# Potential Antitumor Agents. 50. In Vivo Solid-Tumor Activity of Derivatives of *N*-[2-(Dimethylamino)ethyl]acridine-4-carboxamide

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The synthesis, physicochemical properties, and antitumor activity of a series of *N*-[2-(dialkylamino)alkyl]-acridine-4-carboxamides are reported. The compounds bind to DNA by intercalation, but exist under physiological conditions as monocations due to the weakly basic acridine chromophore ($pK_a$ = 3.5–4.5). The acridine-4-carboxamides show very broad structure–activity relationships (SAR) for antileukemic activity, with substituents at nearly all acridine positions proving acceptable. The compounds also show remarkable activity against the Lewis lung solid tumor in vivo, with several analogues capable of effecting 100% cures of the advanced disease. The broad SAR and high solid-tumor activity of the 9-acridine-4-carboxamides imply they should be considered as a completely new class of antitumor agent.

The primary problem in cancer chemotherapy is the treatment of solid tumors, most of which respond much less well to chemotherapy than do the disseminated diseases such as leukemias and lymphomas. There are several reasons for this, including a higher "intrinsic resistance" of many carcinoma cell lines to common agents compared to leukemia cell lines,[1,2] the problems of hypoxic, noncycling cells in solid tumors[3] ("environmental resistance"), and enhanced genetic instability leading to "acquired resistance".[4] An important additional factor is the requirement for drugs to penetrate poorly vascularized solid-tumor masses.

The DNA-intercalating agents are an important class of antitumor drugs, with several new examples now in clinical trials.[5-7] For compounds of this broad class, experimental antitumor activity (usually determined in the first instance against mouse leukemia screens) is correlated with high DNA binding,[8] and this in turn can be achieved by the use of polycationic materials.[9] However, such compounds are often limited in their spectrum of activity, particularly against solid tumors, a major reason for this probably being the poor distribution of such highly charged species. A recent exemplification of this point is the introduction into clinical trial of a new amsacrine analogue.[10] A major reason for choosing this compound from many derivatives was its greatly extended spectrum of activity against solid tumors compared to that of amsacrine, attributed in part to the better distributive properties of its more weakly basic acridine nucleus.[9]

We have recently[11] described a new class of DNA-intercalating antitumor drugs based on the parent compound *N*-[2-(dimethylamino)ethyl]-9-aminoacridine-4-carboxamide (1). Structure–activity relationships for in vivo antileukemic activity have shown a clearly defined requirement for a highly basic, cationic nitrogen at a fixed disposition with respect to the acridine chromophore,[11] and these structure–activity relationships have been related to the kinetics of dissociation from DNA.[12] Evaluation of a limited number of acridine-substituted derivatives showed[13] considerable positional effects, with only 5-substituted compounds consistently possessing in vivo antileukemic activity. However, although acridine substitution provided significant alterations in the pattern of in vitro activity of these compounds,[13] none proved active in vivo against the advanced, remotely sited Lewis lung (LL) carcinoma used as an initial solid-tumor screen, possibly due to the poor distributive properties of these essentially dicationic derivatives (the acridine $pK_a$ of 1 is 8.30).[14] Efforts to lower the $pK_a$ and thus provide a higher proportion of neutral acridine species by substitution of the chromophore with electron-withdrawing groups were successful;[14] the combined electronic and steric effects of groups such as 5-$SO_2CH_3$ and 5-$CF_3$ lowered the acridine $pK_a$ of the corresponding compounds (2 and 3) to 5.15 and 5.89, respectively.[14] These weakly basic 5-substituted derivatives did show an expanded spectrum of activity, with excellent P388 antileukemic activity (clearly superior to that of 1) and in addition moderate activity against the LL carcinoma.[14] Encouraged by these results, we have investigated an alternative way of reducing the $pK_a$ of the acridine chromophore, by replacing the strongly basic 9-aminoacridine system ($pK_a$ = 9.99)[15] with that of acridine itself ($pK_a$ = 5.6). This paper discusses the synthesis, physicochemical properties, and structure–activity relationships for the general class of weakly basic acridinecarboxamides represented by the general formula 4.

## Chemistry

Acridines can be prepared in high yield by mild, acid-catalyzed cyclization of 2-(phenylamino)benzaldehydes.[15] Reaction of the aldehyde is so facile it is likely that selective ring closure of substituted 2-[(2-formylphenyl)-amino]benzoic acids (I; Scheme I) could be achieved to provide the required substituted acridine-4-carboxylic acids (III). However, alternative routes via the corresponding 9-oxoacridans (II) appeared more attractive in the present

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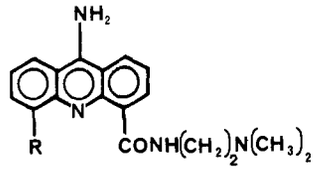
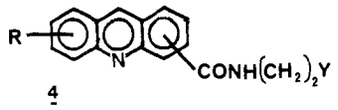


