phoma was passaged in the same way; previously, this tumor had been made resistant to ethyl 5-(3,3-dimethyltriazen-1-yl)-2-phenylimidazole-4-carboxylate and was found to be resistant to the optimum antitumor dose of the dimethyltriazenes used in subsequent studies.[4] Cells were counted with a Model ZBI Coulter counter.

Antitumor Test. Approximately 2×10^5 lymphoma cells were injected subcutaneously in the inguinal region of 20-g female CBA/LAC mice. After 2 days, drugs were administered daily for 5 days by intraperitoneal injection of 0.1 mL of a solution or suspension of drug made by sonication in either 10% acetone–arachis oil or 10% dimethyl sulfoxide–arachis oil. The day of death of the animals, which were in groups of five, was recorded, and the survival time of treated animals was compared with untreated controls. Survival time was shown to be proportional to the number of cells injected.[21]

Bioassay. In vitro–in vivo bioassays were performed as described previously.[21]

Acknowledgment. The authors are grateful to the following agencies for financial support. The Cancer Research Campaign (United Kingdom), The Natural Sciences and Engineering Research Council of Canada, and the Saint Mary's University Senate Research Comittee. The carbon-13 spectrum was provided by the Atlantic Regional Nuclear Magnetic Resonance Centre at Dalhousie University, Halifax, Nova Scotia.

Registry No. **2**, 52416-18-9; **3**, 29168-87-4; **4**, 70346-60-0; **5**, 69467-93-2; **6**, 70303-37-6; **7**, 73779-16-5; **8**, 70346-61-1; **9**, 70346-62-2; **10**, 88392-04-5; **11**, 82647-12-9; **12**, 51029-21-1; **13**, 40643-38-7; **14**, 51029-20-0; **15**, 82647-08-3; **16**, 79868-88-5; **17**, 82647-11-8; **20**, 22305-44-8; methyl anthranilate, 134-20-3; 4-acetylanaline, 99-92-3; methyl 4-aminobenzoate, 619-45-4; ethyl 4-aminobenzoate, 94-09-7; 4-cyanoanaline, 873-74-5; 4-aminobenzamide, 2835-68-9.

Potential Antitumor Agents. 40. Orally Active 4,5-Disubstituted Derivatives of Amsacrine

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The DNA-intercalating agent amsacrine is an effective drug for the treatment of human leukemias and lymphomas but has minimal solid tumor activity. As a first step in identifying analogues with a wider spectrum of activity, a comparison was made of the in vivo antileukemic (P-388) activity of amsacrine analogues given by oral (po) and intraperitoneal (ip) routes. A series of 4-substituted and 4,5-disubstituted derivatives all showed high activity when administered ip against ip-implanted P-388, but activity varied widely when the compounds were given orally. 4-Methoxy and 4-carbamoyl derivatives proved essentially inactive, whereas 4-methyl and 4-methylcarbamoyl derivatives retained activity. Exceptional oral activity was shown by the 4-methyl-5-methylcarbamoyl derivative, making this amsacrine derivative worthy of further testing.

The DNA-intercalating agent amsacrine [*m*-AMSA, 4′-(9-acridinylamino)methanesulfon-*m*-anisidide, **1**] has proved an effective clinical drug for the treatment of various disseminated tumors, especially acute leukemia,[1,2] and certain lymphomas.[3] However, many clinical trials have shown it to be ineffective or only marginally effective against a wide range of solid tumors in man.[4,5]

