Spectroscopy Section of Southern Research Institute who performed most of the microanalytical and spectral determinations.

Registry No. **3**, 6506-86-1; **4**, 86970-41-4; **5**, 86970-42-5; **6**, 86970-43-6; **7**, 86970-44-7; **8a**·$^1/_2$HCl, 86970-45-8; **8b**·HCl, 86970-46-9; **8c**, 86970-47-0; **8d**·HCl, 86970-48-1; **8e**, 86970-49-2; **8f**, 86970-50-5; **8g**, 86970-51-6; **8h**, 86970-52-7; **8i**, 86970-53-8; **8j**, 86970-54-9; **9a**, 86970-55-0; **9b**·HCl, 86970-57-2; **9c**·HCl, 86970-58-3; **9d**, 86970-59-4; **9e**, 86970-60-7; **9f**, 86970-61-8; **12**, 86970-62-9; **13**, 86970-63-0; **13**·HBr, 86970-64-1; α-bromo-3,4,5-trimethoxyacetophenone, 51490-01-8; α-bromo-3,5-dimethoxypropiophenone, 72661-28-0; 2-bromo-1-tetralone, 13672-07-6; potassium thioacetate, 10387-40-3; thiourea, 62-56-6; α-bromo-*p*-chloroacetophenone, 536-38-9; α-bromoacetophenone, 70-11-1; α-bromopropiophenone, 2114-00-3; *N*,*N*-dimethylformamide dimethyl acetal, 4637-24-5; bromoacetic anhydride, 13094-51-4; **7**·Zn, 86970-65-2; **9b**, 86970-56-1.

Potential Antitumor Agents. 38. 3-Substituted 5-Carboxamido Derivatives of Amsacrine

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The synthesis and biological evaluation of a series of 3-substituted 5-carboxamido derivatives of amsacrine (*m*-AMSA) are described. This series was developed as the result of previous quantitative structure–activity relationship (QSAR) studies of the antitumor activity of 9-anilinoacridine derivatives. In agreement with these studies, this class of compounds, possessing a variety of small nonpolar groups at the 3-position, together with very hydrophilic carboxamido groups at the 5-position, have high in vivo activity against animal leukemia models.

The clinically useful antileukemic agent amsacrine (*m*-AMSA)[1-3] is a member of the 9-anilinoacridine class of antitumor agents. Compounds of this class bind tightly to double-stranded DNA[4,5] by intercalation, as shown by helix-unwinding studies with closed circular DNA[6] and by high-field NMR studies with very short DNA fragments.[7] In common with other DNA-binding agents, such as the anthracyclines and the anthracenediones, amsacrine and other 9-anilinoacridines cause DNA breaks[8] and chromosome damage.[9] Studies of 9-anilinoacridines[10] have shown a significant correlation between antitumor activity and DNA association constants, determined in vitro by using either calf thymus DNA or poly(dA-dT). Thus, the DNA-binding ability of these compounds is thought to be an important component in the mechanism of their biological activity.

Work over the last several years on the general class of 9-anilinoacridine antitumor agents has shown the extraordinarily wide range of structural variations permitted to the parent structure while still retaining biological activity.[3] these structural variations, by modulating various aspects of the binding of the drug to DNA, can have a significant effect on biological activity, both in vivo and in vitro. For the several hundred structures examined so far, in vivo potency against the L1210 leukemia (the dose required to give a standard response of a 40% increase in life span) varies by greater than 3000-fold,[11] while in vitro potency against the same tumor varies by more than 60 000-fold.[12]

Recently, we have published an extensive QSAR study for the in vivo antileukemic (L1210) activity of a large number of 9-anilinoacridine derivatives.[11] While overall drug lipopohilicity and pK_a values (determined by summation of the electronic contributions of all substituent groups on the molecule) were both found to influence antitumor potency, by far the most significant influence was the steric effects of groups placed at various positions on the 9-anilinoacridine skeleton. The current[3,4,11] model for the binding of 9-anilinoacridines to DNA was based on the published[12] X-ray crystallographic data for the pseudosymmetric orientation found for 9-aminoacridine bound to iodo-dCpG and assumes intercalation of the acridine chromophore with the 9-anilino group lying in the minor groove and the 4- and 5-positions of the acridine ring oriented toward the major groove. Crystallographic studies of the DNA intercalator ethidium bromide complexed with dinucleotides have shown a similar orientation of the phenyl ring of this compound.[13] X-ray studies of several

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AMSA (1′-methanesulfonamido-9-anilinoacridine) derivatives[14,15] show the anilino ring at a considerable angle to that of the acridine and capable of orientating the acridine ring in such a way that the 1- and 2-positions are severely occluded by the sugar–phosphate chains of the DNA, with the 4- and 5-positions freely accessible from the major groove and the 3-position in a situation of intermediate steric freedom.