1 R = H

2 R = $SO_2CH_3$

3 R = $CF_3$



4

case, since unequivocal and high-yielding routes to the required 9-oxoacridan-4-carboxylic acids have been worked out.[16-18] Conversion of these to acridines can be carried out either by dehalogenation of 9-chloroacridines (catalytically or via the benzenesulfonohydrazides)[15] or by direct reduction of 9-oxoacridans.[15] Both general methods were explored, but the most generally satisfactory route to the methyl-, methoxy-, and chloro-substituted derivatives required for the present work was found to be direct reduction of the corresponding 9-oxoacridan-4-carboxylic acids with aluminum amalgam in alkali, by using a modified version of the procedure first described by Albert and Goldacre.[19] In order to keep the compounds in solution and ensure rapid reduction, KOH in 50% aqueous EtOH was used for many of the compounds (method A). In cases where the K salts were poorly soluble (especially for the chloro analogues where competing dechlorination becomes significant if the starting material remains out of solution and reaction times are prolonged), $Et_3N$ in 50% aqueous EtOH was used as the base (method B). Since in most cases (especially with electron-withdrawing groups) some overreduction to the acridan usually occurs, back-oxidation with acid $FeCl_3$ was carried out.[15] After this the mixture was neutralized with KOAc to precipitate the amphoteric acridine-4-carboxylic acids, leaving the Fe species in solution. For the methyl and methoxy derivatives, the products were obtained in yields of 20–70% after crystallization from suitable solvents. The chloro compounds were preferably purified by crystallization of their Na salts to remove varying amounts of dechlorination products. The acridine acids (Table III) were then coupled with the the appropriate [(dialkylamino)alkyl]amines (IV) in high yield with 1,1′-carbonyldiimidazole[20] as the coupling reagent. The more hindered 3-substituted acid was the most difficult to couple, and in this case it was necessary to use $SOCl_2$ to provide the intermediate acid chloride. An alternative route was used for the 9-methyl derivative **36** (method C, Scheme I), where the amide VI was formed first, followed by acid-catalyzed ring closure.

## Results and Discussion

Tables I and II give physicochemical and biological data for 32 acridinecarboxamide derivatives. Compounds **5–8** explore positioning of the carboxamide side chain at the four available acridine positions. As previously found for the 9-aminoacridinecarboxamides,[11] only the compound with the 4-carbamoyl side chain proved active in vivo. Compounds **9–12** then extend the distance between the cationic dimethylamino group and the chromophore at this position from two to six methylene units, and compounds **13–16** examine the effects of a small range of different alkylamino functions. The remainder of the examples (compounds **17–32**) explore the effects of either a methyl, methoxy, or chloro group at each of the seven available acridine positions 1–3 and 5–9. The methoxy and chloro groups at position 9 are too reactive, but the 9-methyl derivative **36** was examined. As noted previously,[13] these three substituents were chosen to give a reasonable variation of electronic and lipophilic properties, while presenting feasible synthetic efforts.

**Lipophilicity.** Overall drug lipophilicity was determined as before[11,21] by liquid–liquid chromatography of the dications at pH 1–2. Comparison of the $R_m$ values for the parent compound of this series, *N*-[2-(dimethylamino)ethyl]acridine-4-carboxamide (**8**), with the value for the corresponding 9-amino derivative **1** (also listed in Table I for comparison) shows that loss of the 9-amino substituent results in a very large increase in lipophilicity (0.91 $R_m$ unit, corresponding to 3.3 log *P* units[21]). This increase is more than double the $\pi$ value of −1.23 for an $NH_2$ group on an isolated benzene ring[22] and must reflect the considerable difference in the electronic structure of the acridine due to loss of the resonance effects noted above. Comparing differences in $R_m$ values between the compounds of Table I and the corresponding 9-amino derivatives (values taken from ref 11 and 13) shows that, for compounds with an unsubstituted acridine or a substituent in the direction of the short axis of the acridine (compounds **8–19, 24–26, 33–35**), the average $\Delta R_m$ is almost exactly the same (0.91 unit) as that between the two parent compounds. For derivatives with substituents in the direction of the long axis of the acridine (compounds **20–23** and **27–32**) the difference is less (0.83 $R_m$ unit). These compounds are also generally more lipophilic than isomers bearing the same substituent groups off the short axis, a similar pattern to that noted previously for the 9-amino derivatives.[13]

**Acridine Base Strength.** While 9-aminoacridine-4-carboxamide (**1**) will exist as a dication at physiological pH ($pK_a$ = 8.30; all $pK_a$ values determined in water at 25 °C), the chromophore of acridine-4-carboxamide (**8**; $pK_a$ = 3.54) is negligibly protonated (0.02%) under these conditions. Even the most strongly basic member of the series, the 6-methoxy derivative **30** has a $pK_a$ of only 4.59, so all members of the series will exist as monocations at physiological pH. The fact that the 1-, 2-, and 3-carboxamide derivatives **5–7** have $pK_a$ values 0.4–0.8 unit higher than **8** suggests some additional stabilization of the free base form in the latter case (possibly by H-bond donation from the carboxamide NH and/or hindrance of proton approach to the ring nitrogen by the carboxamide group).

**DNA Binding.** This was estimated as before by the ethidium displacement method.[23] Assuming competitive binding of drugs and the ethidium fluorophore to DNA, association constants can be determined from the concentration of drug needed to displace 50% of the ethidium,[24] and these values are recorded in Table I.

