Synthesis, Antitumor Activity, and DNA Binding Properties of a New Derivative of Amsacrine, N-5-Dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4-acridinecarboxamide


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ABSTRACT

The 4-(N-methylcarboxamido)-5-methyl derivative of amsacrine (NSC 249 992) has been synthesized as part of a program aimed at optimizing solid tumor activity in this series. Physicochemical studies of this analogue [N-5-dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4-acridinecarboxamide; NSC 343 499] indicate a slightly increased lipophilicity (estimated log ρ = 1.10), a decreased acridine base strength (pKa 6.40), and a 16-fold-higher association constant for double-stranded calf thymus DNA (Kα 2.1 x 10^6 M^-1 at 0.01 ionic strength). Like amsacrine, the drug binds to DNA by intercalation. Inhibition of cell growth has been monitored by continuous drug exposure assays with a variety of rodent and human cell lines. The concentration for 50% inhibition varied from 6.7 nM (T-47D, a human breast carcinoma line) to 800 nM (P388/ADR, a murine cell line resistant to Adriamycin). N-5-Dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4-acridinecarboxamide was cytotoxic at growth-inhibitory concentrations and also induced cell cycle arrest in the G0 phase. It was active against P388 leukemia following administration by p.o., i.v., or i.p. routes, and it was superior to amsacrine, daunorubicin, and Adriamycin. It was curative towards i.v.-injected Lewis lung tumor in a proportion of animals when treatment was started on Day 1 or Day 5 after tumor inoculation. It also produced highly significant life extensions against advanced tumors (treatment starting Day 9 after i.v. inoculation or on Day 8 after s.c. inoculation) and was comparable to cyclophosphamide in its effectiveness. It is a candidate drug for clinical trial.

INTRODUCTION

Amsacrine (NSC 249 992), a 9-anilinoacridine derivative, was first synthesized and shown to be active in a variety of experimental tumors by Cain and Atwell (11). Clinical trials have demonstrated useful activity against leukemia (1, 28) and, to a lesser extent, against lymphoma (37). Activity in breast cancer is low but significant (27), while activity in other solid tumors is considered insufficient to warrant further clinical trial (26). Studies in this laboratory have been aimed at the identification of analogues of amsacrine which might offer a broader clinical antitumor spectrum.

Amsacrine binds to DNA by intercalation (36) and induces single-stranded and double-stranded breaks in the DNA of treated cells (42). It resembles the anthracycline antibiotic daunorubicin in its action but is less cardiotoxic. Since Adriamycin, differing from daunorubicin in only one substituent, has a markedly broader antitumor spectrum (19), it may be hypothesized that appropriate substitution of amsacrine may increase its activity against clinical solid tumors. To this end, a large series of analogues of amsacrine have been synthesized, and their experimental antitumor activity has been determined (2, 6, 11-13, 15-18).

Many of these new analogues have now been evaluated to identify compounds with superior activity to amsacrine against solid tumors. One approach has involved the comparison of activity in vitro using a panel of human carcinoma cell lines. A second approach has been to investigate the activity of amsacrine analogues against the Lewis lung carcinoma in mice. This is the least responsive mouse tumor in the main NCI panel (23) and is resistant to Adriamycin, actinomycin D, ametantrone, amsacrine, daunorubicin, and mitoxantrone (7, 23).

The results of these studies have been to a large extent convergent, and a disubstituted derivative of amsacrine (CI-921; NSC 343 499) has been identified as the most active derivative of amsacrine in the Lewis lung tumor system. The synthesis, DNA binding properties, in vitro cytotoxicity, and in vivo antitumor activity of this compound form the basis of the present study. A short report of some of these properties has been published previously (3).