Amsacrine is a member of the 9-anilinoacridine series of drugs. A very large number of these now exist,[6] and efficient and flexible synthetic routes have been devised[7,8] that make a much larger number of potential structures easily accessible. Thus, an important goal of current research is to identify analogues of amsacrine that possess enhanced activity against solid tumors. A severe limitation to the effective drug treatment of solid tumors, and one of the principal reasons why many cationic drugs of the DNA-intercalating type are relatively inactive against such tumors notwithstanding high activity against leukemia, is the difficulty of ensuring adequate drug distribution into the solid tumor structure. One approach to selective derivatives of amsacrine with better distribution properties, therefore, is to determine the structural features associated with these properties in biological systems. In earlier work addressing this question for analogues of amsacrine, we employed the L1210 leukemia in vivo, comparing the antitumor effectiveness of compounds when given intraperitoneally (ip) against ip, sc (subcutaneous), and ic (intracerebral) implanted tumors.[9] This system proved to be a severe test for amsacrine derivatives, with many of the ip active compounds proving inactive against sc tumor and only a very few showing activity against ic tumor. This study showed that drugs active against the remotely implanted tumors were more lipophilic than the optimum value for ip activity. A useful structural feature was found to be a 4-CH_3 or 4-OCH_3 group, which greatly enhanced drug distribution to remote sites. In particular, the 4-CH_3 and 4,5-$(CH_3)_2$ derivatives (**2** and **6**; Table I) showed a significant activity against both sc and ic tumors.[9]

A further indication of favorable drug distribution properties is the ability to show activity following oral (po) administration. In addition, antitumor drugs that are effective when given orally are much simpler to administer, so that such a property represents a real clinical advantage. While many uncharged antitumor drugs of the antimetabolite (e.g., 6-mercaptopurine) and alkylating agent (e.g., cyclophosphamide and melphalan) classes are effective when given orally, the DNA-binding agents (usually cationic drugs) are not. In order to have activity when given orally an antitumor drug must be capable of being effi-

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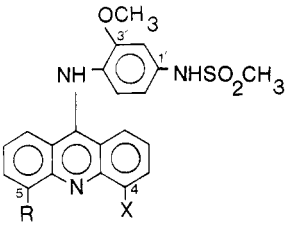
0022-2623/84/1827-0363$01.50/0

Table I. Physicochemical and Biological Data for 4-Substituted and 4,5-Disubstituted Derivatives of Amsacrine



					log K^c		L1210 in vitro:	P388 in vivo			
no.	X	R	Rm[a]	pK_a[b]	AT	GC	ID_{50}[f]	OD[g]	ILS[h]	OD[g]	ILS[b]
1	H	H	0.18	7.43	5.57	5.65	35	13.3	78	65	63
2	CH_3	H	0.25	7.39	6.03	5.96	30	20	143 (2)[i]	65	47 (1)
3	OCH_3	H	0.19	7.39	5.94	6.00	40	30	139 (5)	45	23
4	$CONH_2$	H	−0.27	6.37	5.47	6.13	270	30	113	100	11
5	$CONHCH_3$	H	0.06	6.36	5.54	6.18	270	30	127	100	128 (1)
6	CH_3	CH_3	0.24	6.91	6.14	6.46	110	45	135	100	86
7	CH_3	OCH_3	0.23	6.90	6.21	6.19	30	30	109 (1)	100	69
8	OCH_3	OCH_3	0.32	6.90	6.32	6.40	65	45	99 (4)	100	48
9	CH_3	$CONH_2$	−0.06	6.43	6.62	6.92	47	20	113 (1)	100	25
10	CH_3	$CONHCH_3$	0.34	6.40	6.06	6.64	38	20	200 (3)	50	164 (1)
11	OCH_3	$CONHCH_3$	0.19	6.55	6.24	6.65	17	20	139 (1)	100	34

[a] Rm = a chromatographic measure of drug lipophilicity determined by liquid–liquid chromatography on a cellulose support, as detailed in ref 28. [b] Acridine pK_a values were determined spectrophotometrically in 20% aqueous DMF as detailed in ref 29. [c] Log K values for the binding of drugs to poly[d(A-T)] and poly[d(G-C)] were determined by the fluorometric methods given in ref 15, after allowing for quenching. [d] Drug given by intraperitoneal injection as a solution in 0.1 mL of 30%, v/v, ethanol/water on days 1, 5, and 9 after inoculation of 10^6 P388 leukemia cells. [e] Drug given by gavage directly to the stomach. [f] ID_{50} = the nanomolar concentration of drug that when added to cultures of murine L1210 leukemia cells for a period of 70 h reduces the resultant number of counted cells to 50% of control cultures.[19] [g] OD = optimal drug dose in milligrams per kilogram per day when given on days 1, 5, and 9. [h] ILS = the percentage increase in life span of treated animals over that of control groups bearing tumor, when treated at the optimal dose. The average survival of control mice was 11 days. Values of ILS greater than 20% are considered statistically significant. [i] Numbers in parentheses are the number of animals (out of a group of six) that survived indefinitely.

