# Acridine Derivatives. VI [1]. Redox Chemistry of Novel 9-Anilinoacridines with Antitumor Activities



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In order to elucidate the mechanism of deoxyribonucleic acid (DNA) strand breaks caused by 9-anilinoacridine DNA intercalators, the antitumor activity of L1210, P388 and the reduction-oxidation (redox) reaction of 9-anilinoacridines were studied. The redox reaction induced by two electrons causing structural changes in quinone diimines of 9-anilinoacridines is believed to be an important factor in DNA strand breaks and was examined by means of temperature-dependent nuclear magnetic resonance and cyclic voltammetry. The redox reaction of 9-anilinoacridines is induced by the effect of a low-energy electron transfer from the acridine to the aniline ring. We propose that the redox reaction plays an important role in the DNA strand cleavage of 9-anilinoacridine when it is intercalated into double-strand DNA.

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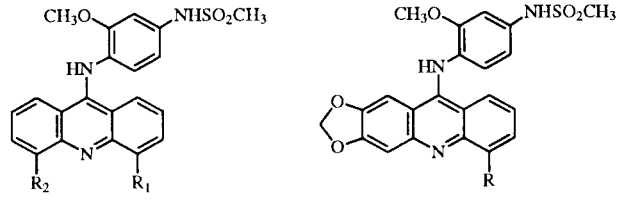
The DNA intercalators 9-anilinoacridines have been extensively investigated as antitumor agents. In particular, *m*-AMSA and CI-921 are actually used clinically for the treatment of leukemia [2-4]. Their biological activities are probably due to the causation of double-strand DNA breaks, and *m*-AMSA is known to be an inhibitor of topoisomerase II, an enzyme required for topological DNA changes such as replication, transcription, recombination, *etc.* [2]. In order to discover new active antitumor compounds, we have synthesized various novel *m*-AMSA-related acridine derivatives and studied their antitumor activities against L1210 and P388 murine leukemia [5-7]. In addition, we also examined the ethidium-DNA fluorescence quenching effect of these compounds and discovered a substance that exhibits a stronger fluorescence quenching effect than *m*-AMSA [7]. Furthermore, we confirmed that the fluorescence quenching effect shows a very good correlation with antitumor activity [7]. In addition, regarding the mechanism of double strand cleavage as recently reported for DNA intercalators, it has been shown that the aniline rings of 9-anilinoacridines are readily and reversibly oxidized either chemically or microsomally to give the two-electron oxidation form, the quinone diimine, and this redox reaction has been shown to play an important role in mammalian metabolism and ability to break DNA strands [8-10].

Scheme 1




*m*-AMSA $R_1 = R_2 = H$
CI-921 $R_1 = CH_3$, $R_2 = CONHCH_3$

A4P73 R = H
A3P166 R = Cl

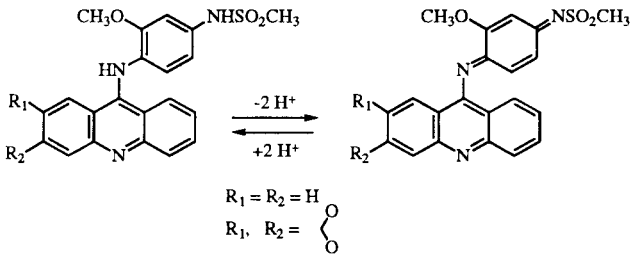
In this study, we report on the synthesis of three acridine derivatives, *m*-AMSA, A4P73, and A3P166 (Scheme 1), and measurements of their antitumor activities and redox reaction. We used *m*-AMSA, A4P73, and A3P166 to analyze the data of temperature-dependent nuclear magnetic resonance (nmr) and cyclic voltammetry, and found new properties related to the redox reaction

Table 1

Summary of L1210 Antitumor Activity and Ethidium Bromide-DNA Fluorescence Quenching

| Compounds | Dose (mg/kg) | Median Survival Days (M.S.D.) | T/C (%) | Ethidium Bromide-DNA Fluorescence Quenching [7] $C_{50}$ (x $10^{-3}$ *mM*) |
|---|---|---|---|---|
| *m*-AMSA | 10 x 2 | 15.55 | 100 | 78 |
| A4P73 | 10 x 2 | 16.23 | 104 | 65 |
| A3P166 | 10 x 2 | 10.71 | 69 | 80 |

Scheme 2




induced by a two-electron oxidation reaction to quinone diimines which we report here.