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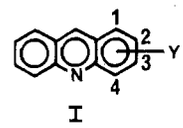
**Table I.** Physicochemical and Biological Data for Acridine-4-carboxamides



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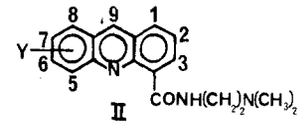

II

| no. | formula | Y | $R_m$[a] | p$K_a$[b] | log $K$[c] AT | log $K$[c] GC | $IC_{50}$[d] L1210 | $IC_{50}$[d] HCT | $IC_{50}$[d] ratio | P388 OD[e] | P388 ILS[f] | LL OD[e] | LL ILS[f] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | | | −1.11 | 8.30 | 7.35 | 7.70 | 15 | 55 | 3.7 | 4.5 | 98 (1)[g] | 4.5 | *h* |
| 5 | I | 1-CONH$(CH_2)_2N(CH_3)_2$ | −0.56 | 4.24 | 6.87 | 7.70 | 17900 | | | 150 | *h* | | |
| 6 | I | 2-CONH$(CH_2)_2N(CH_3)_2$ | −0.57 | 4.37 | 7.17 | 7.59 | 9300 | | | 225 | *h* | | |
| 7 | I | 3-CONH$(CH_2)_2N(CH_3)_2$ | −0.53 | 3.94 | 7.12 | 6.94 | 8100 | | | 150 | *h* | | |
| 8 | I | 4-CONH$(CH_2)_2N(CH_3)_2$ | −0.20 | 3.54 | 6.12 | 6.54 | 105 | 900 | 8.6 | 66 | 91 | 100 | (6)[i] |
| 9 | I | 4-CONH$(CH_2)_3N(CH_3)_2$ | −0.10 | | 6.13 | 6.42 | 370 | 2900 | 7.8 | 150 | 118 | 150 | 84 |
| 10 | I | 4-CONH$(CH_2)_4N(CH_3)_2$ | 0.01 | | 6.47 | 6.40 | 160 | 930 | 5.8 | 100 | 143 (3) | 100 | 100 |
| 11 | I | 4-CONH$(CH_2)_5N(CH_3)_2$ | 0.12 | | 6.16 | 6.38 | 450 | 1800 | 4.0 | 225 | 34 | 150 | 83 (2) |
| 12 | I | 4-CONH$(CH_2)_6N(CH_3)_2$ | 0.28 | | 6.54 | 6.36 | 550 | 1960 | 3.6 | 100 | 32 | 150 | *h* |
| 13 | I | 4-CONH$(CH_2)_2N(Et)_2$ | 0.17 | | 6.51 | 6.38 | 630 | | | 150 | 61 | 150 | 62 |
| 14 | I | 4-CONH$(CH_2)_2NHCH_3$ | −0.27 | | 6.43 | 6.90 | 240 | 620 | 2.6 | 65 | *h* | 65 | *h* |
| 15 | I | 4-CONH$(CH_2)_2NH_2$ | −0.28 | | 7.05 | 7.03 | 275 | 1600 | 5.8 | 13.3 | 24 | | *h* |
| 16 | I | 4-CONH$(CH_2)_2NH(CH_2)_2OH$ | −0.22 | | 6.94 | 7.09 | 240 | 1800 | 7.5 | 13.3 | 22 | 20 | *h* |
| 17 | II | 1-$CH_3$ | 0.10 | | 7.12 | 8.06 | 37 | 400 | 10.8 | 100 | 71 | 100 | 67 |
| 18 | II | 1-$OCH_3$ | −0.13 | 3.81 | 7.05 | 7.96 | 8.8 | 115 | 13.1 | 30 | 205 (1) | 65 | (4)[i] |
| 19 | II | 1-Cl | 0.04 | | 7.18 | 7.20 | 33 | 370 | 11.2 | 100 | 110 | 100 | 120 |
| 20 | II | 2-$CH_3$ | −0.04 | | 6.80 | 7.05 | 130 | 657 | 5.1 | 100 | 120 (1) | 150 | 33 (3) |
| 21 | II | 2-$OCH_3$ | −0.13 | 3.34 | 7.14 | 8.25 | 30 | 720 | 24 | 100 | 99 | 100 | 105 (2) |
| 22 | II | 2-Cl | 0.03 | | 7.12 | 7.31 | 31 | 440 | 14.2 | 100 | 62 (1) | 100 | 219 (4) |
| 23 | II | 3-$CH_3$ | −0.21 | | 5.74 | 6.11 | 12700 | | | 225 | *h* | 100 | *h* |
| 24 | II | 5-$CH_3$ | −0.17 | | 6.99 | 7.42 | 4.3 | 54 | 12.6 | 30 | 87 (1) | 30 | 120 (1) |
| 25 | II | 5-$OCH_3$ | −0.31 | 3.87 | 7.96 | 7.38 | 12 | 180 | 15 | 65 | 70 | 45 | 54 |
| 26 | II | 5-Cl | −0.21 | | 7.63 | 7.20 | 6.9 | | | 20 | 91 | 20 | 92 |
| 27 | II | 6-$CH_3$ | −0.10 | | 6.93 | 7.26 | 121 | 658 | 5.4 | 100 | 82 | 100 | 106 (5) |
| 28 | II | 6-$OCH_3$ | −0.47 | 4.59 | 7.28 | 8.39 | 210 | 470 | 2.2 | 100 | 73 | 100 | 85 |
| 29 | II | 6-Cl | −0.06 | | 6.73 | 7.00 | 6.4 | 350 | 54 | 45 | 29 | 65 | 91 |
| 30 | II | 7-$CH_3$ | −0.05 | | 6.97 | 7.00 | 200 | 1240 | 6.2 | 100 | 68 | 150 | 85 |
| 31 | II | 7-$OCH_3$ | −0.22 | 3.54 | 6.87 | 7.07 | 640 | 1700 | 2.7 | 150 | 91 (2) | 150 | 46 (1) |
| 32 | II | 7-Cl | −0.03 | | 6.87 | 7.14 | 250 | 610 | 2.4 | 100 | 106 | 100 | 94 |
| 33 | II | 8-$CH_3$ | −0.12 | | 7.00 | 7.96 | 140 | 980 | 7.0 | 150 | 61 | 150 | 60 |
| 34 | II | 8-$OCH_3$ | −0.12 | 3.82 | 7.14 | 7.23 | 58 | 520 | 9.0 | 100 | 128 | 100 | 66 |
| 35 | II | 8-Cl | 0.01 | | 7.00 | 6.96 | 160 | 790 | 4.9 | 65 | 102 | 65 | 63 (1) |
| 36 | II | 9-$CH_3$ | −0.33 | 4.34 | 7.75 | 8.48 | 150 | 160 | 1.1 | 45 | *h* | | |

