provided 4-aza-9(10*H*)-acridone.

Results and Discussion

Maximum tolerated doses of the four isomeric aza-AMSA congeners (**7–10**, Table I) could not be reached and, at the highest practically attainable dose (500 mg/kg/day), no significant tumor inhibition was seen in L1210 assays.

There is a discrepancy between the measured base strengths for corresponding pairs of aza and nitro heterocycles, in this series, which suggests that prediction of the pK_a of one partner of the pair, from that of the other, may not always be justified. The 2-, 3-, and 4-aza analogues **8–10** had quite comparable pK_a values to those of the corresponding nitro derivatives **12–14**. However, introduction of a 1-aza function, as in **7**, provided very little base weakening effect, the pK_a of this compound being close to that of the parent **1**. In contrast, a 1-NO_2 group, as in **11**, produced a greater decrease in base strength than did any other isomeric nitro substituent (**12–14**).

As expected the aza variants proved more hydrophilic than the corresponding nitro derivatives, as gauged by R_m values (Table I), measured by reversed-phase chromatography as before.[3] Even more hydrophilic congeners were earlier shown tumor active;[2,3] the lower lipophilic–hydrophilic balance seen with the azalogues **7–10** should not alone provide inactive agents.

While effective dose levels of the azalogues **7–10** could not be reached, earlier research had shown that dose potency, of an agent of this series, was invariably increased by appending a 3′-OCH_3 group.[3,29] This device again proved successful and maximum tolerated doses of aza-AMSA analogues bearing a 3′-OCH_3 group (**17–20**) could be reached and certain members (**19**, **20**) proved significantly tumor inhibitory. For comparison purposes the isomeric nitro-substituted congeners bearing a 3′-OCH_3 group (**21–24**) were also prepared and screened.

There is not complete correspondence between the biologic activity of the isomeric aza and nitro congeners but this is limited to the 1-aza compound **17** which, as before (**7**), has a high pK_a (cf. **1**) and does not provide L1210 activity. The corresponding 1-NO_2 analogue **21** has low base strength and provides significant but low life extension in L1210 tests.

Representative agents of this series have been shown to intercalate into double-stranded DNA[30] and the structure–activity relationships, for varyingly substituted congeners, have been discussed in terms of an intercalation site of action.[1,29] Acridine 2- (7-) substituents proved dystherapeutic and the site–model rationalization was that these substituents would not be tolerated because of the proximity of the DNA sugar–phosphate chains.[1] Thus, within the isomeric nitro variants **21–24** inactivity of **22** might be ascribed to lack of site bulk tolerance for the 2-NO_2 group. Without considering other than steric properties the 2-aza function, as in **18**, appearing even less sterically demanding about the 2 position than the parent **1**, might be expected compatible with L1210 activity. However, if there was hydrogen bond formation from the 2-aza group to solvent water, as is common in most aza heterocycles, the associated water molecule or oriented cluster of water molecules might provide appreciable steric inhibition of site binding, possibly comparable to that provided by some substituents.

The pK_a of the L1210 active 3-aza,3′-OCH_3 variant **19** is as low as that of the tumor inhibitory 3-NO_2 compounds **13** and **23**. It is clear that a low pK_a analogue (**19**) can provide antitumor activity when there is no nitro group present which can suffer reduction to provide more basic intermediates. There is then no necessity to postulate obligatory nitro group reduction, at least for L1210 activity, in the low pK_a nitro-substituted AMSA analogues.

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. Analyses were performed by Dr. A. D. Campbell, Microchemical laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal melting point apparatus with the makers' supplied stem corrected thermometer; melting points are as read. NMR spectra were obtained on a Varian A-60 spectrometer (Me_4Si). IR spectra (KBr) were recorded using a Beckman 237 Infracord. UV spectra were recorded on a Shimadzu UV-200.