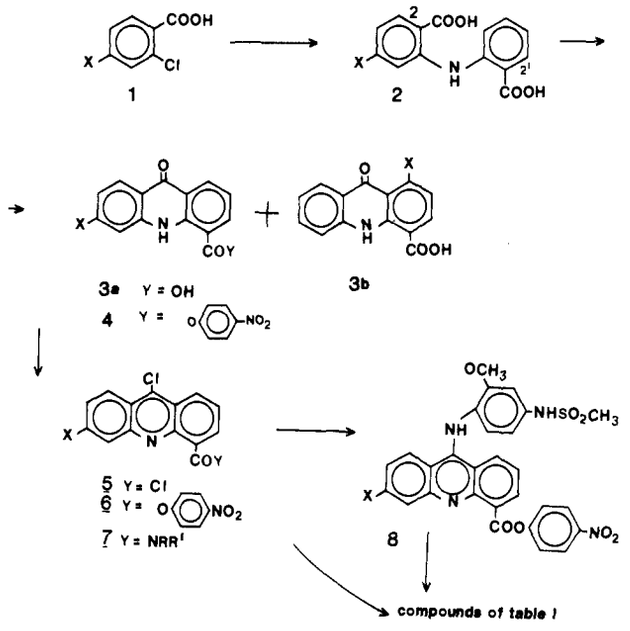
The QSAR study[11] for the in vivo antileukemic activity of 9-anilinoacridine derivatives showed a pattern of steric restraint around the acridine that is entirely consistent with this binding geometry. Groups appended to the 1-position abolished activity, while those at the 2-position were dystherapeutic in direct relationship to their size. In contrast, small groups placed at the 3-position considerably enhanced activity, but there was an optimum group size; groups larger than the optimum again became dystherapeutic. No steric hindrance was observed for groups at the 4-position, as expected if this position faces the major groove. While small groups at the 3-position enhanced activity, 3,6-disubstitution by even small groups was disadvantageous, indicating a fairly constricted binding site.

A comparison of the effects of acridine substitution on DNA association constants for acridine-substituted AMSA derivatives showed the same pattern.[10] Substitution at the 2-position reduced DNA binding, as did very bulky groups in the 3-position, whereas large substituents at the 4-position had essentially no effect.

The results and conclusions of the above studies led to the design of amsacrine analogues with new patterns of substitution in the acridine ring, as part of a continuing search for more active derivatives of the parent drug. The QSAR study cited above[11] demonstrated, in agreement with many other past studies of in vivo drug activity, the importance of drug lipophilic–hydrophilic balance as a determinant of such activity and showed amsacrine itself to be slightly more lipophilic than the optimal value for in vivo antileukemic activity. The study also clearly showed the therapeutic effect of small groups placed at the 3-position of the acridine, although such derivatives (e.g., 3-CH_3, 3-halogen) are even more lipophilic than amsacrine itself. In addition, amsacrine has limited water solubility, and thus any attachment of relatively lipophilic 3-substituents needs to be coupled with concomitant modifications that will reduce the overall lipophilicity and increase water solubility of the resulting compounds to values similar to those for amsacrine itself.

The best place for the attachment of groups to reduce log P is clearly the 4-position (or equivalent 5-position) on the acridine ring, where appended groups project into the major groove with little or no inhibitory effect on DNA binding.[10] It is even possible that polar groups in this position, able to interact with a polar functionality in the major groove, might additionally enhance DNA binding. Placement of the polar group in the 4-position would result in an ortho-disubstituted acridine. It is well-known that electronic and hydrophobic properties are not strictly additive for ortho-disposed groups;[17] the widely accepted reason is that steric effects interfere, with the groups being crowded out of plane. If such were the case, the resulting steric crowding might interfere with the intercalation step

Scheme I



in the binding of such molecules to the DNA. A study of the DNA binding of a limited series of 3,4-disubstituted amsacrine analogues shows some drop in DNA binding over that of the parent 3- or 4-substituted derivatives. More importantly, we have previously shown[18] that 3,4-disubstitution leads to considerable decreases in in vivo activity for amsacrine derivatives. Thus, for the present work the 3,5-disubstitution pattern was considered the most appropriate for employing small groups in the 3-position coupled with a polar functionality at the 5-position. This substitution pattern has already been explored in a preliminary way,[19] when the acceptability of 4-ether and 4-carboxamido functions as polar groups were noted. The 3-iodo-5-(2,3-dihydroxypropoxy) analogue (compound **55** in ref 19) proved exceptionally active against the L1210 leukemia,[19] but the decrease in log P seen with the poly(hydroxy) functions is not as great as might be expected, due to extensive polar interactions.[20] The carboxamido derivatives proved to be much more polar, and thus the present paper presents information on the preparation and antitumor properties of a series of 3-substituted 5-carboxamido derivatives of amsacrine.

Chemistry. The most versatile synthesis of the 9-chloroacridines required for the preparation of the compounds in Table I is by $POCl_3$-induced cyclization of diphenylamine-2-carboxylic acids or by $SOCl_2$ activation of the 9-acridanones, which in turn were produced from diphenylamine-2-carboxylic acids by acid-catalyzed cyclization.[21] The diphenylamine-2-carboxylic acid are most conveniently prepared by the Jourdan–Ullmann condensation of suitable amine and 2-chlorobenzoic acid components as shown in Scheme I.[21,22] This reaction is inhibited by electron-withdrawing substituents on the amine com-

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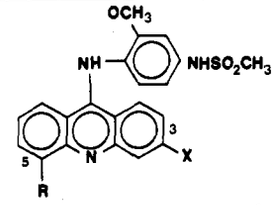
Table I. Physicochemical and Biological Data for 3-Substituted 5-Carboxamido Derivatives of Amsacrine