Table II. Physical and Analytical Data for the New Compounds of Table I

no.	mp, °C	formula	anal.
7	212-214	$C_{23}H_{23}N_3O_4S \cdot HO(CH_2)_2SO_3H$	C, H, N, S
8	263-265	$C_{23}H_{23}N_3O_5S \cdot HCl$	C, H, N, Cl
9	273-275	$C_{23}H_{22}N_4O_4S \cdot HCl \cdot 0.5H_2O$	C, H, N, Cl
10	254-256	$C_{24}H_{24}N_4O_4S \cdot HO(CH_2)_2SO_3H$	C, H, N, S
11	280-282	$C_{24}H_{24}N_4O_5S \cdot HCl$	C, H, N, Cl

ciently absorbed from the gastrointestinal (gi) tract, survive metabolic inactivation by the liver, and possess excellent distributive properties to enable it to reach the tumor site.

In this paper we report a comparison of the results for a number of amsacrine derivatives when tested both ip and po against ip implanted P388 leukemia. In the light of earlier experiments evaluating derivatives against remotely implanted L1210,[9] we have restricted the study to 4-substituted and 4,5-disubstituted derivatives. The results for amsacrine (1) and 10 derivatives are recorded in Table I, together with relevant physicochemical data.

Chemistry. Compounds **1–6** of Table I have been reported previously,[6] as have many of the general procedures for the synthesis of amsacrine congeners.[7,8]

4-Methyl-5-methoxy-9-acridanone, for the preparation of compound **7**, was prepared by the Chapman synthesis, employing a trivial modification of published methods.[10] The 4-methyl- and 4-methoxy-5-carboxy-9-acridanones for compounds **9–11** were elaborated from the respective 3-substituted 2-nitrobenzoic acids by reduction, Jourdan–Ullmann condensation,[11] and H_2SO_4 or PPA ring closure.

DNA Binding. Studies with several series of DNA-intercalating drugs,[12-14] including amsacrine derivatives,[15] have shown a correlation between antitumor activity and the tightness of DNA binding (as determined by binding constants). For the compounds under study, binding to poly(dA-dT) and poly(dG-dC) was determined by an ethidium bromide displacement method,[15] and the values are recorded in Table I. As shown earlier,[14] the 4-CH_3 and 4-OCH_3 groups provide a considerable increase in binding to poly(dA-dT) (2.9- and 2.4-fold, respectively) over that of amsacrine, whereas the carbamoyl and methylcarbamoyl groups provide no such increase (compounds **4** and **5**). The carbamoyl (**4**) and methylcarbamoyl (**5e**) compounds (along with other N-substituted carbamoyl compounds[8]) show increased selectivity for binding to poly(dG-dC).

The generally accepted[6,16] model for amsacrine binding to DNA is with the acridine chromophore intercalated in the same manner as in 9-aminoacridine,[17] with the anilino side chain in the minor groove, as deduced for the phenyl group of ethidium bromide.[18] In this binding mode, 4-

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substituents project into the major groove. For the carboxamides, the alkyl group is likely to project out of the plane of the acridine to relieve nonbonded interactions, and for the alternating poly(dA-dT) copolymer, it would interact sterically with the thymine methyl group. Thus, the observed GC selectivity of these compounds is probably due mainly to lowered binding to AT sites, rather than specific favorable drug–GC interactions.

The disubstituted compounds (6–8) have measured DNA binding constants well approximated by addition of fixed increments for each added methyl or methoxy group, implying a similar geometry and similar environments for both the 4- and 5-substituents. The 4-methyl and 4-methoxy-5-carbamoyl derivatives (9–11) bind somewhat more tightly than expected for simple addition and maintain good G-C selectivity of binding.