To monitor the progress of reactions, purification of products, etc., TLC on SiO_2 (Merck SiO_2, F_{254}) was used. The partition chromatographic methods used in measuring R_m values have been described earlier.[31]

The pK_a values were measured UV spectrometrically in 20% DMF–H_2O employing the methodology described earlier.[33]

2-(3-Pyridylamino)benzoic Acid. A mixture of 2-chlorobenzoic acid (15.6 g, 0.10 M), anhydrous K_2CO_3 (14 g, 0.11 M), 3-aminopyridine (10 g, 0.11 M), catalytic Cu powder (0.1 g), powdered Cu_2O (0.1 g), and 2-ethoxyethanol (15 mL) was heated under reflux conditions in an oil bath maintained at 170 °C for 2 h. The cooled mixture was diluted with warm H_2O, the solution clarified, and crude product precipitated by acidification with HOAc. The black powder was extracted to completion with EtOH (500 mL) in a Soxhlet extractor. Decolorizing charcoal (2 g) was added to the hot solution which, after 15 min of boiling, was clarified through a Celite pad. Concentration of the solution to 200 mL and cooling provided crude 2-(3-pyridylamino)benzoic acid. A further crystallization provided TLC homogeneous product as brown needles of mp 234–236 °C (4.5 g, 20%). Modification of the reaction conditions did not raise the yield above 20%. Earlier described methods[21-23] quote a maximum yield of 11% and melting points of 238[22] and 237 °C.[23]

1-Aza-9(10*H*)-acridone [Pyrido[3,2-*b*]quinolin-10(5*H*)-one]. The preceding compound (4 g, 0.028 M) was heated with commercial polyphosphoric acid (85% P_2O_5, 20 g) at 200 °C, until a homogeneous solution resulted, and then for 30 min longer. The cooled mixture was diluted with H_2O and basified with NH_4OH. The collected solid was well washed with H_2O and crystallized from EtOH to provide pure product as yellow crystals of mp 369–371 °C dec (3.0 g, 75%). Earlier, ring closure employing an $AlCl_3$–NaCl melt provided a 40% yield; product mp[23] 369–371 °C dec. This compound is best characterized by its UV spectra in 0.01 N NaOH and 0.01 N HCl.[23]

1-Aza-9-chloroacridine. The corresponding azaacridone (1.0 g, 5 mM) was suspended in $SOCl_2$ (5 mL) containing DMF (0.02 mL) and the mixture stirred and boiled until homogeneous and then for 10 min longer. Excess $SOCl_2$ was removed in vacuo and the residue dissolved in the minimum necessary volume of EtOH-free $CHCl_3$. The solution was cooled to −5 °C and then poured in a thin stream into a vigorously stirred mixture of chipped ice (100 g) plus concentrated NH_4OH (50 mL). The $CHCl_3$ layer was separated in a prechilled separating funnel and the organic layer washed with ice-cold 2 N NH_4OH (20 mL). The $CHCl_3$ layer was dried over anhydrous Na_2SO_4, at refrigerator temperatures; then solvent was removed in vacuo at 0 °C. TLC demonstrated a single new, less polar product ($CHCl_3$–1% Et_2N) than the starting material had produced. Invariably product was contaminated with the initial azaacridone. On standing or, more rapidly, on heating with water the chloro compound was converted to the initial acridone. The chloro compound could not be obtained completely free of traces of acridone. In view of the instability of this product it was coupled, with minimum delay, with the requisite 4'-aminomethanesulfonanilide.

7 and 17 were prepared by adding ice-cold EtOH solutions of the requisite aminosulfonanilide components (1.05 molar equiv) to freshly prepared samples of the azachloroacridine. The mixtures were stirred at room temperature until homogeneous, then concentrated HCl catalyst (0.02 mL) was added, and the red solutions were maintained at 20 °C for 4 h and then at −15 °C for 15 h. The crystalline hydrochloride salts were collected (68% **7**; 71% **17**) and recrystallized by solution in H_2O at temperatures of not greater than 60 °C and then addition of saturated aqueous NH_4Cl, at the same temperature, until crystallization initiated. Slow cooling provided TLC homogeneous, highly crystalline, deep red products (Table I).

2-(4-Pyridylamino)benzoic Acid (4). A suspension of 4-toluenesulfonic acid hydrate (93 g, 0.49 M) in toluene (150 mL) was heated to boiling under an H_2O entrainment head until all H_2O had been removed and a clear solution resulted. To the cooled solution anthranilic acid (55 g, 0.40 M), 1-(4-pyridyl)-pyridinium chloride (85 g, 0.44 M), and *N*-methyl-2-pyrrolidone (30 mL) were added and the heterogeneous mixture was heated allowing toluene to distill until an internal temperature of 160 °C was reached. After 30 min at this temperature the mixture was cooled to below 100 °C and boiling H_2O (150 mL) added. On addition of 12 N HCl (0.25 vol) to the hot, clarified solution, cooling, and seeding, product crystallized. Recrystallization, by solution in boiling H_2O and addition of 12 N HCl (0.25 vol), provided homogeneous product hydrochloride of mp 279–281 °C (59 g, 59%) (lit.[20,22] mp 260–270, 282–283 °C).