no.	X	R	Rm[a]	pK_a[b]	log K AT[c]	log K GC[d]	L1210 ID_{50}[e]	P388 OD[f]	P388 ILS[g]
11	H	H	0.18	7.43	5.57	5.65	35	13.3	78
12	NH_2	H	0.06	9.50	6.21	6.13	5.5	8.0	116
13	NO_2	H	−0.08	5.52	5.65	6.13	110	5.9	73
14	CH_3	H	0.44	7.70	5.95	6.08	12	10	120 (1)[h]
15	OCH_3	H	0.29	7.81	5.83	5.97	22	8.9	196 (4)
16	Cl	H	0.32	6.84	6.06	5.98	70	20	232
17	Br	H	0.34	6.84	6.29	6.12	50	20	133 (2)
18	I	H	0.38	6.77	6.35	6.20	50	30	137
19	H	$CONH_2$	−0.27	6.37	5.47	6.13	270	30	113
20	H	$CONHCH_3$	0.06	6.36	5.54	6.18	270	30	127
21	H	$CONHCH_2CONH_2$	−0.50	6.18	5.39	6.40	850	100	125 (1)
22	NH_2	$CONHCH_3$	0.00		6.29	6.82	21	3.9	133 (3)
23	NO_2	$CONH_2$	−0.34	4.71	5.96	6.40	350	20	80
24	NO_2	$CONHCH_3$	0.08	(4.71)[i]	5.71	6.65	303	20	77
25	CH_3	$COOC_6H_4$-p-NO_2							
26	CH_3	$CONH_2$	0.05	(4.71)	6.40	6.30	200	30	139
27	CH_3	$CONHCH_3$	0.32	(4.71)	6.22	6.68	170	30	171 (1)
28	CH_3	$CONHCH_2CONH_2$	−0.32	(4.53)	6.00	6.69	460	20	125 (2)
29	OCH_3	$CONHCH_3$	0.19	(4.82)	6.38	6.82	68	30	182 (4)
30	Cl	$COOC_6H_4$-p-NO_2							
31	Cl	$CONH_2$	−0.09	5.68	6.33	6.58	520	65	88 (1)
32	Cl	$CONHCH_3$	0.23	(5.68)	6.29	6.65	217	100	107 (4)
33	Cl	$CONHCH_2CONH_2$	−0.23	(5.50)	6.06	6.83	890	20	129 (1)

[a] Rm in the chromatographic measure of drug lipophilicity determined by liquid–liquid chromatography on a cellulose support, as detailed in ref 31. Rm values are linearly related to log P (octanol) values (ref 11). [b] Acridine pK_a values were determined spectrophotometrically in 20% aqueous DMF as detailed in ref 32. [c] Log K values for the binding of drugs to poly[d(A-T)] were determined by the fluorometric methods given in ref 26, after allowing for quenching. [d] Log K values for binding to poly[d(G-C)]. [e] ID_{50} is the nanomolar concentration of drug that when added to cultures of murine L1210 leukemia cells for a period of 70 h, reduces the resultant counted number of cells to 50% of that of control cultures.[27] [f] OD is the optimal drug dose, in milligrams per kilogram per day, administered intraperitoneally 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. The drug is given as a soluble acid addition salt, usually the hydrochloride. [g] ILS is the percentage increase in life span of treated animals over that of control groups of animals injected with tumor alone. The average survival of control mice was 11 days. Values of ILS greater than 20% are considered statistically significant. [h] Numbers in parentheses are the number of animals (out of a group of six) that survived indefinitely. [i] pK_a values in parentheses are estimated.

ponent.[23] A notable exception to this general rule is the reaction of 2-chlorobenzoic acids with anthranilic acid to provide the diphenylamine-2,2′-dicarboxylic acids (e.g., **3**); these reactions proceed under relatively mild conditions (30 min at 100–120 °C) to give almost quantitative yields of condensation product.

Preparation of 3-substituted 5-carboxy-9-acridanones (**3**) by the method of Scheme I required 2-chloro-4-substituted-benzoic acids (**1**). The 4-chloro, 4-bromo, and 4-nitro acids are commercial products, and the 4-iodo and 4-methoxy derivatives were prepared from the 4-nitro acid by reduction and diazotization. The 4-methyl acid was elaborated from p-toluidine by acetylation, bromination, and diazotization–cyanation, followed by acid hydrolysis of the bromocyanotoluene.

Jourdan–Ullmann condensation of these acids with anthranilic acid at 120–130 °C gave almost quantitative yields of the 5-substituted diphenylamine-2,2′-dicarboxylic acids (**2**). Ring closure of these diacids can proceed in one of two ways to form either 1- or 3-substituted 5-carboxy-acridanones (**3a** or **3b**). The effect of different cyclizing conditions and the steric and electronic effect of the substituent groups on the course of the ring closure have recently been studied.[24] It was found that the conditions of ring closure had little effect on the product composition, which is to be expected as the reaction in all cases is thought to proceed via the acylcarbonium ion.

For the 5-substituted diacids, the most important influence is the electronic nature of the substituent group. Electron-donating groups generally favor ring closure via the 2-acid by stabilizing this carbonium ion intermediate to provide the 3-substituted 5-carboxy-9-acridanone (**3a**) as the main product. In contrast, electron-withdrawing groups favor formation of 1,4-disubstituted 9-acridones (**3b**), although unfavorable steric effects for this direction of ring closure ensure that mixtures of products are always formed.

Since the carboxyacridanones are high-melting, insoluble, poorly crystalline solids, separation of the isomer mixtures resulting from ring closure was tedious. The desired 3,5-disubstituted compounds (**3a**) were the more insoluble, and in the case of the methyl and methoxy analogues, these formed the main product from the ring closure and could be purified by repeated crystallization of the methyl esters. The desired 3-halo-5-carboxy-9-acridones, obtained as minor products of the ring closure,

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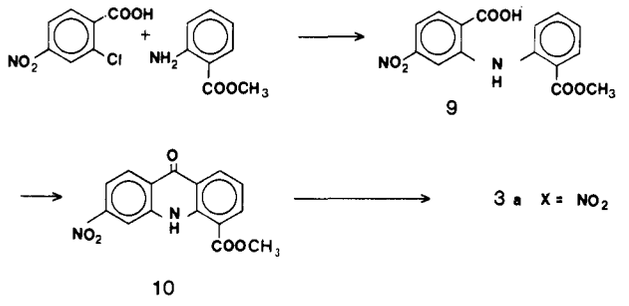
(24) Unpublished data, this laboratory.