Results and Discussion.

The best results of antitumor activities against L1210 and P388 murine leukemia cells of the three acridine derivatives, *m*-AMSA, A4P73 and A3P166, are shown in Tables 1 and 2. When comparing the median survival of the group administered *m*-AMSA with that of the drug-free group, a marked life-extending effect was found.

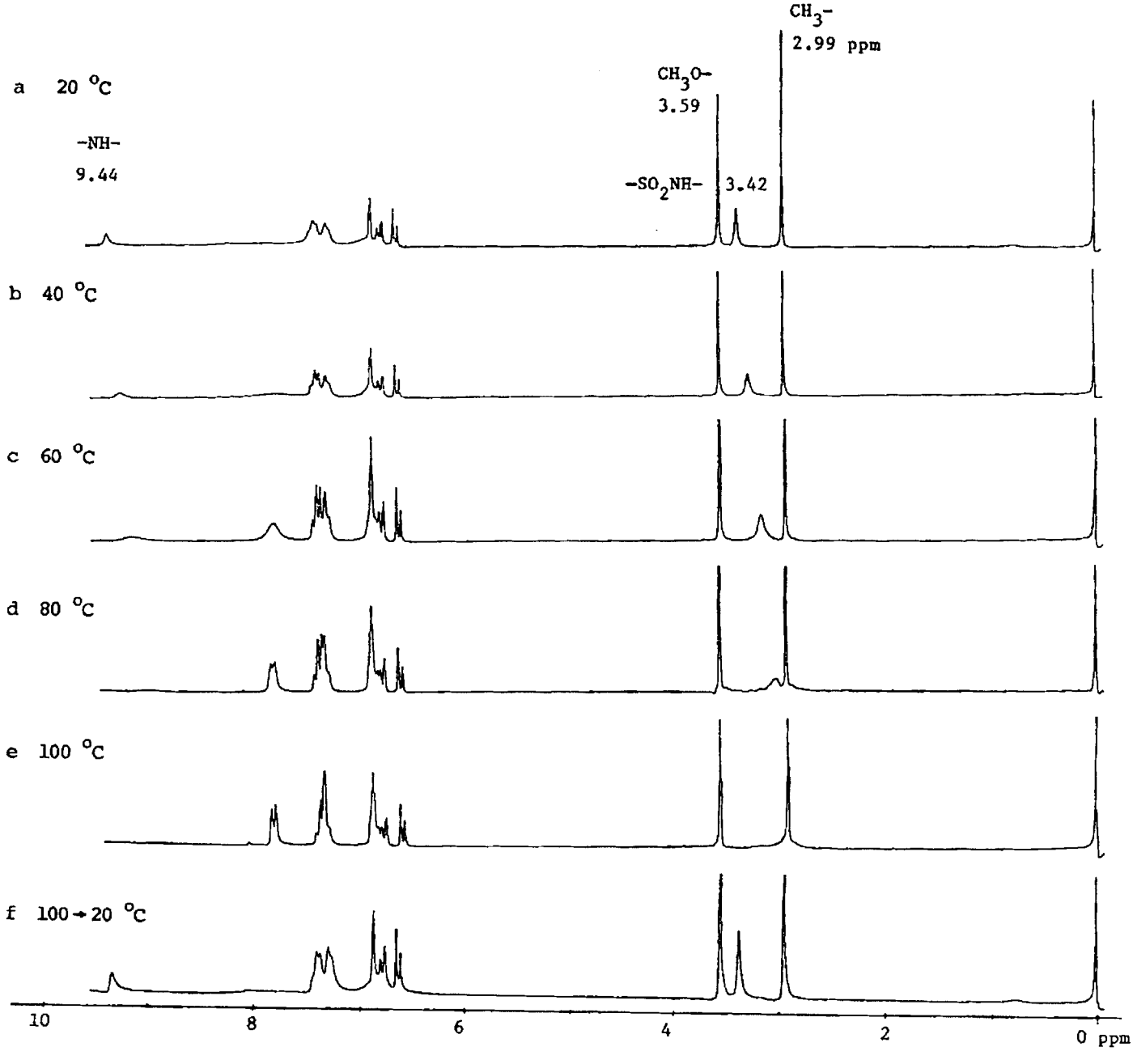



Figure 1. $^1$H-nmr spectra of *m*-AMSA measured in DMSO-$d_6$ at a: 20°, b: 40°, c: 60°, d: 80°, e: 100°, and during temperature cooling from 100° to 20° as described in the Experimental.