Solution of this hydrochloride (10 g, 0.04 M) in H_2O and addition of a solution of crystalline NaOAc (6 g) in H_2O precipitated the free base. Recrystallization from EtOH–H_2O provided pure product as colorless needles of mp 282–284 °C (lit.[22] mp 283–284 °C). Anal. ($C_{12}H_{10}N_2O_2$) C, H, N.

2-Aza-9(10*H*)-acridone [Pyrido[4,3-*b*]quinolin-10(5*H*)-one] (5). Polyphosphoric acid (60 g) was heated to 120 °C and 2-(4-pyridylamino)benzoic acid hydrochloride (20 g, 0.08 M) was added portionwise with stirring, as permitted by HCl evolution. The mixture was heated at 220 °C (internal) until homogeneous and then for 10 min longer. H_2O (60 mL) was added to the cooled mixture; the whole mixture was stirred until homogeneous and then rendered basic with concentrated NH_4OH and further H_2O (70 mL) was added. After overnight refrigeration crude product was collected and then crystallized from 50% EtOH–H_2O. Pure product (14.1 g, 89%) was obtained as yellow needles of mp 339–341 °C dec. Quoted melting points for this product, from various routes, are 339 °C dec[20] and 338–339 °C dec.[26]

2-Aza-9-chloroacridine (6). The marked insolubility of the corresponding azaacridone in either $SOCl_2$ or $POCl_3$ necessitates use of a solvent in conversion to the chloro compound. A sample of the 2-azaacridone (1 g, 5.1 mM) was suspended in pyridine (10 mL) and the mixture boiled and stirred until a solution resulted. To the well-cooled solution $POCl_3$ (2 mL) was added and the mixture heated on a steam bath for 1 h. After removal of volatiles in vacuo on a steam bath the cooled residue was dissolved in dry EtOH-free $CHCl_3$ (50 mL); the resulting solution was cooled to −5 °C and poured into a vigorously stirred mixture of chopped ice (50 g) and concentrated NH_4OH (25 mL). The resulting emulsion was broken by filtering through a precooled Celite pad and the $CHCl_3$ layer was separated, washed once with ice-cold 2 N NH_4OH, dried (Na_2SO_4, 0 °C), and evaporated at 0 °C in vacuo to provide product as a red solid. TLC ($CHCl_3$–1% Et_3N) showed the less polar chloro compound to be contaminated with starting acridone. Brief boiling of a sample with H_2O converted all product to the corresponding azaacridone. Coupling with the requisite 4'-aminomethanesulfonanilide component was carried out as in the case of the 1-aza congener. The deep red crystalline products (8, 18) were crystallized as before from H_2O–NH_4Cl.[1-3] To ensure complete removal of traces of azaacridone from these products it was necessary to convert the hydrochloride salts to the free bases by solution in EtOH–H_2O and addition of the theoretical quantity of 10% aqueous $KHCO_3$. The precipitated, crystalline, red bases were recrystallized from EtOH–H_2O.

3-Phenylaminopyridine-4-carboxylic Acid. A suspension of 3-aminopyridine-4-carboxylic acid (10 g, 0.092 M), anhydrous K_2CO_3 (13 g, 0.095 M), catalytic Cu powder (0.1 g), and powdered Cu_2O (0.1 g) in iodobenzene (14 mL) plus *N*-methyl-2-pyrrolidone (20 mL) was heated in an oil bath maintained at 170 °C for 6 h. The mixture was cooled, then H_2O (75 mL) and ligroine (75 mL) were added, and the whole solution was well mixed. The separated aqueous layer was acidified to pH 5 with HCl and the precipitated crude product recrystallized from DMF until homogeneous to TLC. Pure product was obtained as pale green prisms of mp 304–305 °C (7.0 g, 42%) (lit.[23,27] mp 305–306, 304–305 °C).