MATERIALS AND METHODS

Synthesis of CI-921. The key intermediate for the preparation of CI-921, 5-methylacridine-4-carboxylic acid, was first synthesized from 7-methylisatin (31) (Chart 1, Method A), but this method proved impractical for large quantities. A better overall yield was obtained from 3-methylthranilic acid (Chart 1, Method B) (15), but this is an expensive starting material. We have recently developed a third route (Chart 1, Method C) from the readily obtainable 2-iodosobenzenesulfonic acid.4

Conversion of this compound to 9-chloro-5-methylacridine-4-carbonyl chloride and subsequent selective reaction first with methylamine to provide N-methyl-9-chloro-5-methylacridine-4-carboxamide followed by N-(4-amino-3-methoxyphenyl)methanesulfonanilide gave the hydrochloride salt of the desired compound as reported (15). The hydrochloride (10 g) was dissolved in 50% aqueous ethanol (500 ml) and treated with

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1 This work was supported by the Cancer Society of New Zealand, Inc., by its Auckland Division, and by the Medical Research Council of New Zealand.
2 Dedicated to Professor Bruce F. Cain, who directed the development of the amsacrine series until his death in January 1981.
3 Recipient of a Warmer-Lambert Fellowship.
4 Received January 24, 1984; accepted May 1, 1984.
5 The abbreviations used are: NCI, National Cancer Institute; CI-921, N-5-dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4-acridinecarboxamide; NSC 343 499; 2-hydroxylcasesulfonate (1:1); ID50, drug concentration (μM) required to decrease the cell density at the end of cell culture by 50% relative to that of untreated control cultures; 0.01 AC buffer, 9.3 mM NaCl; 100 μM EDTA; 2 mM sodium acetate (pH 5.0; ionic strength, 0.01); ILS, increase in life span of treated tumor-bearing animals relative to that of untreated control mice; poly(4-deoxyadenylate-4-deoxythymidylate) (alternating); poly(4-deoxyguanylate-4-deoxythymidylate) (alternating).
6 W. A. Denny and W. A. Denny, manuscript submitted for publication.
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Nucleated red blood cells (per liter): Na₂HPO₄ (1.15 g/liter): CaCl₂ (0.1 g/liter): MgCl₂ (0.1 g/liter). Lewis lung cell cultures were inoculated i.p. (10⁶ cells) in frozen stocks in 1977 by the Development Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Bethesda, MD. B6D2F₁ mice under conditions of constant temperature and humidity, with sterile bedding, water, and food were used to establish a breeding unit for the production of C57BL/6J x DBA/2J Fi (hereafter called B6D2F₁) mice. All B6D2F₁ mice were adjusted to pretreatment body weight in groups which were weight matched within 10% of each other. Drug doses were set at 1.5-fold intervals, and the optimal dose was defined as the dose giving the highest ILS without causing significant toxicity. Deaths were recorded daily, and the percent tumor size (measured in 2 dimensions with calipers) on Day 7. Mice were then ear-tagged and divided into groups of 8, tumors being measured thereafter 3 times weekly with vernier slide calipers. Drug treatment was initiated on Day 8. Tumor volume was calculated as 0.52 a²b², where a and b are the minor and major axes, respectively, of the tumor. The percent tumor reduction of each group was calculated for each on the basis of the logarithms of the tumor volumes.

Cell Culture. L1210 and P388 cell lines grown in culture were the same as used for in vivo studies. The Adriamycin-resistant cell line was kindly provided by Mason Research, Inc. The mouse leukemia lines were passaged in DBA/2J carrier mice and adapted to cell culture for 3 weeks before use in growth inhibition assays. All 3 mouse leukemia lines were cultured in 24-well trays in RPMI 1640 medium (Grand Island Biological Co., Grand Island, NY) containing 10% (v/v) heat-inactivated fetal calf serum as described previously (9, 41). The remaining cell lines were cultured in 24-well trays in α-minimal essential medium containing 10% (v/v) heat-inactivated fetal calf serum without antibiotics and is described fully elsewhere (21, 41). Cells were judged Mycoplasma free by cytochemical staining (14). V-79-171b cells were from Dr. W. R. Inch, London, Ontario, Canada. A tissue culture-adapted Lewis lung cell line was provided by Dr. R. C. Jackson, Warner-Lambert Research Laboratories, Ann Arbor, MI, and originated from the Southern Research Institute, Birmingham, AL (39). HCT-8 cells were originally established in culture by Tompkins et al. (35) and were obtained from Dr. R. C. Jackson, Jurkat leukemia cells (30) were provided by Dr. J. D. Watson, Department of Pathology, University of Auckland Medical School. Descriptions of the other human tumor cell lines are given in the following references: HT-29 (22); LoVo (20); MCF-7 (32); MDA-MB-231 (10); T-47D (25); and MM-96 (38).