[a] $R_m$ values were determined as in ref 29 with 4′-(9-acridinylamino)methanesulfonanilide as a standard. [b] Acridine p$K_a$ values were determined in water at 25 °C by the spectrophotometric method detailed in ref 29. [c] log $K$: binding constant to poly(dA-dT) or poly(dG-dC), determined by ethidium bromide displacement; see ref 23 and 24. [d] $IC_{50}$: concentration of drug in nM to inhibit growth of murine leukemia (L1210) or human colon tumor (HCT-8) cells in culture by 50%. See ref 30 and 31. [e] OD: optimal dose of drug in mg/kg$^{-1}$ day$^{-1}$, administered intraperitoneally as a solution in 0.1 mL of 30% v/v ethanol/water on days 1, 5, and 9 after intraperitoneal inoculation of $10^6$ P388 leukemia cells or on days 5, 9, and 13 after intravenous inoculation of $10^6$ Lewis lung carcinoma cells. See ref 31. [f] $ILS_{max}$: the percentage increase in lifespan of drug-treated, tumor-bearing animals compared to nontreated, tumor-bearing controls when treated at the optimal dose; values above 20% for P388 and above 40% for Lewis lung are considered statistically significant. [g] Numbers in parentheses indicate the number of animals in a group of six that were long-term survivors (50 days for P388, 60 days for Lewis lung). [h] Compound inactive at all dose levels up to toxic ones. [i] All 100% long-term survivors; no ILS could be computed.

As might be expected with loss of the resonant 9-amino group, binding constants for the acridinecarboxamides are considerably lower than those for the corresponding 9-amino derivatives. The parent compound **8** has binding constants to poly[d(AT)] and poly[d(GC)] 1.23 and 1.16 log units, respectively, lower than that of the 9-amino analogue **1**. For the acridine-substituted derivatives **17–37**, binding to poly[d(AT)] averages half a log unit lower than that of the corresponding 9-amino analogues.[13] It should be noted, however, that the DNA-binding measurements were carried out in pH 5 buffer. In previous work with the 9-aminoacridinecarboxamides[11,13,14] this ensured that all compounds were completely in the dicationic form (the side chain (dimethylamino)ethyl cationic center has a p$K_a$ of 8.6,[11] while that of the acridine chromophores of substituted 9-aminoacridine-4-carboxamides have p$K_a$ values[14] in the region of 8.0–8.5). However, the substituted acridine-4-carboxamides have acridine p$K_a$ values of 3.5–4.5 (Table I), which implies a variable but significant proportion of free base form at pH 5. Since the free base form will bind to DNA less strongly than the cation, the lower binding of the acridinecarboxamides may be due to this fact alone and not from loss of the 9-amino group. Since it is not feasible to carry out the binding measurements at lower pH values, binding of the dicationic forms of the acridinecarboxamides cannot be determined. The parent compound **8** showed an unwinding angle of 12° in the helix-unwinding assay using closed circular supercoiled DNA.[27] This compares well to the value of 16° determined[11] for the corresponding 9-aminoacridine derivative **1** and clearly shows that loss of the 9-$NH_2$ group does not alter the mode of binding.

**In Vitro Cytotoxicity.** As seen previously with the 9-aminoacridine series,[11] placement of the cationic side chain with respect to the chromophore appears to be im-

