# Acridine Derivatives. IV [1]. Synthesis, Molecular Structure, and Antitumor Activity of the Novel 9-Anilino-2,3-methylenedioxyacridines

Michio Kimura*

Department of Pharmacy, Kyoto University Hospital,
Faculty of Medicine, Kyoto University,
Sakyo-ku, Kyoto 606, Japan

Akira Kato and Ichizo Okabayashi

Niigata College of Pharmacy, 5-13-2 Kamishin'ei-cho,
Niigata 950-21, Japan

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In the biological and physical investigation of a new class of deoxyribonucleic acid (DNA)-intercalating antitumor agents, novel 9-anilino-2,3-methylenedioxyacridines (twelve compounds) have been synthesized and evaluated for the activity against L1210 leukemia *in vivo*. A few of them possessed the same potency of the antitumor activity as 4'-(9-acridinylamino)methanesulfonyl-*m*-anisidine (amsacrine, *m*-AMSA), which is an important antitumor agent in clinical use. The molecular structure of a typical one, **9a** in this series have been determined by the X-ray diffraction method using a single crystal. The results of this X-ray investigation have shown that the new class of acridine derivatives have the methylenedioxy group fused at the 2- and 3-positions of the acridine ring.

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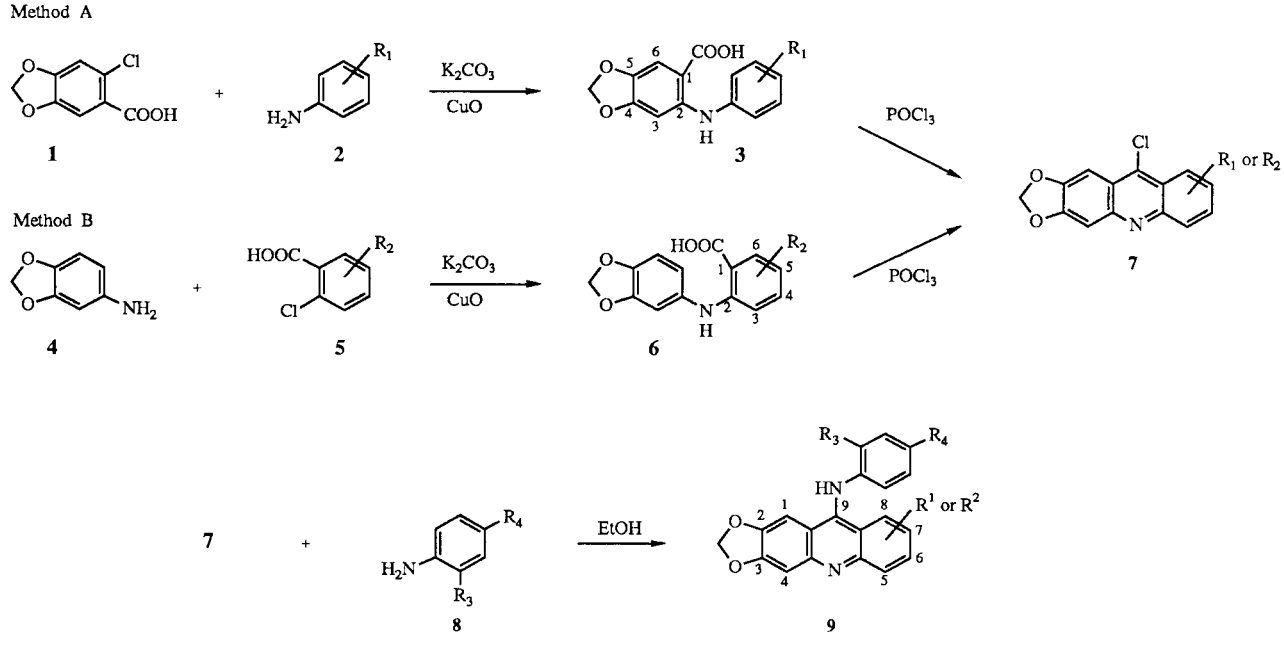
We have been studying the properties of DNA-binding drugs possessing antitumor activities to develop potential antitumor agents. In the previous paper [1], we have reported that novel acridinyl-substituted uracils were synthesized by the enamine reaction between 6-aminouracil and 9-chloroacridines, and they had antitumor activity as well as antibacterial and antifungal activity. It can fairly be argued that most aspects of the total cancer problem revolve around the abnormal behavior of DNA, and antitumor agents suppress it by interacting with DNA. The DNA-binding drugs developed are known to have a wide variety of binding modes, but they may be divided into two main classes; (1) intercalative and (2) non-intercalative DNA binding drugs. Within each class, some compounds interact non-covalently with DNA, and others bind first reversibly, and then interact covalently with DNA. DNA has the reversible binding ability to include planar aromatic molecules which can be inserted between the base pairs of the double helix [2-4]. On the other hand, the intercalation of some molecules within DNA is considered to be important in mutagenesis, carcinogenesis, and the medicinal activities of antibacterial, antiparasitic, and