Scheme II



were purified by fractional crystallization of their potassium salts from aqueous EtOH. This procedure was not suitable for separation of the nitro isomers, which were unstable to base. Although the mixture of isomers from the ring closure of the 5-nitrodiphenylamine-2,2′-dicarboxylic acid could be separated by repeated fractional crystallization of the methyl esters, the desired 3,5-isomer, as the minor product of the ring closure, was obtained in only very low yield. Thus, the use of suitably protected anthranilic acids in the Jourdan–Ullmann reaction was examined. Such a protective group needs to withstand the severe conditions of the reaction and yet be readily removed from the sterically hindered 5-acid afterwards. An added problem is that most acid-protecting groups are electron withdrawing, thus acting to inhibit the reaction. The route shown in Scheme II was eventually used, employing methyl anthranilate as the amine components, together with considerable changes in the conditions of the Jourdan–Ullman reaction, particularly including the use of organic bases as combined solvent and acid acceptor, to prevent competing hydrolysis of the methyl anthranilate.

Ring closure of the half ester (**9**) was effected with polyphosphate ester (prepared from phosphorus pentoxide and diethyl ether) to give the 9-acridone ester (**10**), which was hydrolyzed slowly (90% H_2SO_4, 100 °C, 7 h) but quantitatively to give the desired product (**3a**, X = NO_2).

As noted previously,[19] several alternative routes to carboxamido derivatives of *m*-AMSA are available. Treatment of the 3-substituted 5-carboxy-9-acridones with $SOCl_2$–DMF provided the 9-chloro-5-(chloroformyl) derivatives, which react selectively with aliphatic amines at low temperatures under basic, anhydrous conditions to give excellent yields of the 9-chloro-5-carboxamido derivatives. For the production of 5-$CONH_2$ and 5-$CONHCH_3$ derivatives, a more covenient approach was to add dropwise a solution of the acid chloride in CH_2Cl_2 to an ice-cold, stirred, aqueous solution of excess amine. A small amount (less than 10%) of acid was also produced when this method was used, but this was readily separated. The 9-chloro carboxamides so produced could be coupled with the amsacrine side chain under the usual, mildly acid conditions.

Where a number of different carboxamide derivatives of a single 9-acridanone were needed, a more convenient method was via formation of the 3-substituted 5-(*p*-nitrophenyl) ester (**4**), followed by the usual activation and coupling to provide the protected amsacrine derivatives **8** (e.g., compounds **25** and **30** of Table I). Displacement of the *p*-nitrophenyl ester with a variety of amines in DMF provided the required carboxamides of Table I. The 3-NH_2 derivative (**22**) was prepared from the corresponding 3-nitro derivative (**24**) by reduction. Attempts to use suitably protected derivatives of 3-amino-5-carboxy-9-acridanone failed, due to the intractable nature of this zwitterion.

One unexpected problem was the lability of the halogen groups of the 3-halo-5-carboxy-9-acridanones to replacement by chlorine during activation with $SOCl_2$. We had previously observed that the isomeric 1-halo-4-carboxy-9-acridanones possessed very labile halogen groups. One method for separation of isomers was to form the methyl esters by treatment with $SOCl_2$, followed by MeOH, with subsequent hydrolysis of the 9-chloro group. Fractional crystallization then provided the more polar 1,4-isomer pure. However, for the bromo and iodo analogues it was found that quantitative replacement of the halogen by chlorine had occurred during the $SOCl_2$ treatment.

The 3-bromo- and 3-iodo-5-carboxy-9-acridanones were later prepared, for the work described in this paper, by fractional crystallization of the potassium salts, as described above. Usual $SOCl_2$ activation and coupling provided derivatives of amsacrine, but elemental analysis again showed that replacement of halogen by chlorine had occurred, and it proved impossible to prepare the 3-bromo- and 3-iodo-5-(methylcarboxamido) compounds by these methods. This unexpected lability of the halogen group of 3-halo-5-carboxy-9-acridanones was confirmed by subjecting 3-bromo-5-carboxy-9-acridanones to usual activation with $SOCl_2$. Subsequent hydrolysis of the 9-chloro-5-(chloroformyl) compound (**5**) gave a quantitative yield of the 3-chloro derivative (**3a**, X = Cl).

Results and Discussion

DNA Binding. Table I records the physicochemical and biological data for the new series of amsacrine derivatives (**22** and **23**), together with the same data for the parent compound amsacrine (**11**) and the appropriate monosubstituted derivatives (**12–21**). Use is made of the ethidium displacement assay[25,26] to determine selectivity of DNA binding to the two alternating copolymers poly(dA-dT) and poly(dG-dC). 3-Substitution of the acridine ring with lipophilic groups enhances DNA binding. The 3-halogen compounds (**16–18**) showed a slight but consistent selectivity for poly(dA-dT), whereas the 3-nitro group provided a selective increase in binding to poly(dG-dC) (compound **13**). This selective increase in binding was also observed with the 4-carboxamido derivatives (**19–21**), the degree of selectivity increasing with the size of the N-substituent.