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Scheme I

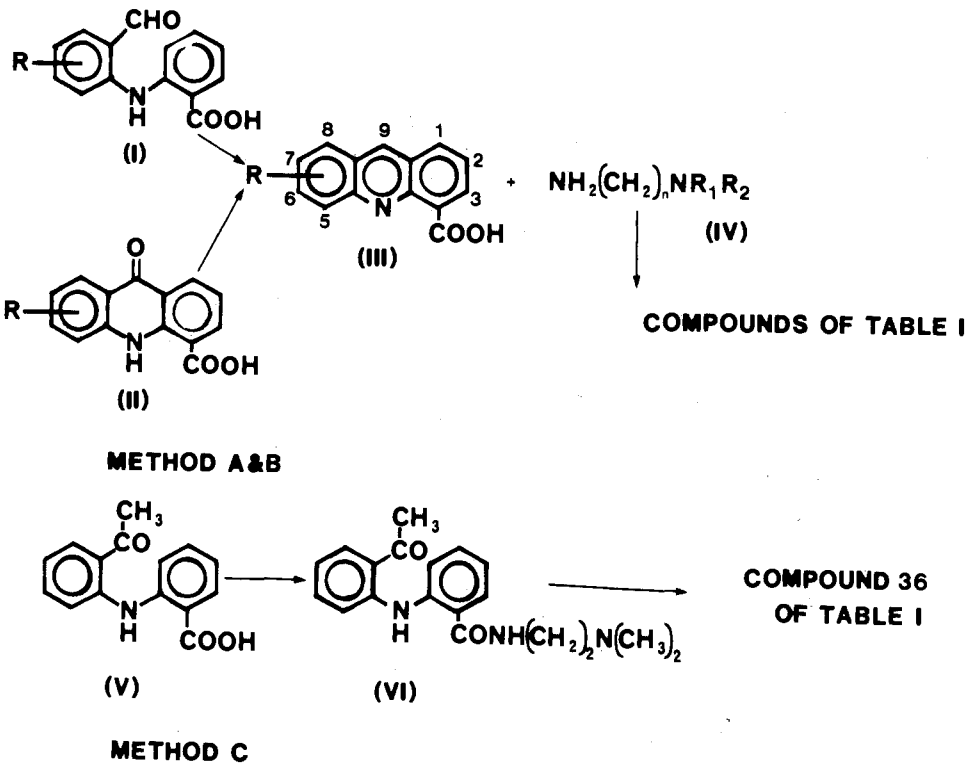


Table II. Physicochemical Properties for the New Compounds of Table I

| no. | mp, °C | formula | anal. |
|---|---|---|---|
| 5 | 215–216 | $C_{18}H_{19}N_3O{\cdot}2HCl{\cdot}H_2O$ | C, H, N, Cl |
| 6 | 217–220 | $C_{18}H_{19}N_3O{\cdot}2HCl{\cdot}H_2O$ | C, H, N, Cl |
| 7 | 265–267 | $C_{18}H_{19}N_3O{\cdot}2HCl$ | C, H, N, Cl |
| 8 | 162–164 | $C_{18}H_{19}N_3O{\cdot}2HCl$ | C, H, N, Cl |
| 9 | 149–151 | $C_{19}H_{21}N_3O{\cdot}2HCl$ | C, H, N, Cl |
| 10 | 192–194 | $C_{20}H_{23}N_3O{\cdot}HCl$ | C, H, N, Cl |
| 11 | 179–180 | $C_{21}H_{25}N_3O{\cdot}HCl$ | C, H, N, Cl |
| 12 | 212–213 | $C_{22}H_{27}N_3O{\cdot}HCl$ | C, H, N, Cl |
| 13 | 196–198 | $C_{20}H_{23}N_3O{\cdot}2HCl$ | C, H,[b] N, Cl |
| 14 | 161–163 | $C_{17}H_{17}N_3O{\cdot}2HCl$ | C, H, N, Cl |
| 15 | 262–265 | $C_{16}H_{15}N_3O{\cdot}2HCl$ | C, H, N |
| 16 | 213–216 | $C_{18}H_{19}N_3O_2{\cdot}2HCl$ | C, H, N, Cl |
| 17 | 251–252 | $C_{19}H_{21}N_3O{\cdot}2HCl$ | C, H, N, Cl |
| 18 | 177–178 | $C_{19}H_{21}N_3O_2{\cdot}2HCl$ | C, H, N, Cl |
| 19 | 80–82 | $C_{18}H_{18}ClN_3O{\cdot}2HCl$ | C, H, N, Cl |
| 20 | 222–223 | $C_{19}H_{21}N_3O{\cdot}2HCl$ | C, H, N, Cl |
| 21 | 231–232 | $C_{19}H_{21}N_3O_2{\cdot}2HCl$ | C, H, N, Cl |
| 22 | 266–268 | $C_{18}H_{18}ClN_3O{\cdot}'2HCl$ | C, H, N, Cl |
| 23 | 244–245 | $C_{19}H_{21}N_3O{\cdot}2HCl$ | C, H, N, Cl |
| 24 | 205–208 | $C_{19}H_{21}N_3O{\cdot}2HCl$ | C, H, N, Cl |
| 25 | 195–198 | $C_{19}H_{21}N_3O_2{\cdot}HCl$ | C,[c] H, N |
| 26 | 169–170 | $C_{18}H_{20}ClN_3O{\cdot}2HCl$ | C, H, N, Cl |
| 27 | 219–220 | $C_{19}H_{21}N_3O{\cdot}2HCl$ | C, H, N, Cl |
| 28 | 222–223 | $C_{19}H_{21}N_3O_2{\cdot}2HCl$ | C,[a] H, N, Cl |
| 29 | 170–173 | $C_{18}H_{18}ClN_3O{\cdot}HCl$ | C, H, N |
| 30 | 220–222 | $C_{19}H_{21}N_3O{\cdot}2HCl$ | C, H, N, Cl |
| 31 | 236–238 | $C_{19}H_{21}N_3O_2{\cdot}2HCl$ | C, H, N, Cl |
| 32 | 190–192 | $C_{18}H_{18}ClN_3O{\cdot}2HCl$ | C, H, N, Cl |
| 33 | 228–230 | $C_{19}H_{21}N_3O{\cdot}2HCl{\cdot}H_2O$ | C, H, N, Cl |
| 34 | 212–215 | $C_{19}H_{21}N_3O_2{\cdot}HCl$ | C,[c] H, N, Cl |
| 35 | 246–247 | $C_{18}H_{18}ClN_3O{\cdot}2HCl$ | C, H, N, Cl |
| 36 | 178–180 | $C_{19}H_{21}N_3O{\cdot}2HCl$ | C, H, N, Cl |