Scheme 1



antineoplastic drugs [5-8]. The acridine derivatives known as DNA intercalators, such as acridine orange [8], quinacrine [9], terpyridine platinum [10], ethidium [11], 9-aminoacridine [12], proflavine [13], and ellipticine [14] are effective in chromosome binding studies in cytogenecis. Furthermore, the intercalation and the subsequent unwinding of the DNA double helix has been shown to be an important tool in the study of the superhelicity [15-19]. A series of 4'-(9-acridinylamino)methanesulfonyl-*m*-anisidine (amsacrine, *m*-AMSA) [20-22] analogues has been shown to exhibit excellent anticancer activity in clinical use an intercalator within the DNA duplex. It is thus of great interest to find new types of intercalators possessing potent antitumor activities and to understand in detail the manner of their binding modes within the confines of the double-helical structure of DNA.

In this work, we have synthesized new types of acridines fused with a methylenedioxy group at the 2- and 3-positions of the acridine ring, which have shown the same potency of antitumor activities as *m*-AMSA in the L1210 antitumor test (as in biological section). The molecular structure of the typical product (**9a** in Scheme 1) was established by X-ray diffraction studies using a single crystal. We further synthesized its derivatives which vary in substituent size and electronic character in order to examine their biological activities and to know how they are perturbed by substituent changes on the acridine nucleus. The novel 9-anilino-2,3-methylenedioxyacridine **9** were synthesized by the reaction process shown in Scheme 1. The diphenylamines **3** and **6** used as intermediates were prepared by two different methods (method A and B in Scheme 1). The 2-anilino-4,5-methylenedioxybenzoic acid derivatives **3** were prepared by direct nucleophilic displacement (Ullmann reaction) [23,24] of 2-chloro-4,5-methylenedioxybenzoic acid (**1**) by substituted anilines **2** (method A). Similarly, alternative diphenylamines **6** were also obtained by the Ullmann reaction between 3,4-methylenedioxyaniline (**4**) and substituted 2-chlorobenzoic acids **5** (method B). These reaction processes are outlined in Scheme 1. Method A proceeded with a range of 15-25% yields to obtain the desired 2-anilino-4,5-methylenedioxybenzoic acids **3**, but method B gave **6** in lower yield (less than 20%). In addition, the diphenylamines **3** were easily purified by the steam distilation after the reaction of method A, and further purification processes were unnecessary for the next reaction step. On the other hand, the 3',4'-methylenedioxyanilinobenzoic acids **6** in the method B were obtained as a mixture of the decomposed tar **4**, which required further purification processes for pure products **6**. The synthesis of the starting material **1** requires two reaction steps [25] from piperonal whose overall yield is less than 20%. Method B has the advantage that 3,4-methylenedioxyaniline (**4**) is commercially available, but the cost of **4** is high. Considering these reasons, in this report, method A is mainly used to obtain 2-anilino-4,5-methylenedioxybenzoic acids **3**. The ten methylenedioxybenzoic acids synthesized by the method A and B are listed in Table 1. By using **3** and **6**, the acridine ring formation was effected in many cases with phosphoryl chloride or thionyl chloride to provide a 2,3-methylenedioxy-9-chloroacridines **7** directly without isolation of the acridines prepared as an intermediate [26]. In many cases, this ring closure proceeded rapidly with refluxing in phosphoryl chloride solution by taking 2 hours and changed to a green color to give the desired 9-chloro-2,3-methylenedioxyacridines **7** in high yields. When the ring formation with phosphoryl chloride was not desirable, this reaction was effected with either sulfuric acid, PPA or PPE to give the 9(10*H*)-acridones, which were then converted to the desired 9-chloroacridines with thionyl chloride/DMF as usual [1]. These reaction product 9-chloro-2,3-methylenedioxyacridine derivatives **7** can be used for the next step without further purification. The 9-chloro-2,3-methylenedioxyacridine **7** was then coupled with the appropriate arylamine bearing $CH_3O$, $NHSO_2CH_3$ or $CH_3$ groups as side chains to provide the desired new type of acridines **9**.