The influence of the carboxamide groups on DNA binding behavior was most marked in the 3,5-disubstituted compounds bearing a 3-methyl, 3-methoxy, or 3-chloro group. The 5-carboxamido increased binding to both polymers (**26** and **31**), and substitution at the amide nitrogen considerably increased the G-C selectivity of binding (**27**, **28**, **32**, and **33**). The glycinamide substituent had little effect on binding to poly(dA-dT) but increased binding to poly(dG-dC) by almost 10-fold. In our current model of DNA binding, the 5-substituent projects into the major groove of the DNA; disubstitution may have the effect of limiting movement within the intercalation site and increasing selectivity against A-T pairs because of interaction of the substituted carboxamide with the thymine methyl group. It will be interesting to determine what effects these different substituents have on the kinetics of DNA binding.

Inhibition of Growth of Cultured L1210 Cells. The methods used for the assay have been previously published.[27] For this limited series there is no significant

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(26) Baguley, B. C.; Denny, W. A.; Atwell, G. J.; Cain, B. F. *J. Med. Chem.* **1981**, *24*, 170.

correlation of in vitro activity with DNA binding or hydrophobicity, which were significant variables with a larger series. However, there is a good correlation ($r = 0.80$) with acridine base pK_a:

$$\log (1/ID_{50}) = 0.37pK_a - 4.39$$

the most basic derivatives being most potent.

In a larger series of amsacrine derivatives, this dependence on pK_a was also observed,[28] and in the present limited series, this term may mask the dependence on other parameters. The dependence on pK_a is probably a result of a dependence on thiolytic half-life, which is a function of pK_a.[10] Amsacrine derivatives are known to react with thiols[29] and probably break down in culture in response to the presence of sulfhydryl-containing components of the growth medium.

In Vivo Antitumor Activity. With the exception of the 2-NO_2 derivative **13** (which may be metabolized in vivo), the more weakly basic compounds are somewhat less dose potent than the others. This dependence is less marked than in the culture system, as reported previously, for a larger series of derivatives, since biological half-life in vivo is determined at least in part by pathways of degradation other than thiolysis.[30]

The P388 leukemia is less responsive to amsacrine than is the L1210 leukemia, and thus the large increases in P388 antitumor activity provided by monosubstitution of amsacrine at the 3- or 4-position (Table I) is particularly noteworthy. As measured by increased life span, the activity of the carboxamides increases in the order carboxamide, *N*-methylcarboxamide, glycinamide. This pattern is seen for the monosubstituted compounds, as well as for the combinations with 3-methyl and 3-chloro substituents. Since selectivity for binding to poly(dG-dC) increases in the same order for these compounds, it is quite possible that activity in the P388 leukemia is a function of sequence selectivity of DNA binding. The most selective agents are among the most active compounds so far found in the amsacrine series. The nitro compounds (**23** and **24**) are exceptions in that they are selective but only moderately active. However, these compounds may be rapidly metabolized in vivo.

Conclusions

The biological results listed in Table I generally bear out the conclusions and predictions made from previous QSAR studies of amsacrine derivatives. The carboxamido groups provide an excellent way of producing very hydrophilic derivatives (as measured by Rm values), with good water solubility for formulation, while the small, nonpolar 3-substituents retain high antitumor activity, in spite of lower pK_a values. As a class, the 3-substituted 5-carboxamido compounds are among the most active derivatives of amsacrine against laboratory leukemia models, which suggests that this pattern of acridine substitution may be close to optimal. They provide a group of compounds, with very high in vitro and in vivo activity, where a number of important physicochemical parameters (log *K*, Rm, and pK_a) are widely varied. Thus, they are of potential use for wider testing against a panel of tumor lines, particularly solid tumor models, and this work is in progress.

Table II. Analytical Details for New Amsacrine Derivatives

no.	mp, °C	formula	anal.
22	>360	$C_{23}H_{23}N_5O_4S\cdot HCl$	C, H, N, Cl
23	286–289	$C_{22}H_{19}N_5O_6S\cdot HCl$	C, H, N
24	238–240	$C_{23}H_{21}N_5O_6S\cdot HCl$	C, H, N
25	239–241	$C_{29}H_{24}N_4O_7S\cdot HCl$	C, H, N
26	302–305	$C_{23}H_{22}N_4O_4S\cdot HCl$	C, H, N, Cl
27	311 dec	$C_{24}H_{24}N_4O_4S\cdot HCl$	C, H, N, Cl
28	254–256	$C_{25}H_{25}N_5O_5S\cdot HCl$	C, H, N, Cl
29	255–257	$C_{24}H_{24}N_4O_5S\cdot HCl$	C, H, N
30	253–255	$C_{28}H_{21}ClN_4O_7S\cdot HCl$	C, H, N, Cl
31	290–291	$C_{22}H_{19}ClN_4O_4S\cdot HCl$	C, H, N, Cl
32	300–303	$C_{23}H_{21}ClN_4O_4S\cdot HCl$	C, H, N, Cl
33	248–251	$C_{24}H_{22}ClN_5O_5S\cdot HCl$	C, H, N, Cl

Experimental Section

For analyses indicated by symbols of the elements, analytical results obtained for these compounds 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 maker's supplied stem-corrected thermometer; melting points are as read.

2-[[2-(Methoxycarbonyl)phenyl]amino]-4-nitrobenzoic Acid (9). 2-Chloro-4-nitrobenzoic acid (7.0 g, 35 mmol), methyl anthranilate (9.0 g, 60 mmol), and cupric acetate (6.3 g, 35 mmol) were suspended in a mixture of *N*-methylpyrrolidone (5 mL) and *N,N*-diisopropylethylamine (10 mL). The mixture was stirred at 160 °C for 2 h under N_2, cooled, diluted with water, and acidified to pH 2. The gummy precipitate was collected by decantation and triturated with MeOH to give a red solid suitable for the next step (3.7 g, 34%). Recrystallization from EtOAc gave red needles: mp 243–245 °C. Anal. ($C_{15}H_{12}N_2O_6$) C, H, N.