[a] C out by 0.5%. [b] H out by 0.5%. [c] C out by 0.6%.

portant, since the 1-, 2-, and 3-carboxamides **5–7** are much less potent than the 4-derivative **8**. Even this compound, with an $IC_{50}$ of 106 nM against the leukemia, proved much less cytotoxic than the corresponding 9-aminoacridine-4-carboxamide derivative **1**, which has an $IC_{50}$ of 15 nM. This is in broad agreement with results from other weakly basic intercalating agents (notably 9-anilinoacridines), where cytotoxicity is strongly correlated with the base strength of the chromophore.[26] As the length of the side chain was increased (compounds **8–12**), the cytotoxicity decreased, but not as abruptly as for the 9-aminoacridinecarboxamide analogues.[11] Thus the ratio of $IC_{50}$ values (acridinecarboxamide/9-aminoacridinecarboxamide) against L1210 leukemia steadily decreased from 7.0 for the $(CH_2)_2$ derivatives (**1** and **8**) to only 0.26 for the $(CH_2)_6$ derivatives. The much greater relative cytotoxicity of the higher members of the acridinecarboxamide series may be due to their much higher lipophilicity, which is known to contribute to in vitro cytotoxicity.[25] However, a comparison of the relative cytotoxicity of the acridine-substituted derivatives of the two series shows a less regular relationship. Thus, the 6-methoxyacridinecarboxamide **28** is 14-fold less potent than its 9-aminoacridinecarboxamide analogue, which has an $IC_{50}$ of 15 nM,[13] but the opposite is true for the 6-Cl derivative, where **29** has an $IC_{50}$ of 6.9 nM and the corresponding 9-aminoacridinecarboxamide an $IC_{50}$ of 105 nM.[13] These significant variations in relative cytotoxicity between the two series suggest that the 9-aminoacridinecarboxamides and the acridinecarboxamides are two quite distinct classes of compound.

The relative cytotoxicity of the acridinecarboxamides between the L1210 leukemia and the HCT-8 human colon carcinoma lines (the L1210/HCT-8 ratio) varies widely (from 54.6 for the 6-Cl derivative **29** to 2.1 for the 8-OMe derivative **34**), but none showed selectivity for the colon line.

**In Vivo Antitumor Activity.** Of the four positional isomers **5–8**, only the 4-substituted derivative **8** showed in vivo antileukemic activity, following the same pattern found for the 9-aminoacridinecarboxamide series.[11] Although compound **8** is 15-fold less potent than its 9-aminoacridinecarboxamide analogue **1**, tumor-cell selectivity (measured by % ILS) is about the same. Unlike the 9-amino series where lengthening of the side chain by one methylene unit abolished activity, the corresponding acridinecarboxamides remain active, with the $(CH_2)_4$

**Table III.** Substituted Acridine-4-carboxylic Acids

| substituent | method[a] | % yield[b] | mp, °C | formula | anal. |
|---|---|---|---|---|---|
| 1-$CH_3$ | B | 71 | 264–266 | $C_{15}H_{11}NO_2$ | C, H, N |
| 1-$OCH_3$ | B | 21 | 227.5–228.5 | $C_{15}H_{11}NO_3$ | C, H, N |
| 1-Cl | B | 27 | 273–274 | $C_{14}H_8ClNO_2$ | C, H, N, Cl |
| 2-$CH_3$ | A | 63 | 234–235 | $C_{15}H_{11}NO_2$ | C, H, N |
| 2-$OCH_3$ | A | 56 | 264–266 | $C_{15}H_{11}NO_3$ | C, H, N |
| 2-Cl | B | 39 | 317–319 | $C_{14}H_8ClNO_2$ | C, H, N, Cl |
| 3-$CH_3$ | B | 41 | 195–198 | $C_{15}H_{11}NO_2$ | C, H, N |
| 5-$CH_3$ | A | 44 | 300–302 | $C_{15}H_{11}NO_2$ | C, H, N |
| 5-$OCH_3$ | B | 42 | 273–275 | $C_{15}H_{11}NO_3$ | C, H, N |
| 5-Cl | B | 38 | 337–340 | $C_{14}H_8ClNO_2$ | C, H, N, Cl |
| 6-$CH_3$ | A | 31 | 119–201 | $C_{15}H_{11}NO_2$ | C, H, N |
| 6-$OCH_3$ | A | 69 | 245–246 | $C_{15}H_{11}NO_3$ | C, H, N |
| 6-Cl | B | 65 | 302–304 | $C_{14}H_8ClNO_2$ | C, H, N, Cl |
| 7-$CH_3$ | A | 68 | 214–216[c] | $C_{15}H_{11}NO_2$ | C, H, N |
| 7-$OCH_3$ | A | 72 | 249–256 | $C_{15}H_{11}NO_3$ | C, H, N |
| 7-Cl | B | 69 | 309–312 | $C_{14}H_8ClNO_2$ | C, H, N, Cl |
| 8-$CH_3$ | A | 92 | 285–286 | $C_{15}H_{11}NO_2$ | C, H, N |
| 8-$OCH_3$ | A | 28 | 310–311 dec | $C_{15}H_{11}NO_3$ | C, H, N |
| 8-Cl | B | 79 | 328–329 dec | $C_{14}H_8ClNO_2$ | C, H, N, Cl |