The ID₅₀ assay procedure involves the addition of drugs at the appropriate dilution to exponentially growing cultures in 24-well trays and further growth of cells for 4 to 5 generations. Cells were counted at the end of the incubation period using an electronic particle counter (Coulter). The clonogenicity of one of the cell lines (T-47D) was assessed by trypanozinizing (0.07% trypsin for 10 min), washing with growth medium, and replating in 60-mm Petri dishes containing 5 ml of medium. Colonies of greater than 30 cells were counted 17 days later after staining with 50% (v/v) aqueous ethanol containing 0.5% methylene blue.

Flow Cytometry. L1210 cell cultures (1 ml) were prepared for flow cytometry by addition of phosphate-buffered saline (1 ml) containing 0.4% Triton X and 10 μl of an ethanolic solution of diamidinophenylindole (0.2 mg/ml) according to the method of Taylor (34). Analysis was carried out using an Ortho Instruments ICP 22A cytometer with a Model 2103 multichannel analyzer. Proportions of cells in different phases of the cell cycle were estimated by fitting symmetrical curves to the G₀ and G₂/M phase peaks and measuring areas.

DNA Binding. DNA binding isotherms were measured by equilibrium dialysis using a Dianorm apparatus (Diachema A. G., Zurich, Switzerland) with two 1/ml compartments separated by Diachema membranes (M, cut-off, 10,000). Calf thymus DNA (1 to 15 Åₑ units/ml) and CI-921 were dissolved in 0.01 AC buffer. The cells were rotated at 16 rpm for 15 to 20 hr at 25° (equilibrium was attained well before this time), and the contents of each cell were then assayed spectrophotometrically. The spectrum of the DNA-containing cell was measured using the DNA-free cell as a reference, and the bound drug concentration was calculated from the extinction coefficient of DNA-bound drug (measured in a 1 mg/ml solution of DNA).

Viscometry. The extent of DNA unwinding induced by drug binding was determined by measuring the viscosity of closed circular DNA at 25° using methods published previously (36). The DNA was prepared from a mini-col E1 plasmid PML-21 (24), kindly provided by Dr. H. E. D. Lane, Department of Cell Biology, University of Auckland. Equilibrium sedimentation in ethidium bromide:cesium chloride was used for purification of the DNA. Small aliquots of drug solution (0.4 mg/ml in H₂O) were added to an Ostwald viscometer containing 1.1 ml of the DNA solution (65 μg/ml) in 0.01 AC buffer. The helix unwinding angle was calculated as 26° x rₐ/rₑ, where rₑ and rₐ are the binding ratios at the

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**Materials.** Amsacrine was either synthesized in this laboratory as described previously (12) or obtained from Warner-Lambert as the isethionate. Cyclophosphamide (Bristol-Myers), 5-fluorouracil (Roche), Adriamycin (Farmitalia), and daunorubicin (May & Baker) were obtained as clinical formulations. Calf thymus DNA (type V) was from Calbiochem, and calf thymus DNA (1 to 15 Åₑ units/ml) and CI-921 were dissolved in 0.01 AC buffer. The cells were rotated at 16 rpm for 15 to 20 hr at 25° (equilibrium was attained well before this time), and the contents of each cell were then assayed spectrophotometrically. The spectrum of the DNA-containing cell was measured using the DNA-free cell as a reference, and the bound drug concentration was calculated from the extinction coefficient of DNA-bound drug (measured in a 1 mg/ml solution of DNA).