In Vitro Activity. For any study comparing the efficacy of antitumor drugs when administered by different protocols, establishment of some measure of base line or intrinsic activity of the compounds against the tumor is desirable. An obvious method is to use the same cell line in culture, and data have been presented previously for amsacrine derivatives, comparing their in vitro and in vivo activity against L1210 leukemia.[19]

The monosubstituted 4-methyl and 4-methoxy derivatives (2 and 3) have ID_{50} values similar to that of amsacrine, which probably reflects their true relative intrinsic activity since these compounds are about as stable as the parent drug to thiol attack (a major cause of drug breakdown in culture). The disubstituted methyl and methoxy compounds (6–8) have somewhat lower intrinsic activity, possibly due to their higher lipophilicities. Interestingly, the methylcarbamoyl-containing disubstituted analogues (10 and 11) have excellent in vitro activity comparable to amsacrine, in spite of higher rates of thiolytic cleavage.[20]

In Vivo Activity. All of the compounds in Table I, show high (in many cases curative) levels of activity against ip implanted P388 leukemia. This uniformly high activity makes it difficult to rank the compounds in terms of efficacy; however, the 4-methyl-5-methylcarbamoyl derivative (10) appears to be more active than the others and is considerably more active than amsacrine.

The activities of the compounds when dosed orally against the same tumor system vary over a much greater range. Amsacrine itself (1) is moderately active, albeit at an optimum dose nearly 5-fold higher than that for ip delivery. Since the optimum dose in this testing is fairly close to the effective LD_{10}, such a great increase in tolerated dose must be due either to significant first-pass liver metabolism prior to entering the general circulation or poor distribution of drug from the gi tract to sites of dose-limiting toxicity. Among the monosubstituted derivatives, the inactivity of the 4-methoxy compound (3) is striking, when compared to its exemplary activity when given ip. The low po activity of the disubstituted compounds 7, 8, and especially 11 suggests that the presence of a 4-methoxy group is the dominant factor. The carbamoyl compounds 4 and 5 show the striking effect of simple N-methylation. While the methylcarbamoyl compound (5) has similar activity whether given ip or po (although at a lowered potency), the nonmethylated derivative (4), equally active ip, is inactive when given po. Again, the effect of this group is dominant, as shown by the disubstituted compounds 9 and 10.

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Discussion

The results of Table I, show that, while all of the derivatives tested show high antitumor activity when dosed ip, activity against the same tumor varies markedly if the drug is given po. The presence of methoxy or carbamoyl substituents, either singly or in combination with other groups, effectively abolish activity. Apart from the possibility of specific metabolism of these compounds in the highly acid stomach, the general reasons for low activity of drugs given po is poor absorption due to excessive hydrophilicity, poor aqueous solubility, and a significant degree of ionization.[21]

The first factor may be a contribution reason for the very poor oral activity of compounds such as 4 and 9, which contain the very hydrophilic carbamoyl group. The same criteria may explain the previously observed[9] beneficial effect of a 4-methyl group on the distributive properties of amsacrine derivatives.

Amsacrine and derivatives are amphiphilic compounds. For the derivatives of Table I, the aqueous pK_a of the acridine nitrogen varies between about 6.6 and 8.0 (on average, 0.59 unit higher than values recorded in Table I, which are determined in 20% DMF)[6], while pK_b for the sulfonamide is about 9.3.[22] Since absorption of the neutral species is assumed to be dominant,[23] the compounds will not be absorbed from the stomach, where they will exist solely as cations. Furthermore, their rate of absorption from the intestine (where the pH varies from about 5 in the duodenum to 8 in the ileum) will be determined significantly by the percentage of neutral species available over that pH range. Substituents that reduce the acridine pK_a provide compounds that have a higher proportion of neutral species over a wider range of pH and, thus, will favor rapid absorption from the gi tract.