[a] See Experimental Section. [b] Yield of product suitable for coupling; not optimized. [c] Literature[32] mp 206–207 °C.

analogue **10** showing clearly superior results to the parent against P388 leukemia and even the $(CH_2)_6$ analogue showing statistically significant activity. However, the most striking contrast with the 9-aminoacridine-4-carboxamide series is the extraordinary activity of the parent compound **8** against the remotely sited Lewis lung (LL) carcinoma used to evaluate solid-tumor activity. At the optimum dose of 100 mg/kg, the compound is repeatedly able to provide close to 100% cures of advanced disease (drug treatment on days 5, 9, and 13). In contrast, the 9-aminoacridinecarboxamide derivative **1**, amsacrine, and adriamycin are all essentially inactive against this tumor with this protocol.[27,28] Activity against LL is retained for the derivatives **9–11** of longer chain length, but at a reduced level. Replacement of the dimethylamino side chain group by more polar functions (e.g., compounds **14–16**) results in loss of LL activity, although P388 activity is retained (albeit at a low level).

For the acridine-substituted derivatives **17–36**, completely different structure–activity relationships are seen compared to those for the corresponding 9-aminoacridine-4-carboxamides.[13] While in the latter series P388 activity is restricted to the 2-OMe and 5-substituted derivatives (and none of the compounds show LL activity), in the present case all compounds except those with 3-substituents show in vivo activity against both test systems. The inactivity of the 3-substituted compound may be due to distortions in side-chain geometry introduced by the ortho substitution. It is difficult to discern any clear structure–activity relationships from the results of Table I, but a few comments can be made. As with the 9-aminoacridine-4-carboxamides, the most important influence appears to be the position rather than the nature of the substituent group. Overall, the 2-substituted derivatives **20–22** are the most active against LL, with all three compounds producing about 50% cures. Other exceptionally active compounds include the 1-OMe and 6-Me derivatives, which also produce a majority of cures. The 1-OMe compound also showed the best P388 activity and was among the more dose-potent derivatives. The complete inactivity of the 9-methyl derivative **36** was surprising, but serves to emphasize the importance of alterations at the 9-position among the broad class of acridinecarboxamides.

## Conclusions

The substituted acridine-4-carboxamides presented here represent a logical development of the 9-aminoacridine-4-carboxamide series that were discovered during attempts to devise new classes of antitumor drugs from electron-deficient tricyclic chromophores.[11] Removal of the resonant 9-amino group provided compounds with greatly reduced acridine $pK_a$ values, where the chromophore is uncharged at physiological p$K$. However, the extraordinary solid-tumor activity of the resulting acridine-4-carboxamide and the very different in vitro and in vivo structure–activity relationships seen for these compounds suggest that they are not merely weakly basic 9-aminoacridinecarboxamides. Removal of the 9-amino group drastically alters many electronic properties such as dipole moment and electronic charge distribution, and the compounds must be considered as a completely new class of agent.

The very high antileukemic and solid-tumor activity shown by the parent compound **8** and several substituted derivatives makes them candidates for advanced evaluation. However, the lack of clear structure–activity relationships among the acridine-substituted compounds (**17–36**) makes it uncertain as to what direction should be followed in the further development of this particular series. It seems probable, though, that the general class of electron-deficient, tricyclic chromophores still has much to offer in terms of the development of novel anticancer drugs.

## Experimental Section

Where analyses are indicated by the symbols of the elements, analyses within ±0.4% of theoretical were obtained. Analyses were carried out by Professor A. D. Campbell, Microchemical Laboratory, University of Otago, Dunedin, New Zealand. Melting points were determined on an Electrothermal apparatus using the manufacturer's stem-corrected thermometer and are as read. TLC on $SiO_2$ ($F_{254}$) was used to follow the course of reactions and to monitor product purity. For the very polar products of Table I, TLC on cellulose using the top phase of a mixture of *i*-BuOH, HOAc, $H_2O$, and DMF (30:6:24:2.25) was used.

**General Procedure for Reduction of 9-Oxoacridan-4-carboxylic Acids. Method A. Acridine-4-carboxylic Acid (III, R = H).** Portions (0.1–0.2 g each) of Al foil (total 5 g) were

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amalgamated with Hg by immersing them for 1 min in a solution of mercuric chloride (18 g) in EtOH (200 mL). After being washed in EtOH, these were added over 30 min to a refluxing, stirred solution of 9-oxoacridan-4-carboxylic acid (II; R = H) (6 g, 0.025 mol) dissolved in 50% aqueous EtOH (200 mL) containing KOH (1.6 g, 0.029 mol). When the reaction was complete, as indicated by TLC, 1 N aqueous KOH (50 mL) was added, the hot mixture was filtered, and the solids were washed with hot 50% aqueous EtOH (150 mL). The combined filtrates were strongly acidified with 12 N HCl, treated with $FeCl_3$ (10 g), heated under reflux until homogeneous, and clarified by filtration. Sufficient aqueous KOAc was then added to just complete precipitation of the crude product, which was immediately collected by filtration and washed well with water. It was then dissolved in dilute aqueous KOH, filtered, diluted while hot with EtOH, and then treated slowly with HOAc, when acridine-4-carboxylic acid separated as a pale yellow, granular solid (3.6 g, 64%), suitable for further use. Crystallization of a sample from aqueous $Me_2CO$ gave crystals, mp 202–204 °C (lit.[19] mp 189–190 °C).