Methyl 3-Nitro-9-oxoacridan-5-carboxylate (10). The above half ester (1.0 g, 3.2 mmol) was heated with polyphosphate ester at 100 °C for 1 h. The cooled product was diluted with water and basified to pH 9. The insoluble product was collected and crystallized from ethanol as yellow prisms: mp 252–253 °C. Anal. ($C_{15}H_{10}N_2O_5$) C, H, N.

3-Nitro-5-carboxy-9(10*H*)-acridanone (3a, X = NO_2). The above acridone ester (1.5 g, 5.0 mmol) was heated in sulfuric acid (20 mL; 92%, v/v) for 7 h 100 °C. The cooled mixture was diluted with water, and the product was collected and washed. Trituration with 2 N aqueous Na_2CO_3, followed by removal of insoluble products by filtration and acidification of the filtrate, gave the acid (1.29 g, 90% yield). A sample was crystallized from a large volume of EtOH, mp 375 °C. Anal. ($C_{14}H_8N_2O_5$) C, H, N. Attempted ester hydrolysis under basic conditions gave impure, deeply colored products.

***p*-Nitrophenyl 3-Nitro-9-oxoacridan-5-carboxylate (4, X = NO_2).** The above acid (3.5 g, 12.3 mmol) and *p*-nitrophenol (1.8 g, 12.9 mmol) were suspended in dry pyridine (60 mL). PCl_3 (0.6 mL, 6.8 mmol) was added, and the mixture was heated rapidly to boiling to dissolve all solids. (Filter if necessary.) On cooling, the product separated and was then collected and washed well with EtOH: yield 3.3 g (63%). A sample was recrystallized from DMF as yellow needles, mp >360 °C . Anal. ($C_{20}H_{11}N_3O_7$) C, H. N.

2-[(2-Carboxyphenyl)amino]-4-methylbenzoic Acid (2, X = CH_3). A suspension of 2-bromo-4-methylbenzoic acid (43 g, 0.2 mol), 2-aminobenzoic acid (30 g, 0.22 mol), and dry K_2CO_3 (42 g, 0.30 mol) in 2-ethoxyethanol (250 mL) was gently heated until gas evolution ceased. Copper powder/cuprous oxide (1:1, 0.5 g) was added, and the stirred mixture was heated to 120 °C. At this temperature, gas evolution began, and the mixture eventually solidified. After 30 min, the cooled mixture was diluted with water and then filtered, and the filtrate was acidified to provide the diacid (52 g, 96% yield). A sample was crystallized from EtOAc as prisms: mp 302–304 °C. Anal. ($C_{15}H_{13}NO_4$) C, H. N.

3-Methyl-5-carboxy-9(10*H*)-acridanone (3a, X = CH_3). The above diacid (50 g, 0.18 mol) was finely powdered, and the

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Table III. Analytical Details for New 3,5-Disubstituted 9(10*H*)-Acridanones

substituents	mp, °C	formula	anal.
3-NH_2, 5-COOH	348–349	$C_{14}H_{10}N_2O_3 \cdot H_2O$	C, H, N
3-NO_2, 5-COOH	>360	$C_{14}H_8N_2O_5$	C, H, N
3-NO_2, 5-$COOCH_3$	252–253	$C_{15}H_{10}N_2O_5$	C, H, N
3-NO_2, 5-$COOC_6H_4$-*p*-NO_2	>360	$C_{20}H_{11}N_3O_7$	C, H, N
3-CH_3, 5-COOH	337–338	$C_{15}H_{11}NO_3$	C, H, N
3-CH_3, 5-$COOC_6H_4$-*p*-NO_2	279–281	$C_{21}H_{14}N_2O_5$	C, H, N
3-OCH_3, 5-COOH	322–324	$C_{15}H_{11}NO_4$	C, H, N
3-Cl, 5-COOH	>360	$C_{14}H_8ClNO_3$	C, H, N, Cl
3-Cl, 5-$COOC_6H_4$-*p*-NO_2	311–312	$C_{19}H_{11}ClN_2O_5$	C, H, N, Cl
3-Br, 5-COOH	>360	$C_{14}H_8BrNO_3$	H, N, Br; C[a]
3-I, 5-COOH	>360	$C_{14}H_8INO_3$	H, N; C[a]

[a] C out by 0.6%.

well-dried powder was added slowly to stirred concentrated H_2SO_4 (150 mL). The eventual solution was heated at 90 °C for 2 h and poured slowly into stirred boiling water. After the solution was cooled, the product was collected by filtration and washed well with water. Repeated crystallization of the mixture of isomers **3a** and **3b** from large volumes of EtOH gave pure **3a** (the major, less soluble and more polar isomer): yield 19.0 g (41%); mp 337–338 °C. Anal. ($C_{15}H_{11}NO_3$) C, H, N.

***p*-Nitrophenyl 3-Methyl-9-oxoacridan-5-carboxylate (4, X = CH_3).** This was prepared by the method given above to yield needles, mp 279–281 °C. Anal. ($C_{21}H_{14}N_2O_5$) C, H, N.

2-[(2-Carboxyphenyl)amino]-4-methoxybenzoic acid (2, X = OCH_3), mp 270–271 °C. [Anal. ($C_{15}H_{13}NO_5$) C, H, N], and **3-methoxy-5-carboxy-9(10*H*)-acridanone (3a, X = OCH_3),** mp 322–324 °C. [Anal. ($C_{15}H_{11}NO_4$) C, H, N], were prepared by trivial modifications of the above procedures.