While it is possible to accurately calculate the pK_a of acridine derivatives from Hammett constants for 2- and 3-substituted compounds, 4-substituted compounds are weaker bases than predicted, due to varying degrees of steric shielding of proton approach.[6] This is also seen with 4,5-disubstituted derivatives (6–11, especially 10 and 11), which have the advantageous properties of being weak bases. Thus, exceptional oral activity of the 4-methyl-5-methylcarbamoyl derivative (10) may be due to its possession of two metabolically stable substituted groups, which provide a greater percentage of the highly lipophilic free base form.

Conclusions

When searching for "more active" analogues of a proven clinical drug, the test systems used need to be more stringent than the normal screening assays used to detect new classes of potentially useful compounds. This is clearly seen from the data of Table I; the 4-substituted and 4,5-disubstituted amsacrine derivatives studied have such uniformly high activity against ip implanted P388 leukemia that ranking of the compounds on this basic is virtually impossible.

The choice of a suitable "second line" screen is not easy. The data presented here suggest that po administration of drug against the same tumor system is one possibility. The po dosing protocol is well-suited for selecting ana-

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logues with adequate metabolic stability and distributive properties: attributes of importance for solid tumor activity. Thus, for the amsacrine derivatives studied here, the uniformly high activity seen with ip dosing has been effectively diversified by the discovery that methoxy and unsubstituted carbamoyl groups destroy po activity. This result, not predictable from the usual structure–activity studies, clearly selects the 4-methyl-5-(methylcarbamoyl)amsacrine (**10**) as the most worthy analogue for further advanced testing against solid tumor systems.

Experimental Section

Chemistry. Where analyses are indicated only by symbols for the elements, results obtained for these elements were within ±0.4% of the theoretical values. Analyses were carried out at the Microchemical Laboratory, University of Otago, Dunedin, New Zealand, under the direction of Professor A. D. Campbell. Melting points were determined on an Electrothermal apparatus, using the manufacturer's stem-corrected thermometer, and are as read.

4-Methyl-5-methoxy-9-acridanone. This was prepared from methyl *o*-cresotinate and *N*-(2-methoxyphenyl)benzimidoyl chloride by trivial modifications of the general procedures given in ref 10. The crude imidoyl chloride from 64 mmol of *o*-anisidine benzoate was dissolved in 1,1-dimethoxyethane and added dropwise at 20 °C to a suspension of the Na salt of methyl *o*-cresotinate (60 mmol). After 20 h at 20 °C, the solvents were removed under vacuum, and the residue was shaken with aqueous $KHCO_3$ to precipitate an oily material. This was crystallized from benzene–petroleum ether to give colorless prisms of **2-(methoxycarbonyl)-6-methylphenyl *N*-(2-methoxyphenyl)benzimidate** (10.6 g, 45% yield), mp 114–116 °C. Anal. ($C_{23}H_{21}NO_4$) C, H, N.

The above product (28 mmol) was rearranged by heating in Dowtherm A at reflux for 2 h to give **methyl *N*-benzoyl-*N*-(2-methoxyphenyl)-3-methylanthranilate** (9.0 g, 89% yield) as colorless prisms from benzene–petroleum ether, mp 134–135 °C. Anal. ($C_{23}H_{21}NO_4$) C, H, N.

Hydrolysis of the above ester (24 mmol) with KOH, followed by acid precipitation and azeotropic removal of water, gave ***N*-benzoyl-*N*-(2-methoxyphenyl)-3-methylanthranilic acid** (8.2 g, 99% yield), as colorless prisms from benzene–petroleum ether, mp 134–136 °C. Anal. ($C_{22}H_{19}NO_4$) C, H, N.

The above compound (16 mmol) was heated in PPA until all dissolved and then for an additional 1 h at 150 °C, to give **4-methyl-5-methoxy-9-acridanone** (2.6 g, 69% yield) as yellow needles from aqueous EtOH, mp 179–180 °C. Anal. ($C_{15}H_{13}NO_2$) C, H, N.