Table 1

Physical Data of Methylenedioxybenzoic Acids 3 and 6

| No. | $R_1$ or $R_2$ | $^1$H-NMR ($Me_2SO$-$d_6$) -O-$CH_2$-O- | NH | COOH | $CH_3$ or $CH_3O$ | IR (KBr) ($cm^{-1}$) |
|---|---|---|---|---|---|---|
| **3a** | H | 5.95 | 4.82 | 9.43 | | 3260 1660 1630 |
| **3c** | 2'-$CH_3$ | 6.03 | 2.34 | 9.83 | 2.26 | 3300 1670 1640 |
| **3d** | 3'-$CH_3$ | 6.02 | 4.85 | 9.52 | 2.33 | 3300 1670 1630 |
| **3e** | 4'-$CH_3$ | 5.88 | 4.90 | 9.65 | 2.32 | 3250 1660 1635 |
| **3f** | 4'-Cl | 6.03 | 5.02 | 9.82 | | 3320 1660 1640 |
| **3h** | 2'-$CH_3O$ | 6.05 | 5.09 | 9.83 | 3.80 | 3300 1670 1630 |
| **3j** | 3'-$CH_3O$ | 6.03 | 4.83 | 9.58 | 3.92 | 3350 1680 1650 |
| **6b** | 4-Cl | 5.99 | 4.83 | 9.45 | | 3300 1700 1650 |
| **6g** | 3-Cl | 6.05 | 4.93 | 9.35 | | 3250 1680 1630 |
| **6i** | 5-$CH_3O$ | 6.05 | 4.80 | 9.90 | 3.95 | 3300 1700 1630 |

Table 2

Physical Data of 9-Anilino-2,3-methylenedioxyacridines **9**

| No. [a] | $R_1$ or $R_2$ | $R_3$ | $R_4$ | $^1$H-NMR ($Me_2SO$-$d_6$) acridine ring $-O-CH_2-O-$ | phenyl ring $CH_3O$ | phenyl ring $SO_2CH_3$ | $CH_3$ or $CH_3O$ | IR (KBr) ($cm^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| **9a** | H | $CH_3O$ | $NHSO_2CH_3$ | 6.29 | 3.51 | 3.08 | | 3830 3740 3400 1640 1610 1570 |
| **9b** | 6-Cl | $CH_3O$ | $NHSO_2CH_3$ | 6.32 | 3.48 | 3.10 | | 3820 3730 3450 1650 1620 1580 |
| **9c** | 5-$CH_3$ | $CH_3O$ | $NHSO_2CH_3$ | 6.30 | 3.51 | 3.09 | 2.51 | 3850 3740 3450 1650 1630 1600 |
| **9d** | 6-$CH_3$ | $CH_3O$ | $NHSO_2CH_3$ | 6.29 | 3.39 | 3.09 | 3.04 | 3850 3740 3430 1700 1640 1610 |
| **9e** | 7-$CH_3$ | $CH_3O$ | $NHSO_2CH_3$ | 6.28 | 3.48 | 3.08 | 2.33 | 3850 3740 3430 1650 1640 1610 |
| **9f** | 7-Cl | $CH_3O$ | $NHSO_2CH_3$ | 6.32 | 3.51 | 3.09 | | 3850 3740 3450 1650 1610 1560 |
| **9g** | 5-Cl | $CH_3O$ | $NHSO_2CH_3$ | 6.31 | 3.51 | 3.07 | | 3850 3730 3420 1640 1610 1550 |
| **9h** | 5-$CH_3O$ | $CH_3O$ | $NHSO_2CH_3$ | 6.23 | 3.40 | 2.99 | 2.42 | 3830 3730 3500 1650 1610 1580 |
| **9i** | 7-$CH_3O$ | $CH_3O$ | $NHSO_2CH_3$ | 6.21 | 3.44 | 2.99 | 2.52 | 3850 3740 3520 1650 1620 1550 |
| **9j** | 6-$CH_3O$ | $CH_3O$ | $NHSO_2CH_3$ | 6.30 | 3.43 | 3.01 | 2.31 | 3850 3740 3430 1630 1600 1520 |
| **9k** | H | H | $CH_3$ | 6.30 | | | 3.05 | 3830 3750 3400 1645 1590 1570 |
| **9l** | H | H | H | 6.30 | | | | 3825 3750 3390 1640 1600 1570 |