This general reduction procedure was modified in cases where the starting 9-oxoacridan acids had poorly soluble K salts and/or where partial dehalogenation of chloro-substituted compounds occurred during the reaction. These modifications are illustrated (method B) for the synthesis of 1-chloroacridine-4-carboxylic acid.

**Method B. 1-Chloroacridine-4-carboxylic Acid (III, R = 1-Cl).** A suspension of 1-chloro-9-oxoacridan-4-carboxylic acid (5.47 g, 0.02 mol) in 50% aqueous EtOH (400 mL) containing $Et_3N$ (4.1 mL, 0.03 mol) was heated under reflux until homogeneous. Portions of Al foil (total 4 g; freshly amalgamated as detailed above) were added to the vigorously stirred boiling solution over 30 min, and after the reaction was complete (TLC), the mixture was filtered, and the solids were washed with a solution of KOH in 50% aqueous EtOH. The combined filtrates were strongly acidified with 12 N HCl, treated with $FeCl_3$ (12 g) under reflux for 45 min, concentrated, and cooled. The solid that separated was extracted with hot 2 N $NH_4OH$, and addition of HOAc to this clarified solution precipitated the crude product, together with a quantity of the dechlorinated (parent) acid. Two crystallizations of the K salt from aqueous KOH/KCl followed by crystallization of the free acid from acetone gave pure product as yellow needles (1.37 g, 27%), mp 273–274 °C.

In many cases, crude products obtained by this method could be purified simply by crystallization from an appropriate solvent (e.g., $Me_2CO$ or DMF) or by crystallization of HCl salts.

**2-[*N*-[2-(Methylcarbonyl)phenyl]amino]benzoic Acid (V).** A mixture of 2-bromoacetophenone (2 g, 10 mmol), anthranilic acid (2 g, 15 mmol), CuCl (0.1 g),and *N*-ethylmorpholine (3.45 g, 30 mmol) in butane-2,3-diol (20 mL) was stirred at 140 °C for 4 h. The cooled mixture was diluted with 1 N $NH_4OH$, filtered, acidified with 2 N HCl, and extracted into EtOAc. The organic layer was extracted into 1 N $NH_4OH$, and the solution was slowly acidified at 10 °C to give 2-[*N*-[2-(methylcarbonyl)phenyl]-amino]benzoic acid (1.21 g, 47%), mp (EtOH) 280–283 °C. Anal. ($C_{15}H_{13}NO_3$) C, H, N.

**Compound 36 of Table I.** A solution of the above keto acid in dry DMF (20 mL) was treated with 2.3 g (1.2 equiv) of diethyl phosphorocyanidate, and an excess of *N,N*-diethylethylenediamine (2 g) was added dropwise. After being warmed to 90 °C for 30 min, the reaction mixture was basified with aqueous $K_2CO_3$ and the solvent was removed under vacuum. The residue was extracted with EtOAc to give the crude amide VI as an oil. This was dissolved in 25 mL of a mixture of HOAc/$H_2SO_4$ (20:1). After the mixture was heated under reflux for 1 h, the HOAc was removed under vacuum and the residue was dissolved in water. The aqueous fraction was washed with $CH_2Cl_2$, then basified with aqueous NaOH, and extracted into EtOAc. The EtOAc layer was washed with water and saturated NaCl and evaporated to give *N*-[2-(dimethylamino)ethyl]-9-methylacridine-4-carboxamide (2.1 g, 60%) as an oil. This was dissolved in MeOH/EtOAc and treated with dry HCl gas to provide compound **36** as a crystalline dihydrochloride salt, mp (EtOH) 178–180 °C (Table I).

**General Procedure for Coupling of the Acridine-4-carboxylic Acids. *N*-[2-(Dimethylamino)ethyl]acridine-4-carboxamide (Compound 8 of Table I).** Acridine-4-carboxylic acid (2 g, 9 mmol) was suspended in dry DMF (20 mL) with 1,1′-carbonyldiimidazole (2.9 g, 2 equiv), and the mixture was stirred at 20 °C with exclusion of moisture until all solids had dissolved. The mixture was then cooled to 0 °C, *N,N*-dimethylethylenediamine (5 equiv) was added, the mixture was stirred for another 5 min, and all volatiles were removed under vacuum. The residue was partitioned between $CH_2Cl_2$ and 1 N aqueous $Na_2CO_3$, and the organic layer was washed with water and evaporated. The residue was crystallized from hot benzene/petroleum ether to give the pure free base (90% yield). This was dissolved in MeOH and dry HCl gas was added to pH 2. Slow dilution of the warmed solution with EtOAc gave the dihydrochloride as yellow crystals, mp 162–164 °C, in quantitative yield (Table I).

This general coupling method was also suitable for preparation of the other substituted acridinecarboxamides listed in Table I. Some of the acridine acids required several hours of stirring with the 1,1′-carbonyldiimidazole before completely dissolving, but all were converted to the desired carboxamides in yields of 80–95%. Use of $SOCl_2$ generally gave lower yields and in some cases led to nuclear chlorination of the acridine acids.

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