4,5-Dimethoxy-9-acridanone. 2-Nitro-3-methoxybenzoic acid (51 mmol; prepared by the method of ref 24 as the minor isomer of the nitration of *m*-methoxybenzoic acid) was hydrogenated over Pd/C in aqueous $NaHCO_3$. The filtered solution was treated with AcOH to give 2-amino-3-methoxybenzoic acid (8.3 g, 97% yield), mp 169–171 °C (lit.[25] mp 170–173 °C). This was converted by diazotization to 2-bromo-3-methoxybenzoic acid (73% yield), mp 154–155 °C (from water) (lit.[26] mp 157–158 °C). The bromo acid (68 mmol), *o*-anisidine (72 mmol), K_2CO_3 (68 mmol), and CuO (0.05 g) were heated in 2-ethoxyethanol (20 mL) at 140 °C for 1 h. Dilution with water and acid precipitation gave ***N*-(2-methoxyphenyl)-3-methoxyanthranilic acid** (11.0 g, 59% yield) as yellow leaflets from aqueous EtOH, mp 169–170 °C. Anal. ($C_{15}H_{15}NO_4$) C, H, N.

Treatment of the above acid with PPA at 100 °C for 2 h gave **4,5-dimethoxy-9-acridanone** (6.6 g, 87% yield) as yellow crystals from aqueous DMF, mp 268–269 °C. Anal. ($C_{15}H_{13}NO_3$) C, H, N.

4-Methyl-9-oxoacridan-5-carboxylic Acid. 3-Methylanthranilic acid potassium salt (0.22 mol, generated from the corresponding nitro acid by catalytic hydrogenation), 2-chlorobenzoic acid (0.22 mol), K_2CO_3 (0.25 mol), and Cu/CuBr (1:1, 0.6 g) were heated in a mixture of 2-ethoxyethanol (45 mL) and *N*-methylpyrrolidinone (45 mL) at 150 °C for 2 h. The cooled mixture was diluted with water and acidified with AcOH. The resulting crude product was redissolved in aqueous Na_2CO_3 (500 mL), decolorized with charcoal, and filtered. The filtrate was diluted with EtOH (500 mL), heated to boiling, and neutralized with AcOH to give crystalline ***N*-(2-carboxyphenyl)-3-methylanthranilic acid** (48.9 g, 82% yield), mp 258–259 °C. Anal. ($C_{15}H_{13}NO_4$) C, H, N.

The above product (0.16 mol) was stirred with H_2SO_4 (*d* 1.84, 120 mL) at 90 °C for 4 h and poured into water. The dried crude product was dissolved in DMF (200 mL), EtOH (200 mL), and water containing 12 N NH_4OH (30 mL). After clarification, the boiling solution was acidified with AcOH to give yellow prisms of **4-methyl-9-oxoacridan-5-carboxylic acid** (34.5 g, 85% yield), mp 356–357 °C. Anal. ($C_{15}H_{11}NO_3$) C, H, N.

4-Methoxy-9-oxoacridan-5-carboxylic Acid. This was prepared from 2-amino-3-methoxybenzoic acid and 2-chlorobenzoic acid by the procedures detailed above, via ***N*-(2-carboxyphenyl)-3-methoxyanthranilic acid**, mp 257 °C. Anal. ($C_{15}H_{13}NO_5$) C, H, N. The **acridanone** was crystallized from aqueous DMF as yellow needles, mp 335–337 °C. Anal. ($C_{15}H_{11}NO_4$) C, H, N.