[a] For the hydrochlorides of compounds **9a-l**.

After the coupling reaction, the novel compounds **9** were obtained in the crystalline state which were purified by recrystallization from methanol or ethanol by the addition of a small amount of water. The molecular structure of a typical product **9a** of this novel acridine series was determined by X-ray analysis using a single crystal of **9a**. For twelve compounds listed in Table 2, the analytical and spectroscopic data are in good agreement with the structures.

In the proton nuclear magnetic resonance ($^1$H-nmr) spectra measured at 200 MHz (in deuteriodimethyl sulfoxide), the singlet lines at 6.0-6.5 are assignable to the methylenedioxy group on the acridine ring, the sharp singlet signals of the methoxy group at 3.0-3.5, and the multiplet signals at 7.3-8.6 assignable to the acridine ring protons were characteristic. The infrared spectra ir (potassium bromide) indicated a broad absorption band at 3800 and 3700 $cm^{-1}$ with a shoulder at 3500 $cm^{-1}$, and very strong peaks at 1700, 1600 and 1500 $cm^{-1}$ (Table 2). The mass spectra (ms) of products **9** exhibited a strong molecular ion $M^+$ corresponding to the molecular weight, and a typical peak due to $M^+$-$SO_2CH_3$, and $M^+$-$SO_2CH_3$-NH.

Biological Activity and Molecular Structure.

In the series of 9-anilino-2,3-methylenedioxyacridines **9**, the substituent effects on overall molecular lipophilic-hydrophilic balance and the electronic and steric effects of substituent groups are important factors for the antitumor biological activity. In consideration of these effects, twelve

Table 3

Antitumor Activities *in vivo*

| No. | Dose (mg/kg) | Median survival days (M. S. D.) | T/C (%) |
|---|---|---|---|
| Control | 10 x 2 | 10.67 | 100 |
| *m*-AMSA | 10 x 2 | 20.50 | 192 |
| **9a** | 10 x 2 | 16.23 | 152 |
| **9b** | 10 x 2 | 14.38 | 135 |
| **9c** | 10 x 2 | 13.57 | 127 |
| **9d** | 10 x 2 | 13.43 | 126 |
| **9e** | 10 x 2 | 10.71 | 100 |
| **9f** | 10 x 2 | 10.29 | 96 |
| **9g** | 10 x 2 | 10.71 | 100 |
| **9h** | 10 x 2 | 9.43 | 88 |
| **9i** | 10 x 2 | 10.00 | 94 |
| **9j** | 10 x 2 | 15.14 | 142 |

Mice were given the compound (10 mg/kg) intraperitoneally twice a day, and on day 2, 4 and 6 after intraperitoneal injection of L1210 ($2 \times 10^5$/mouse).