3-Chloro-5-carboxy-9(10*H*)-acridanone (3a, X = Cl). 2-[(2-Carboxyphenyl)amino]-4-chlorobenzoic acid (**2**, X = Cl; 50 g) (made from 2,4-dichlorobenzoic acid and 2-aminobenzoic acid by the above method) was dissolved in concentrated H_2SO_4 (150 mL) and kept at 100 °C for 2 h. The mixture was poured slowly into hot water and boiled briefly. The precipitate was collected and washed well with water to give a mixture of carboxyacridones (**3a** and **3b**, X = Cl; 45 g, 96%).

This crude mixture (70 g) was suspended in boiling EtOH (1200 mL), and a hot solution of KOH (70 g) in water (1200 mL) was added rapidly. All the solids dissolved, followed by precipitation of the potassium salt of 1-chloro-4-carboxy-9(10*H*)-acridanone (**3b**, X = Cl).

The suspension was allowed to cool to 30 °C, and the solid was collected (62 g = 53 g of free acid; 76% yield). The filtrate was concentrated to 1000 mL and kept at 20 °C for 24 h, when the potassium salt of 3-chloro-5-carboxy-9(10*H*)-acridanone (**3a**, X = Cl) precipitated and was collected (12.5 g = 10.3 g of free acid, 15% yield).

The free acids were liberated from the above potassium salts by treatment of the aqueous solution with 2 N HCl, followed by crystallization from DMF. **1-Chloro-4-carboxy-9(10*H*)-acridanone**: yellow needles; mp 321–323 °C. Anal. ($C_{14}H_8ClNO_3$) C, H, N, Cl. **3-Chloro-5-carboxy-9(10*H*)-acridanone**: yellow microcrystals; mp >360 °C. Anal. ($C_{14}H_8ClNO_3$) C, H, N, Cl.

The other 3-halo-5-carboxy-9(10*H*)-acridanones were prepared by the same general method.

***p*-Nitrophenyl 3-chloro-9-oxoacridan-5-carboxylate (4, X = Cl)** was prepared by the method given above to yield yellow prisms, mp 311–312 °C. Anal. ($C_{19}H_{11}ClNO_3$) C, H, N, Cl.

Compound 23 of Table I. The acridone acid (**3a**, X = NO_2; 1.3 g, 4.6 mmol) was finely powdered and heated under reflux in $SOCl_2$ (50 mL) with a trace of DMF. After 1 h, the clear solution was evaporated to dryness, and residual $SOCl_2$ and HCl were removed by azeotroping with drying benzene. The residue was dissolved in CH_2Cl_2 and added slowly to ice-cold concentrated NH_4OH. The CH_2Cl_2 layer was washed with water, dried, and evaporated to give the crude 9-chloro compound (1.1 g). This was suspended in MeOH (50 mL) with 4-aminomethanesulfon-*m*-anisidide (1.05 g, 4.9 mmol), and the mixture was heated to boiling with a trace of HCl. MeOH was then distilled off to half volume, an equal amount of EtOAc was added, and distillation continued until crystallization began. The mixture was cooled well and filtered to give compound **23** of Table I as red needles (1.7 g, 76% yield), mp 286–289 °C. Anal. ($C_{22}H_{19}N_5O_6S \cdot HCl$) C, H, N, Cl.

Compounds **24**, **29**, **34**, and **28** of Table I were prepared from the appropriate acridone acids by trivial modifications of the above procedure.

Compounds 25 and 26 of Table I. The acridone ester (**4**, X = CH_3; 6 g, 16 mmol) was refluxed gently in $SOCl_2$ (50 mL) with a trace of DMF for 30 min. The clear solution was evaporated to dryness and azeotroped with dry benzene. The solid residue was treated with ice-cold 2 N aqueous $KHCO_3$ with stirring, until all gas evolution ceased. The solid was filtered, washed well with water, and dried over KOH to provide crude 9-chloro compound (**6**, X = CH_3). This was suspended in MeOH (100 mL) with 4-aminomethanesulfon-*m*-anisidide (3.5 g, 16.2 mmol) and heated to boiling with a trace of HCl. After 15 min, the mixture was diluted with an equal quantity of EtOAc, and the volume was reduced by distillation until crystallization began. After thorough cooling, the product hydrochloride (**25**) was collected as orange needles (7.5 g, 81% yield), mp 239–241) °C. Anal. ($C_{29}H_{24}N_4O_7S \cdot HCl$) C, H, N.

This compound (2.0 g, 3.5 mmol) was suspended in DMF (20 mL) and treated with concentrated NH_4OH at 20 °C for 3 h. Slow dilution with saturated aqueous NaCl gave the free base of **26** as a red powder. Crystallization from MeOH–EtOAc–HCl gave the hydrochloride salt (1.2 g, 71% yield) as red needles, mp 302–305 °C. Anal. ($C_{23}H_{22}N_4O_4S \cdot HCl$) C, H, N, Cl.

Compounds **27**, **28**, and **30–33** of Table I were prepared by trivial modifications of the above procedure.

Biological Testing. Cell culture methods for determining ID_{50} values for L1210 leukemia are given in ref 27. P388 leukemia cells were obtained as frozen stock from Mason Research Inc., U.S.A., and passaged intraperitoneally according to standard methods (ref 33) in DBA-2 mice of either sex. Groups of six hybrid mice (DBA-2 male × C57BL female, weight 20 ± 1 g) were injected intraperitoneally with 10^6 cells on day 0.