Table 2
Antitumor Activity Against Murine P388 Leukemia

| Compounds | Dose (mg/kg) | Mean Survival Day±SD | ILS (%) | 30-Day Survivors |
|---|---|---|---|---|
| Control | | 9.6 ±0.5 | 0 | 0/5 |
| *m*-AMSA | 6.25 | 15.2 ±0.8 | 58 | 0/5 |
| | 12.5 | 18.2 ±3.6 | 90 | 0/5 |
| | 25.0 | 17.6 ±1.1 | 80 | 0/5 |
| A4P73 | 6.25 | 13.8 ±0.8 | 44 | 0/5 |
| | 12.5 | 17.0 ±1.4 | 77 | 0/5 |
| | 25.0 | 18.4 ±0.5 | 92 | 0/5 |
| A3P166 | 6.25 | 9.6 ±0.5 | 0 | 0/5 |
| | 12.5 | 9.8 ±0.4 | 2 | 0/5 |
| | 25.0 | 10.0 ±0.9 | 4 | 0/5 |
| MMC | 2.0 | 17.6 ±1.1 | 80 | 0/5 |
| | 4.0 | 14.8 ±1.3 | 33 | 0/5 |

On the other hand, we tested the life-extending value of A4P73 and A3P166 in comparison with *m*-AMSA and found that the activity of A4P73 was higher (1.04) than that of *m*-AMSA, while that of A3P166 was lower (0.69) than that of *m*-AMSA. Furthermore, according to a previous report [7], the DNA-ethidium bromide quenching effect was in the order of A4P73, *m*-AMSA and A3P166; these data show the order of intercalation strength with double strand DNA. Recent reports [8,9,11] related to the mechanism of cleavage of double-stranded DNA by acridine derivatives having antitumor activity propose a process in which the acridine derivatives first intercalate between DNA base pairs, after which divalent metals such as $Mg^{2+}$ and $Cu^{2+}$ then become involved, causing a redox