Table 4a

Atomic Parameters for Non-hydrogen Atoms

Estimated standard deviations are given in parentheses. Beq is isotropic equivalent of the anisotropic thermal parameter (Hamilton, 1959) [36]

| atom | x | y | z | Beq |
|---|---|---|---|---|
| C1 | 0.8818(2) | 1.3133(2) | 0.3050(2) | 2.38(7) |
| C2 | 0.8047(2) | 1.3012(2) | 0.2162(2) | 2.49(7) |
| C3 | 0.8430(2) | 1.3234(2) | 0.0795(2) | 2.64(7) |
| C4 | 0.9584(2) | 1.3591(2) | 0.0304(2) | 2.35(7) |
| C5 | 1.0419(2) | 1.3789(2) | 0.1251(2) | 2.31(7) |
| N6 | 1.1583(3) | 1.4284(3) | 0.0869(3) | 2.33(7) |
| C7 | 1.2436(2) | 1.4676(2) | 0.1799(2) | 2.46(7) |
| C8 | 1.3595(3) | 1.5318(3) | 0.1412(3) | 4.77(7) |
| C9 | 1.4466(2) | 1.5710(2) | 0.2335(2) | 3.45(7) |
| C10 | 1.4200(2) | 1.5474(2) | 0.3684(2) | 3.19(7) |
| C11 | 1.3077(2) | 1.4847(2) | 0.4068(2) | 2.80(7) |
| C12 | 1.2152(2) | 1.4434(2) | 0.3160(2) | 2.38(7) |
| C13 | 1.0962(2) | 1.3742(2) | 0.3480(2) | 2.31(7) |
| C14 | 1.0063(2) | 1.3536(2) | 0.2615(2) | 2.26(7) |
| O15 | 0.6835(2) | 1.2671(2) | 0.2366(2) | 1.24(7) |
| C16 | 0.6408(2) | 1.2782(2) | 0.1195(2) | 3.10(7) |
| O17 | 0.7472(2) | 1.3052(2) | 0.0151(2) | 2.93(7) |
| N18 | 1.0732(2) | 1.3365(2) | 0.4667(2) | 2.44(7) |
| C19 | 0.9815(2) | 1.2288(2) | 0.4831(2) | 2.16(7) |
| C20 | 0.9739(2) | 1.1025(2) | 0.3698(2) | 2.33(7) |
| C21 | 0.8942(2) | 0.9970(2) | 0.3953(2) | 2.55(7) |
| C22 | 0.8228(2) | 1.0188(2) | 0.5369(2) | 2.51(7) |
| C23 | 0.8263(2) | 1.1439(2) | 0.6474(2) | 2.89(7) |
| C24 | 0.9075(2) | 1.2486(2) | 0.6201(2) | 2.73(7) |
| O25 | 1.0474(2) | 1.0910(2) | 0.2371(2) | 3.18(7) |
| C26 | 1.0494(2) | 0.9611(2) | 0.1262(2) | 3.86(7) |
| N27 | 0.7514(3) | 0.9036(3) | 0.5579(3) | 3.44(7) |
| S28 | 0.6328(3) | 0.8956(3) | 0.6789(3) | 2.93(7) |
| O29 | 0.6562(2) | 0.9953(2) | 0.8262(2) | 5.52(7) |
| O30 | 0.5234(3) | 0.9297(3) | 0.6150(3) | 8.01(7) |
| C31 | 0.5955(2) | 0.7623(2) | 0.6603(2) | 2.04(7) |
| O32 | 0.3254(2) | 1.1721(2) | 0.9378(2) | 15.00(7) |
| C33 | 0.3365(2) | 1.0618(2) | 0.9106(2) | 10.52(7) |
| Cl34 | 1.2232(2) | 1.3695(3) | 1.7307(2) | 2.94(7) |

Table 4b

Fractional Coordinates and Isotropic Thermal Parameters (A**2) for Hydrogen Atoms