Preparation of the Compounds in Table I. The general procedures for activation of the acridanones to 9-chloroacridines and their mild acid-catalyzed coupling with *N*-(4-amino-3-methoxyphenyl)methanesulfonamide in anhydrous media have been recorded.[27] A modified procedure was used for the 4-substituted 9-oxoacridan-5-carboxylic acid; 1.5 g (6 mmol) was suspended in $SOCl_2$ (20 mL) with a drop of DMF and refluxed gently for 45 min. The reaction mixture was concentrated to a small volume (but not to dryness) and diluted with dry benzene (30 mL) to provide a fine suspension of 4-methoxy-9-chloroacridine-5-carbonyl chloride hydrochloride. This was evaporated to dryness. During the evaporation, the suspension dissolved, indicating loss of the hydrochloride. The residue was dissolved in dichloromethane and added to a cold aqueous solution of excess methylamine. The organic layer was separated, washed with water once to remove traces of unreacted acid, and then dried and evaporated at 40 °C under vacuum to provide *N*-methyl-4-methoxy-9-chloroacridine-5-carboxamide (1.4 g, 4.9 mmol) as a yellow powder. This was suspended in dry MeOH (50 mL) and *N*-(4-amino-3-methoxyphenyl)methanesulfonamide (5.1 mmol) and concentrated HCl (1 drop) were added. The reaction mixture was heated slowly to boiling and then reduced to half volume by evaporation at the boiling point. Hot EtOAc was added slowly until crystallization began. After the mixture was cooled at 20 °C for 4 h, the hydrochloride of compound **11** was collected as red prisms (85% yield), mp 280–283 °C (Table II).

Biological Testing. Cell culture methods for determining ID_{50} values for L1210 leukemia are given in ref 19. P388 leukemia cells were obtained as frozen stock from Mason Research Inc. and passaged intraperitoneally according to standard methods in DBA-2 mice of either sex. Groups of six hybrid mice (DBA-2 male × C_{57} B1 female, weight 20 ± 1 g) were injected intraperitoneally with 10^6 cells on day 0.

Acknowledgment. The authors thank Antoinette Kernohan, Cherry Grimwade, Julie Harris, and Rosalee Nash for their skilled technical assistance and Sally Hill for preparing the manuscript. This work was supported by the Auckland Division of the Cancer Society of New Zealand (Inc.) and by the Medical Research Council of New Zealand.

Registry No. **1**, 51264-14-3; **2**, 51963-57-6; **3**, 76708-48-0; **4**, 76708-54-8; **5**, 76708-55-9; **6**, 53478-42-5; **7**, 88377-18-8; **8**, 80258-05-5; **8** (base), 88377-19-9; **9**, 88377-20-2; **9** (base), 88377-21-3; **10**, 80841-48-1; **11**, 88377-22-4; **11** (base), 88377-23-5; 4-methyl-5-methoxy-9-acridanone, 88377-24-6; methyl *o*-cresotinate,

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23287-26-5; *N*-(2-methoxyphenyl)benzimidoyl chloride, 59386-96-8; 2-(methoxycarbonyl)-6-methylphenyl *N*-(2-methoxyphenyl)-benzimidate, 88377-25-7; methyl *N*-benzoyl-*N*-(2-methoxyphenyl)-3-methylanthranilate, 88377-26-8; *N*-benzoyl-*N*-(2-methoxyphenyl)-3-methylanthranilic acid, 88377-27-9; 4,5-dimethoxy-9-acridanone, 88377-28-0; 2-nitro-3-methoxybenzoic acid, 4920-80-3; 2-amino-3-methoxybenzoic acid, 3177-80-8; 2-bromo-3-methoxybenzoic acid, 88377-29-1; *o*-anisidine, 90-04-0; *N*-(2-methoxyphenyl)-3-methoxyanthranilic acid, 88377-30-4; 4-methyl-9-oxoacridan-5-carboxylic acid, 24782-66-9; 3-methylanthranilic acid, potassium salt, 59425-36-4; 2-chlorobenzoic acid, 118-91-2; *N*-(2-carboxyphenyl)-3-methylanthranilic acid, 80841-45-8; 4-methoxy-9-oxoacridan-5-carboxylic acid, 88377-31-5; *N*-(2-carboxyphenyl)-3-methoxyanthranilic acid, 88377-32-6; 4-methoxy-9-chloroacridine-5-carbonyl chloride hydrochloride, 88377-33-7; *N*-methyl-4-methoxy-9-chloroacridine-5-carboxamide, 88377-34-8; *N*-(4-amino-3-methoxyphenyl)methanesulfonamide, 57165-06-7.