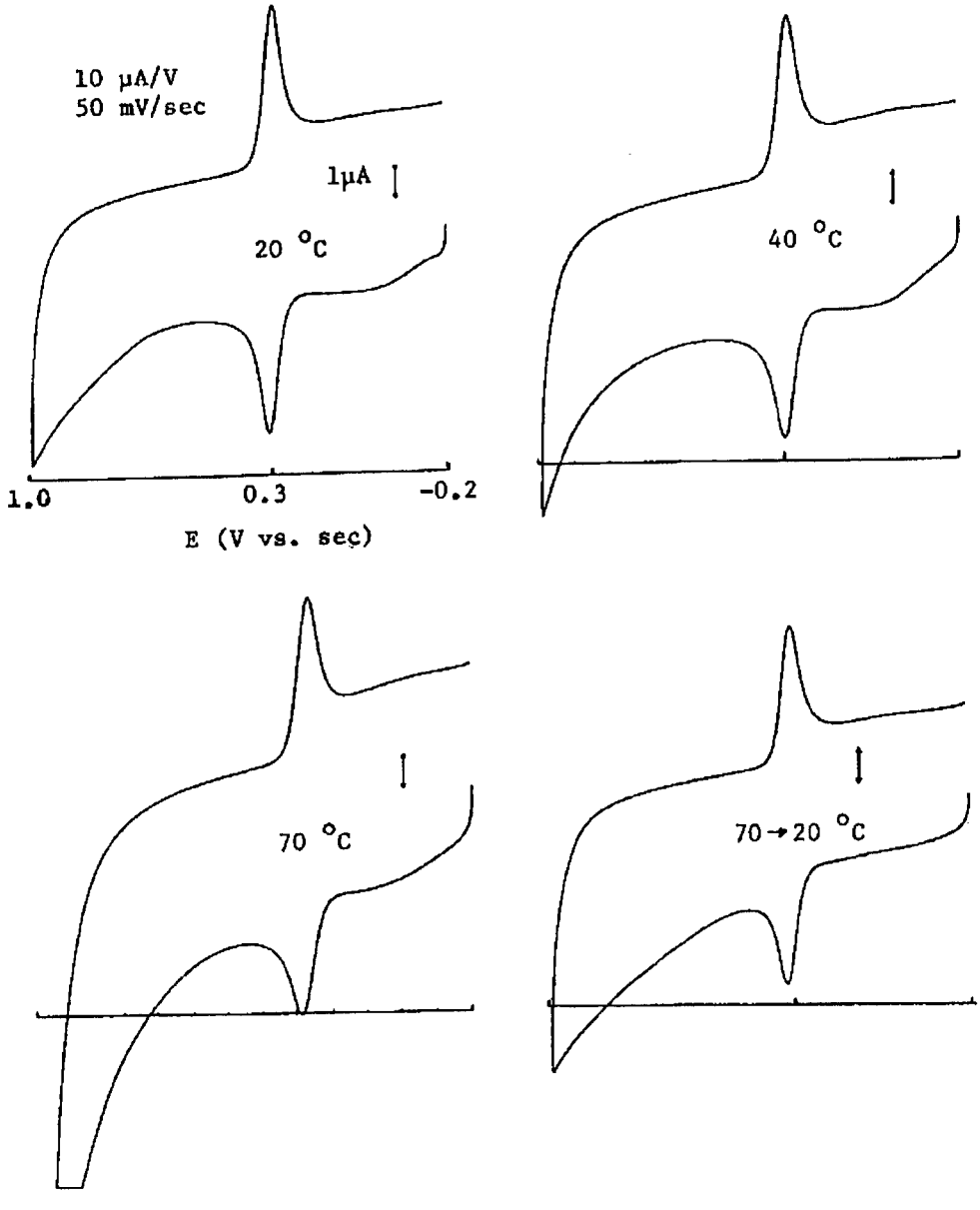



Figure 2. Temperature-dependent cyclic voltammetry of A4P73 showing reversibility of the redox reaction.

reaction induced by a two-electron oxidation reaction of the acridine derivative (Scheme 2) breaking the double strand DNA. Therefore, we conducted a detailed study of the redox reaction involving *m*-AMSA-related acridines using *m*-AMSA, A4P73 and A3P166 at various temperatures (20, 40, 60, 100°, and cooling from 100° to 20°), nmr measurements, and cyclic voltammetry over various temperature ranges (20, 40, 100°, and cooling from 70° to 20°). Analyses of the nmr data for *m*-AMSA (Figure 1) revealed that during a temperature increase from 20° to 100°, the two NH-group protons, *i.e.*, protons at the NH-phenyl (9.44 ppm) and phenyl-$NHSO_2$- (3.42 ppm) regions, shift to higher magnetic fields as the temperature rises, and these protons return to their original states (are reversible) when the temperature is gradually lowered from 100° to 20°. These data suggest that the two readily transferred protons (labile protons) are involved in a redox reaction induced by a two-electron oxidation during double strand DNA cleavage.

When cyclic voltammetry of A4P73 was performed at various temperatures (20, 40, 70 and 70-20°), the results shown in Figure 2 were obtained. These data confirm that redox reactions involving two-electron oxidation of A4P73 occurred, and confirmed that the reversible redox reaction is promoted by temperature increases.

The results of pharmacological tests on L1210 and P388 antitumor activity and the results of $^1$H-nmr measurements and cyclic voltammetry confirmed that the *m*-AMSA-related acridines, A4P73 and A3P166, are intercalated into double strand DNA, causing a redox reaction induced by two-electron oxidation, which enabled double strand DNA cleavage and stabilization.

## EXPERIMENTAL

### Spectroscopy.

The $^1$H-nmr spectra were measured in deuteriodimethyl sulfoxide (DMSO-$d_6$) solution in 5 mm tubes on a JEOL FX-200 spectrometer (JEOL Ltd., Tokyo, Japan). Chemical shifts were recorded as units relative to tetramethylsilane (δ 0.0) as the internal standard. Measurements were made at 20, 40, 60, 80 and 100°, and after cooling to 20° following heating to 100°. The ir spectra were measured on a JASCO A-3 spectrometer. The melting points were measured on a Yanagimoto micromelting point apparatus, and were uncorrected.