3-Aza-9(10*H*)-acridone [Pyrido[3,4-*b*]quinolin-10(5*H*)-one]. A sample of the aforementioned acid (8 g, 0.043 M) was suspended in a mixture of polyphosphoric acid (80 g) and $POCl_3$ (8 mL) and the whole mixture heated at 100 °C for 1 h. To the cooled reaction chopped ice was added followed by Na_2CO_3 until the mixture was basic. The precipitated solid was crystallized from small volumes of EtOH until homogeneous to TLC. Pure product was obtained as yellow prisms of mp 327–329 °C (5.1 g, 64%) (lit.[23] mp 328–330 °C).

2-Phenylaminopyridine-3-carboxylic Acid. A suspension of 2-chloronicotinic acid (16.9 g, 0.107 M), anhydrous K_2CO_3 (15 g, 0.109 M), aniline (11 g, 0.119 M), catalytic Cu powder (0.1 g), and powdered Cu_2O (0.1 g) in 2-ethoxyethanol (20 mL) was boiled under reflux conditions in an oil bath for 2 h. The cooled mixture was brought to pH 5 with HOAc and volatiles were removed in vacuo. The residue was partitioned between warm C_6H_6 (250 mL) and H_2O and the organic layer washed several times with warm H_2O. On thorough cooling product crystallized from the wet C_6H_6 solution. Recrystallization provided pure product as yellow needles of mp 152–153 °C (14.0 g, 61%) (lit.[28] mp 154–156 °C).

4-Aza-9(10*H*)-acridone [Pyrido[2,3-*b*]quinolin-5(10*H*)-one]. The preceding acid (10 g, 0.054 M) was heated with polyphosphoric acid (100 g) at 190 °C (internal) until a homogeneous solution resulted and then for 30 min further. Product was precipitated by addition of H_2O and basification with NH_4OH. Repeated crystallization from EtOH provided pure product as pale yellow needles of mp 277–279 °C (9.0 g, 85%) (lit.[28] mp 278–279 °C).

Both the 3- and 4-aza-9(10*H*)-acridones could be converted to the corresponding 9-chloro compounds, by the methods employed for the 1-aza isomer, and these hydrolyzed to return the initial acridones. The chloro compounds were invariably contaminated with the starting acridone. Further reaction of the chloro compounds, with the requisite aminosulfonanilides, was carried out by the modified coupling procedure described above.

Biologic Testing. Animals were implanted intraperitoneally (ip) with 10^5 L1210 cells on day 0 and drug was administered ip daily, starting day 1 and continuing once daily for 5 days. Detailed description of the testing protocols has been provided earlier.[1-3,32]

Acknowledgment. This work was supported by the Auckland Division of the Cancer Society of New Zealand and, in part, by the Medical Research Council of New Zealand. We are indebted to Miss Carolin West and her capable assistants for performance of the many biological tests.

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Synthesis of Nitrosourea Derivatives of Sucrose as Central Nervous System Anticancer Agents

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Received January 10, 1977

Nitrosourea derivatives of sucrose have been synthesized for the purpose of obtaining anticancer agents with activity against brain cancer. Two such compounds, 6,6′-dideoxy-6,6′-di(3-methyl-3-nitrosoureido)sucrose (**13**) and 1′,-6,6′-trideoxy-1′,6,6′-tri(3-methyl-3-nitrosoureido)sucrose (**14**), and their respective acetylated derivatives **15** and **16** have been prepared from sucrose. Compounds **13** and **14** have demonstrated antitumor activity against both L1210 leukemia and ependymoblastoma brain tumor in mice.

The treatment of human brain tumors by chemotherapy has led to minimal success up to this time. Compounds such as 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU) have been used in the clinic,[1,2] but toxic side effects can pose a problem when using these compounds over long periods of time. Physical characteristics such as high lipid solubility, small molecular size, and a low degree of ionization which enable these compounds to penetrate the blood–brain barrier[3] also allow them to easily penetrate into all cells of the body where they might inflict various toxic manifestations.

In 1970, Bakay reported on some studies he had undertaken on sucrose uptake in humans with brain tumors.[4] He found that whereas sucrose did not penetrate into normal brain tissue, the concentration of sucrose found in the tumorous tissue was in direct relation to its concentration in plasma. These results demonstrated that whereas the blood–brain barrier prevents entry of sucrose into the extracellular fluid surrounding normal brain tissue, there was a complete absence of this barrier to sucrose in brain tumors. Bakay's data further indicated that sucrose penetrated the cell membranes of human brain tumor cells.

The penetration of sucrose through cell membranes is

Chart I