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equivalence point for ethidium and CI-921, respectively. Binding ratios were calculated from the drug association constants determined by equilibrium dialysis using the McGhee and von Hippel equation (29).

RESULTS

Physicochemical Properties. The lipophilicity of CI-921 was determined using a thin-layer chromatographic system to measure relative mobilities \([Rm = \log \left(\frac{1}{Rf} - 1\right)]\), which are linearly related to the logarithms of the partition coefficients between n-octyl alcohol and water (log \(p\) values) (17). The \(Rm\) values determined for the cationic forms of CI-921 and amsacrine are, respectively, 0.34 and 0.18, corresponding to log \(p\) values of 1.10 and 0.60 (17, 18) and indicating that the analogue is slightly more lipophilic (0.5 log \(p\) units) than amsacrine. Acridine \(pK_a\) values for amsacrine and analogues have been determined previously (2) in 20% aqueous dimethyl formamide. Under these conditions, amsacrine has a value of 7.43, while CI-921 is a considerably weaker base, with a \(pK_a\) of 6.40 (15).

DNA Binding Properties. Binding of CI-921 to double-stranded calf thymus DNA in 0.01 AC buffer was accompanied by a bathochromic shift in the wavelength of maximal absorption from 448 nm to 464 nm and a decrease in the extinction coefficient at the wavelength of maximal absorption from 12,740 to 8,360 cm\(^{-1}\) M\(^{-1}\). Equilibrium dialysis was carried out in this buffer using calf thymus DNA (Chart 2). The scatter of the data reflects the low concentrations of drug in the DNA-free compartment. A theoretical curve was calculated using the equation derived by McGhee and von Hippel (29) for a model with neighboring site exclusion and assuming a binding site size of \(n = 2\) base pairs. The association constant obtained was \(2.1 \times 10^6\) \(M^{-1}\), 16-fold higher than that of \(1.3 \times 10^5\) \(M^{-1}\) obtained (at pH 6.0) with amsacrine at the same ionic strength (40).

The base sequence binding selectivity was examined using poly(dA-dT) and poly(dG-dC) in 0.01 AC buffer using an ethidium displacement method as described previously (5). Competition binding equations developed by McGhee and von Hippel (29) were combined with a correction for quenching of DNA-bound ethidium fluorescence by bound drug (39). This quenching effect, which may be due to an electron transfer phenomenon (8), was 45% greater with CI-921 than with amsacrine at the same binding ratio. Binding constants for poly(dA-dT) and poly(dG-dC) were, respectively, \(1.15 \times 10^6\) \(M^{-1}\) and \(4.4 \times 10^5\) \(M^{-1}\), as compared to \(3.7 \times 10^5\) \(M^{-1}\) and \(4.5 \times 10^5\) \(M^{-1}\) for amsacrine (4). CI-921 therefore demonstrated greater selectivity than did amsacrine for poly(dG-dC), as opposed to poly(dA-dT).

The binding mode of CI-921 was investigated by viscometric titration with closed circular duplex DNA (Chart 3). As the drug:DNA phosphate (D:P) ratio increased, the viscosity first increased and then decreased, indicative of intercalative binding. The unwinding angle of 18° (corrected for the proportion of unbound drug) is slightly less than that of amsacrine (20.5°) and less than that of ethidium (26°).

In Vitro Inhibition of Tumor Cell Growth. CI-921 was compared to amsacrine in growth inhibition assays using a variety of rodent and human tumor cell lines (Table 1). CI-921 had a potency similar to that of amsacrine in the mouse leukemia lines but was more active against the Lewis lung carcinoma and against all the human tumor cell lines tested. Adriamycin and daunorubicin, 2 other DNA binding drugs which differ only minimally in structure but show different spectra of clinical activity, were also compared (Table 1). It was of interest to determine whether differences in patterns of sensitivity between CI-921 and amsacrine were mirrored by differences between Adriamycin and daunorubicin. The ratios of ID\(_{50}\) values for each pair of drugs are therefore compared in Table 1 and indicate similar patterns.