### Cyclic Voltammetric Measurements.

A YANACO (yanagimoto Seisaku-sho, Inc., Kyoto, Japan) P1100 (voltage capability 100 V, accuracy 1 mV) was used for the cyclic voltammetric measurements. The sample solutions were adjusted to a concentration of 0.1 *mM* with a 40% acetonitrile-acetate buffer (*p*H 4.5). A sample solution (30 ml) was placed in the measurement cell and purged with nitrogen gas for 10 minutes before measurement. At the time of measurement, a glassy carbon electrode was well polished, and a cyclic voltammogram was obtained after two measurements made at a 10 μA/V sensitivity and scan rate of 50 mV/sec. Measurements were made at 20, 40, 70° and after cooling to 20° from 70°.

### 4'-(9-Acridinylamino)methanesulfonyl-*m*-anisidide (*m*-AMSA).

*m*-AMSA was prepared according to the method described by Cain, *et al.* [12]. The melting point, nmr and ir spectra supported the structure of *m*-AMSA, mp 196-198°; ir (potassium bromide): ν 3500-2800, 2350, 1640, 1500 $cm^{-1}$; $^1$H-nmr (DMSO-$d_6$): δ 2.99 (s, 3H, $CH_3$), 3.42 (s, 1H, $SO_2NH$), 3.59 (s, 3H, $CH_3O$), 7.80-6.40 (m, 11H, aromatic protons), 9.44 (s, 1H, NH).

*Anal.* Caled. $C_{21}H_{19}N_3O_3S$: C, 61.59; H, 4.69; N, 10.26: S, 7.83. Found: C, 61.73; H, 4.70; N, 10.42; S, 7.95.

### 4'-[9-(2,3-Methylenedioxyacridinylamino)]methanesulfonyl-*m*-anisidide (A4P73).

A4P73 was prepared according to the method described by Kimura, *et al.* [6]. The melting point, nmr and ir spectra supported the structure of A4P73, mp 300° dec; ir (potassium bromide): ν 3830-3400, 1640, 1610, 1570 $cm^{-1}$; $^1$H-nmr (DMSO-$d_6$): δ 3.08 (s, 3H, $SO_2CH_3$), 3.51 (s, 3H, $CH_3O$), 6.29 (s, 2H, O-$CH_2$-O), 7.00-8.20 (m, 9H, aromatic protons).

*Anal.* Calcd. for $C_{22}H_{19}N_3O_5S$: C, 60.39; H, 4.39; N, 9.61; S, 7.33. Found: C, 60.13; H, 4.23; N, 9.44; S, 7.12.

### 4'-[9-(2,3-Methylenedioxy-5-chloroacridinylamino)]methanesulfonyl-*m*-anisidide (A3P166).

A3P166 was prepared according to the method described by Kimura, *et al.* [6]. The melting point, nmr and ir spectra supported the structure of A3P166, mp 300° dec; ir (potassium bromide): ν 3850-3420, 1640, 1610, 1550 $cm^{-1}$; $^1$H-nmr (DMSO-$d_6$): δ 3.07 (s, 3H, $SO_2CH_3$), 3.51 (s, 3H, $CH_3O$), 6.31 (s, 2H, O-$CH_2$-O), 7.04-8.02 (m, 8H, aromatic protons).

*Anal.* Calcd. for $C_{22}H_{18}ClN_3O_5S$: C, 55.99; H, 3.85; Cl, 7.51; N, 8.91; S, 6.79. Found: C, 55.79; H, 3.78; C1, 7.76; N, 8.79; S, 6.98.

### Antitumor Activity Tests.

The antitumor activity tests were conducted according to the previously reported method using murine leukemia cells L1210 [7].

P388 leukemia and B16 melanoma tests, kindly supplied by the National Cancer Institute, Bethesda, MD, were carried out in DBA/2 and C57BL/6 male mice, respectively. For drug testing, P388 cells (1 x $10^6$/mouse) and B16 cells (0.5 ml/mouse of 10% homogenate) were inoculated i.p. into BALB/c x DBA/2 $F_1$ and C57BL/6, respectively, and drug therapy was initiated 24 hours later. In order to verify that tumor responsiveness did not change from one series of experiments to another, mitomycin C (MMC) and *m-AMSA* were used in each series as a positive control. Drug activity for these tumor models was calculated by the ILS (increase in life span) as compared with the control group.

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