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Registry No. **2** (X = CH_3), 63329-46-4; **2** (X = Cl), 63329-53-3; **2** (X = OCH_3), 86611-38-3; **3a** (X = NO_2), 86611-39-4; **3a**·K (X = Cl), 86187-38-4; **3a** (X = Cl), 24782-72-7; **3a** (X = OCH_3), 86611-40-7; **3a** (X = CH_3), 78847-67-3; **3b** (X = Cl), 80258-99-7; **3b**·K (X = Cl), 86202-29-1; **3b** (X = CH_3), 86611-41-8; **4** (X = NO_2), 86611-42-9; **4** (X = CH_3), 78847-68-4; **5** (X = CH_3), 86611-43-0; **6** (X = CH_3), 78847-69-5; **9**, 86611-44-1; **10**, 86611-45-2; **11**, 51264-14-3; **12**, 76708-34-4; **13**, 64895-35-8; **14**, 53478-40-3; **15**, 79453-41-1; **16**, 58658-30-3; **17**, 57164-79-1; **18**, 76708-40-2; **19**, 76708-54-8; **20**, 76708-55-9; **21**, 76708-63-9; **22**·HCl, 86611-46-3; **22**, 86611-47-4; **23**·HCl, 86611-48-5; **23**, 78847-63-9; **24**·HCl, 86611-49-6; **24**, 86632-07-7; **25**·HCl, 86611-50-9; **25**, 78847-70-8;

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Potential Antitumor Agents. 39. Anilino Ring Geometry of Amsacrine and Derivatives: Relationship to DNA Binding and Antitumor Activity

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The clinical antileukemic drug amsacrine and analogues are thought to exert their biological activity by binding tightly but reversibly to DNA, with the acridine chromophore intercalated and the anilino group making additional binding contact in the minor groove of the double helix. In this binding model the steric environment around the 3′- and 5′-positions of the anilino ring is crucial. Two 3′,5′-disubstituted analogues of amsacrine have been prepared, and their conformation, DNA binding properties, and antitumor activity were determined and compared with corresponding unsubstituted and 3′-substituted compounds. Addition of 3′- and 3′,5′-substituents have little effect on minimum-energy conformations of the anilino side chain but have significant effects on DNA binding and biological activity. Monosubstitution lowers binding constants several-fold, but intercalative binding with extensive drug–base pair overlap is retained. Disubstitution lowers binding further, and although the binding is still intercalative as assessed by unwinding angles, it appears to occur with little drug–base pair overlap, as determined by high-field NMR studies of DNA imino proton shifts. These changes in DNA binding are accompanied by an abrupt change in biological activity, with the 3′,5′-disubstituted analogues proving inactive and nontoxic even though other physicochemical properties, such as lipophilicity and stability, remain within acceptable limits. This study provides further evidence that the binding of drugs to DNA has a critical influence on their biological activity.

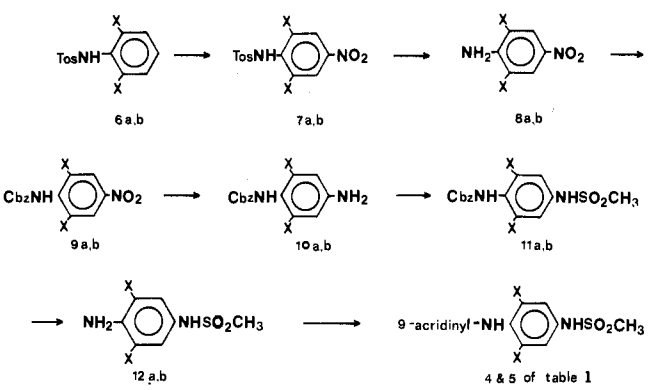
In recent years, DNA-intercalating[1,2] agents have been intensively studied and developed as potential antitumor agents. Intercalative binding initiates events leading to cell death by inhibition of nucleic acid synthesis and/or by the induction of strand breaks.[3] However, a great many DNA-intercalating ligands that bind equally tightly possess no antitumor activity.[4] In fact, elucidation of the structural features that distinguish active and inactive drugs of this general class, as well as an understanding of the underlying physical phenomena for this dichotomy, is a current central problem in antitumor drug development.

Recent studies[4] of a large series of DNA intercalators suggest that an important phenomenon is long residence times of a particular drug molecule *at a particular site* on the polymer. It can be envisaged how this could provide long-lived blocks to the passage of replication and/or transcription enzymes.

A majority of the tumor-active intercalating agents have attached to the chromophore a side chain bearing a cationic function. This side chain is essential for biological activity, and it is reasonable to suggest that the additional electrostatic binding of this group results in slower dissociation rates from particular sites on the DNA.[4,5]

A smaller group of tumor-active ligands (e.g., actinomycin D, the phenylphenanthridinium cations, and the 9-anilinoacridines) possesses a sterically demanding, rather than a cationic, side chain which is again essential for biological activity.

Scheme I



The 9-anilinoacridines form an important class of antitumor agents of this general type and include the clinical antileukemic drug amsacrine (*m*-AMSA, compound **3** in Table I).[6] The exact geometry of the binding of amsacrine and related 9-anilinoacridines to DNA has yet to be determined, but the available experimental results are consistent with the proposal that these compounds bind by intercalation of the acridine chromophore between the base pairs, with the anilino ring lodging in the minor groove.[6] In this binding mode, the 1- and 2-positions of the acridine ring are occluded by the sugar–phosphate chains of the DNA, while the 4- and 5-positions are oriented toward the major groove, free of steric restraint. The anilino 1′- and 2′-positions lie in the minor groove, also reasonably free from occlusion by the DNA in the binding model. A recent extensive QSAR study of the antileukemic activities of 9-anilinoacridine derivatives suggested that this binding

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