Relationship of Growth Inhibition to Cytotoxicity. In order to determine whether growth inhibition was a result of cytotoxicity or merely cytostasis, T-47D cells, at the end of the ID\(_{50}\) assay, were plated to determine the number of clonogenic cells. The relationship between total cell number and number of clonogenic cells is shown for various concentrations of amsacrine (3 to 50
Table 1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Origin</th>
<th>IDso (nM)</th>
<th>CI-921 Amsacrine</th>
<th>IDso (nM)</th>
<th>Adriamycin</th>
<th>Daunorubicin</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1210</td>
<td>Mouse leukemia</td>
<td>38</td>
<td>33</td>
<td>1.2</td>
<td>30</td>
<td>21</td>
</tr>
<tr>
<td>P388</td>
<td>Mouse leukemia</td>
<td>12</td>
<td>14</td>
<td>0.86</td>
<td>14</td>
<td>18</td>
</tr>
</tbody>
</table>
| P388/ADR  | Mouse leukemia       | 800       | 550               | 1.5       | 145        | 55           | 2.64
| V-79      | Chinese hamster fibroblast | 9.5 | 16 | 0.59 | 19 | 18 | 1.1 |
| Lewis     | Mouse lung carcinoma| 12.6      | 27                | 0.47      | 30         | 150          | 0.20
| HCT-8     | Human colon carcinoma| 25       | 70                | 0.36      | 63         | 220          | 0.29 |
| HT-29     | Human colon carcinoma| 20       | 72                | 0.28      | 25         | 83           | 0.30 |
| LoVo      | Human colon carcinoma| 12       | 47                | 0.26      | 13         | 75           | 0.17 |
| MCF-7     | Human breast carcinoma| 18      | 79                | 0.23      | 13         | 56           | 0.23 |
| MDA-231   | Human breast carcinoma| 31      | 77                | 0.40      | 20         | 73           | 0.27 |
| T-47D     | Human breast carcinoma| 6.7     | 28                | 0.24      | 2.1        | 22           | 0.10 |
| MM-96     | Human melanoma       | 16        | 50                | 0.32      | 6.3        | 48           | 0.13 |
| Jurkat    | Human T-cell leukemia| 3.6      | 12                | 0.30      | 9.2        | 38           | 0.24 |

Chart 4. Comparison of cell killing and growth inhibition by CI-921 (○) and amsacrine (○), using cultured T-47D human mammary carcinoma cells. At the end of the standard IDso assay procedure, cells were counted to determine total cell density or replated at the appropriate dilution to assess clonogenicity. Clonogenicity is expressed as a percentage of that of untreated cells.

nm) and CI-921 (0.5 to 10 nm) in Chart 4. The plating efficiency of treated cells decreased with increasing drug concentrations, indicating increasing cytotoxicity. The decrease was similar for both drugs.

Cell Cycle Effects. The effects of CI-921 on the cell cycle were investigated by flow cytfluorometry using cultured L1210 leukemia cells. The compound caused a dose- and time-dependent accumulation of cells in G2 phase. At a concentration of 100 nm, an increase in G2-phase cells was evident from 4 hr onwards. Exposure for 24 hr to a drug concentration of 6 nm produced a significant increase in G2-phase cells (27% as compared to the control value of 16%), and at higher concentrations, a high proportion of the total population could be trapped in G2 phase (Chart 5).

Activity towards P388 Leukemia in Vivo. Although having a therapeutic range similar to that of amsacrine (active over a 10-fold dose range), CI-921 provided a considerably higher life extension at the optimal dose (Chart 6). This superiority was manifested when the drug was given i.p., p.o., or i.v. (Chart 6; Table 2). CI-921 was also therapeutically superior to Adriamycin and daunorubicin, although its dose potency was lower (Table 2).

Activity towards the Lewis Lung Carcinoma in Vivo. Amsacrine had only marginal activity towards this tumor when injected i.p. on Days 1, 5, and 9 after i.v. injection of tumor cells (Table 3). Adriamycin and daunorubicin were inactive with this route and schedule of administration. In 3 experiments, CI-921 provided 14 of 17 sixty-day survivors and provided highly significant life extensions in the remainder.