Potential Antitumor Agents. 41. Analogues of Amsacrine with Electron-Donor Substituents in the Anilino Ring

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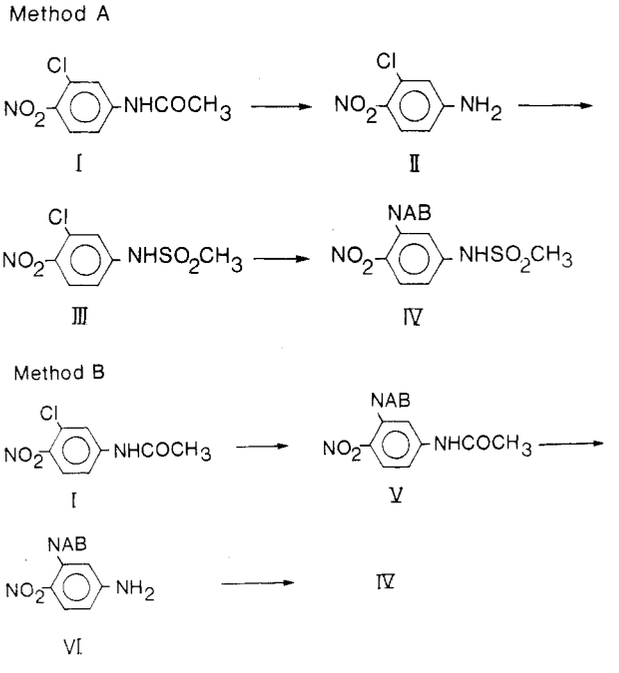
The preparation and antitumor activity of a series of 3′-alkylamino and 3′-dialkylamino analogues of amsacrine are reported. The results support previous work suggesting that the presence of electron-donating groups in the 3′-position of the anilino ring substantially enhance the antitumor activity of amsacrine analogues, possibly by the provision of high levels of electron density at the 6′-position. The alkylamino derivatives generally possess tighter DNA binding, higher levels of in vitro and in vivo antileukemic activity, and greater aqueous solubility than the corresponding amsacrine analogues.

During the early work that resulted in development of the clinical antileukemic agent amsacrine (*m*-AMSA, **6**)[1] from the general class of 9-anilinoacridine antitumor agents, the importance of electron-donor substituents on the anilino ring was noted.[2,3] For 1′-substituted derivatives of 9-anilinoacridine, there is an absolute requirement for electronically neutral or electron-donating groups at this position in order to preserve biological activity. The biochemical reasons for such a requirement are not entirely clear, for several important drug properties, such as acridine pK_a and drug–DNA binding constants, vary in a collinear fashion with the electronic properties of the 1′-substituent.[4] Similar structure–activity relationships were found for derivatives of the parent compound AMSA (**1**), where the effect of other aniline substituents on antitumor activity was examined.[3] For a series of 3′-substituents of varying electronic properties, those compounds (**2–4**) bearing electron-withdrawing groups proved inactive and nontoxic against the L1210 leukemia, both in vivo and in vitro, whereas those (e.g., **6**, **10**, and **11**) bearing small electron-donating groups at the 3′-position proved equally active to the parent compound. A hypothesis[3] that high electron density in the 6′-position of the anilino ring was required for antitumor activity was supported by the two AMSA analogues **12** and **13**. Both compounds have generally electron-deficient anilino rings, but the 2′-analogue **13**, where the electron-rich pyridyl nitrogen can act as a substitute for an electron-rich 6′-position, has appreciable biological activity.[3]

However, the substituent in the 3′-position has to be small enough to allow normal DNA binding of the drug to occur. An increase in the size of the 3′-substituent (e.g., **6–9**) results in steady decrease in biological activity and in the association constant (K) for the binding of the drugs to poly[d(A-T)][5,6] (Table I). This is compatible with current models of the drug–DNA complex, where the acridine chromophore binds by intercalation and the anilino ring makes further binding contacts in the minor groove.[7,8]

These results prompted the examination of other small 3′-substituents with powerful electron-donating properties,

Scheme I



and the 3′-OH and 3′-NH_2 compounds (**10** and **11**) have already been reported.[3] While possessing high activity, the dose potency of these compounds was no better than that

†Deceased 1981

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