In order to provide a larger number of target cells, the Lewis
Properties of a New Amsacrine Derivative

Chart 6. Dependence on dose of the percentage of ILS of animals inoculated i.p. on Day 0 with 10⁶ P388 leukemia cells. CI-921 (○, ●); amsacrine (○, △). Drugs were administered on Days 1, 5, and 9 either i.p. (○, ●) or p.o. (●, △).

Table 2
Activity of CI-921 and other agents towards i.p.-inoculated P388 leukemia

<table>
<thead>
<tr>
<th>Compound</th>
<th>Route</th>
<th>Optimal dose (mg/kg)</th>
<th>% of ILS at optimal dose</th>
<th>Survivors (50 day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI-921</td>
<td>i.p.</td>
<td>20</td>
<td>200</td>
<td>3/6</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>50</td>
<td>164</td>
<td>1/6</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>30</td>
<td>136</td>
<td>1/6</td>
</tr>
<tr>
<td>Amsacrine</td>
<td>i.p.</td>
<td>8.9 or 13.3</td>
<td>78</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>65</td>
<td>62</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>30</td>
<td>72</td>
<td>0/6</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>i.p.</td>
<td>3.9</td>
<td>79</td>
<td>1/6</td>
</tr>
<tr>
<td>Daunorubicin</td>
<td>i.p.</td>
<td>2.7</td>
<td>60</td>
<td>0/6</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>i.p.</td>
<td>225</td>
<td>216</td>
<td>5/6</td>
</tr>
</tbody>
</table>

* Drug administered on Days 1, 5, and 9 after tumor inoculation, except for cyclophosphamide, which was administered on Day 1 only. Data (i.p.) for amsacrine represent an average of multiple determinations, and the optimal dose was either 8.9 or 13.3 mg/kg.

Table 3
Activity of CI-921 and other agents towards i.v.-inoculated Lewis lung carcinoma

<table>
<thead>
<tr>
<th>Compound</th>
<th>Route</th>
<th>Schedule (days of administration)</th>
<th>Optimal dose (mg/kg)</th>
<th>ILS (%)</th>
<th>Survivors (60 day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI-921</td>
<td>i.p.</td>
<td>1</td>
<td>20</td>
<td>61</td>
<td>1/6</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>1, 5, 9</td>
<td>20</td>
<td>168</td>
<td>14/17</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>5</td>
<td>20</td>
<td>38</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>5, 9, 13</td>
<td>30</td>
<td>197</td>
<td>9/21</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>9, 13, 17</td>
<td>30</td>
<td>86</td>
<td>0/10</td>
</tr>
<tr>
<td></td>
<td>p.o.</td>
<td>5, 9, 13</td>
<td>30</td>
<td>132</td>
<td>0/6</td>
</tr>
<tr>
<td></td>
<td>i.v.</td>
<td>5, 9, 13</td>
<td>30</td>
<td>127</td>
<td>5/6</td>
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<tr>
<td>Amsacrine</td>
<td>i.p.</td>
<td>1, 5, 9</td>
<td>8.9 or 13.3</td>
<td>38</td>
<td>0</td>
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<td></td>
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<td>5, 9, 13</td>
<td>8.9 or 13.3</td>
<td>42</td>
<td>0</td>
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<td></td>
<td>p.o.</td>
<td>5, 9, 13</td>
<td>75</td>
<td>38</td>
<td>0/6</td>
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<tr>
<td></td>
<td>i.v.</td>
<td>5, 9, 13</td>
<td>30</td>
<td>59</td>
<td>1/6</td>
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<tr>
<td>Adriamycin</td>
<td>i.p.</td>
<td>5, 9, 13</td>
<td>2.6</td>
<td>25</td>
<td>0/6</td>
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<tr>
<td>Daunorubicin</td>
<td>i.p.</td>
<td>5, 9, 13</td>
<td>3.9</td>
<td>12</td>
<td>0/6</td>
</tr>
<tr>
<td>Cyclophosphamide</td>
<td>i.p.</td>
<td>1</td>
<td>225</td>
<td>216</td>
<td>5/6</td>
</tr>
<tr>
<td></td>
<td>i.p.</td>
<td>5</td>
<td>225</td>
<td>185</td>
<td>5/6</td>
</tr>
</tbody>
</table>

*Average of multiple experiments; optimal dose was either 8.9 or 13.3 mg/kg.*

The primary aim of our studies with amsacrine analogues has been to develop compounds with activity against solid tumors. This development can be regarded theoretically as an optimization of pharmacokinetics and drug stability (to allow maximal exposure of tumor cells to drug) and optimization of intrinsic activity towards tumor cells (cell cycle selectivity, transport, metabolism) as compared to normal cells. CI-921, developed as a result of previous structure-activity studies (6, 18) which indicated the optimal physicochemical properties for this class of (7), the solvent for i.p. dosage was 30% (v/v) aqueous ethanol. However, distilled water (optimal dose, 30 mg/kg; ILS, 203% with 5 of 6 sixty-day survivors) and 50% dextrose in water (optimal dose, 30 mg/kg; ILS, 161% with 4 of 6 survivors) were acceptable solvents, providing full dissolution of the agent. CI-921 thus showed activity comparable to cyclophosphamide (the NCI standard for this tumor) and superior to 5-fluorouracil.

When treatment was initiated at Day 9, at a stage where tumor nodules in the lung were 1 to 2 mm in diameter and the mice were approximately 7 days from death, a highly significant increase in life span was still achieved, although there were no long-term survivors. Advanced s.c. tumors were also responsive to treatment with CI-921 (Chart 7). On the basis of data from several experiments, control tumors grew in a fashion which could be acceptably described by a Gompertzian growth equation (33), with an initial doubling time of 18 hr and a halving of the growth rate every 8 days. At the time of initiation of treatment, the tumor volume was estimated as 1 cu cm, and the doubling time was 36 hr. The delay in tumor regrowth corresponded to an overall 6 log₁₀ cell kill (assuming no reversible growth inhibition) and was comparable to that of cyclophosphamide. Measured tumor volumes after 4 days were all significantly less than those of the control tumors (p < 0.01). In contrast, amsacrine treatment at the highest tolerated dose resulted in no significant reduction in tumor volume over the course of the experiment (data not shown).

DISCUSSION

The primary aim of our studies with amsacrine analogues has been to develop compounds with activity against solid tumors. This development can be regarded theoretically as an optimization of pharmacokinetics and drug stability (to allow maximal exposure of tumor cells to drug) and optimization of intrinsic activity towards tumor cells (cell cycle selectivity, transport, metabolism) as compared to normal cells. CI-921, developed as a result of previous structure-activity studies (6, 18) which indicated the optimal physicochemical properties for this class of...
compound, has shown the highest levels of activity of any amsacrine derivative, particularly against the Lewis lung carcinoma.

The first physicochemical parameter to be investigated in the 9-anilinoacridine series was lipophilicity, and a clear parabolic dependence of antileukemic activity (as measured by increased life span) on lipophilicity was demonstrated (13). More recent studies have shown that measurements of in vitro and in vivo activity towards L1210 leukemia cells are related by a parabolic term in lipophilicity (4), suggesting that this property plays an important role in the pharmacokinetics of drugs in this series. Lipophilicity also appears to be of critical importance in determining the activity of amsacrine derivatives towards the Lewis lung carcinoma, by combining a lipophilic (methyl) and hydrophilic (N-methylcarboxamide) group, possesses near-optimal lipophilicity as suggested by structure-activity relationships (15, 18). These groups also have the additional advantage of conferring greater water solubility than is shown by amsacrine.

Investigation of the other effects of acridine ring substituents of amsacrine was initially carried out by comparing the effect of substitution on antileukemic activity with that expected on the basis of the changes in lipophilicity alone, the latter being ascertained with reference to homologous alkanesulfonamide series containing constant acridine substituent patterns (12, 13). A number of substituents showed increased activity over that expected from changes in lipophilicity alone (12, 16), and the 4-methyl (equivalent to the 5-methyl in CI-921) derivative (11) and a variety of N-substituted 4-carboxamide groups (16) showed large effects. Recently, both the 4-methyl and the 4-N-methyl carboxamide substituents have been identified as providing greatly increased activity towards the Lewis lung carcinoma in mice (7).

Substituents which result in such increases in activity may do so by altering a number of physicochemical properties simultaneously. A number of acridine substituents are known to increase DNA binding (6). Multivariate equations modelling dose potency indicate that both high DNA binding affinity and high acridine base strength (pKᵦ) are associated with high dose potency. This may be rationalized in terms of increased retention in tumor cells (i.e., by drug binding to DNA) and decreased excretion rates (since charged molecules are excreted at lower rates). However, anti-leukemia L1210 selectivity, as measured by the life extension at the optimal dose, cannot be modelled in terms of these parameters for monosubstituted amsacrine derivatives (6). Dose potency and drug solubility are important practical considerations in the design of new agents. It can be argued that a low base strength, ensuring that a significant proportion of the circulating drug is in a neutral form, allows more rapid distribution across biological membranes to provide improved distribution into solid tumors. On the other hand, the resulting decrease in dose potency suggested by the structure-activity equations (6) will lead to problems with drug solubility. CI-921 represents a solution to this dilemma in that the DNA association constant is significantly higher than that of amsacrine, thus compensating for the decrease in acridine base strength, so that the resulting dose potency is only slightly lower than that of amsacrine itself.

A feature of the experimental antitumor results (Tables 3 and 4) of CI-921 is the high activity of p.o.-administered drug. This may be a consequence of the low base strength and comparably high water solubility of this compound.

Acridine substitution of amsacrine has been shown previously to influence the relative activity towards individual cell lines (41). Table 1 represents an attempt to determine the extent of this variation for CI-921 relative to amsacrine by presenting results with a number of different rodent and human cell lines in culture. Ratios of ID₉₀ values vary from 1.5 to 0.23 with the human lines showing the greatest selectivity for CI-921. By comparison, the corresponding range for Adriamycin and daunorubicin is from 2.6 to 0.1, and there is evidence of parallel effects with the 2 pairs of compounds. Although some of these effects may be artifacts of tissue culture techniques (e.g., differences in drug half-life in different culture media), the results suggest strongly that individual cell line sensitivity differences do exist and that CI-921 and Adriamycin show increased activity towards carcinoma cells as compared to amsacrine and daunorubicin.

In conclusion, CI-921 represents an analogue of amsacrine which was selected from in vitro and in vitro screening of several hundred members (15, 18) of the 9-anilinoacridine series. While it resembles amsacrine in its mode of action on cultured cells (G₂ arrest), its activity against P388 leukemia and Lewis lung carcinoma is superior to that of amsacrine and to all other derivatives of amsacrine tested so far. A particular feature is its activity towards advanced Lewis lung carcinoma, growing either in the lung or s.c., where it is similar to cyclophosphamide in its efficacy. It is active against a number of other tumors including L1210 leukemia, B16 melanoma, the CDBF1 mammary tumor, and the colon 26 and 38 tumors. This activity, in combination with the selective activity towards human carcinoma cell lines in culture, strongly suggests that it may be superior to amsacrine in the treatment of human carcinomas. It has now been selected as a candidate drug for clinical trial.

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REFERENCES

7. Baguley, B. C., Kemohan, A. R., and Wilson, W. R. Divergent activity of...
Synthesis, Antitumor Activity, and DNA Binding Properties of a New Derivative of Amsacrine, *N*-5-Dimethyl-9-[(2-methoxy-4-methylsulfonylamino)phenylamino]-4-acridinecarboxamide


*Cancer Res* 1984;44:3245-3251.

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