| atom | x | y | z | Biso |
|---|---|---|---|---|
| HC1 | 0.851(4) | 1.298(4) | 0.409(4) | 2.5 |
| HC4 | 0.987(4) | 1.372(4) | -0.074(4) | 2.6 |
| HN6 | 1.182(4) | 1.433(4) | -0.018(4) | 9.2 |
| HC8 | 1.379(4) | 1.550(4) | 0.041(5) | 3.6 |
| HC9 | 1.536(4) | 1.621(5) | 0.207(5) | 3.1 |
| HC10 | 1.487(5) | 1.580(5) | 0.437(5) | 2.6 |
| HC11 | 1.290(4) | 1.468(4) | 0.507(4) | 2.8 |
| HC16A | 0.590(4) | 1.192(4) | 0.066(4) | 8.6 |
| HC16B | 0.575(4) | 1.328(4) | 0.167(4) | 7.0 |
| HN18 | 1.140(5) | 1.368(5) | 0.540(5) | 3.7 |
| HC21 | 0.887(4) | 0.900(4) | 0.307(4) | 3.3 |
| HC23 | 0.771(4) | 1.160(4) | 0.748(4) | 2.7 |
| HC24 | 0.915(4) | 1.346(4) | 0.704(4) | 2.3 |
| HC26 | 1.112(4) | 0.969(4) | 0.056(4) | 3.6 |
| HN27 | 0.752(5) | 0.810(5) | 0.468(5) | 4.0 |
| HC31A | 0.620(4) | 0.773(4) | 0.767(4) | 3.5 |
| HC31B | 0.624(4) | 0.721(4) | 0.602(4) | 6.1 |
| HC31C | 0.535(4) | 0.737(4) | 0.655(4) | 6.0 |
| HC33A | 0.378(4) | 1.068(4) | 1.003(4) | 3.5 |
| HC33B | 0.262(4) | 0.977(4) | 0.898(4) | 5.0 |
| HC33C | 0.395(4) | 1.026(4) | 0.805(4) | 5.0 |

derivatives **9a-9l** with electron-donating or electron-withdrawing substituents on the acridine ring as shown in Table 2 were prepared. Ten compounds (Table 3) with various substituents on the acridine ring were tested for antitumor activity by measuring the life extension assay with L1210 leukemia (as shown in the Experimental). Some variants **9h** and **9i** provided only fair-potency or less active properties in the antitumor effects. However, the Cl, $CH_3$, or $CH_3O$ groups at the 5- or 6-positions of the acridine ring provided agents with significant activity. It is clear from these biological activities that these variants have the best combinations of 6-Cl and 2,3-methylenedioxy groups, and that less active agents result from other placement of the substituents.

The structure of compound **9a** determined by the X-ray diffraction method has shown the novel acridine derivatives fused by methylenedioxy group, which is obtained by the acridine ring formation reaction using corresponding anilinobenzoic acid and phosphoryl chloride. The molecular structure and the atom labeling of **9a** are presented by the ORTEP [27] drawing in Figure 1. The bond distances and angles are given in Table 5 together with estimated

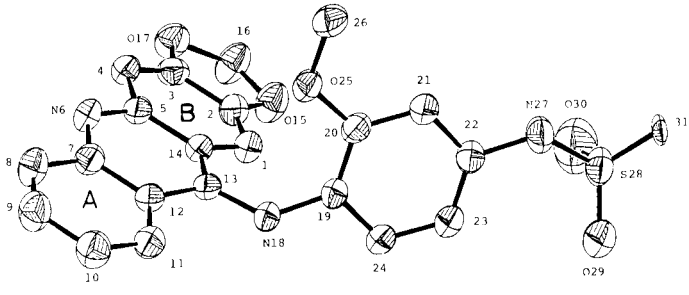



Figure 1. ORTEP drawing of compound **9a**. Thermal ellipsoids are drawn at the 50% probability level.

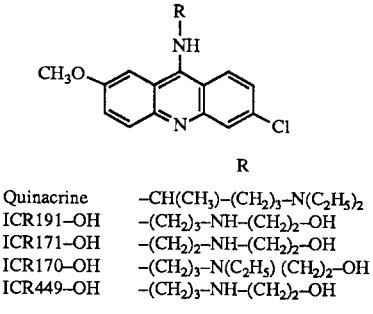



Figure 2. Formulae of ICR series compounds.

Table 5

Bond Lengths (A) and valence angles (degree)

| Bond | Length | Bond | Length |
|---|---|---|---|
| C1 – C2 | 1.344(4) | C1 – C14 | 1.430(3) |
| C2 – C3 | 1.418(3) | C2 – O15 | 1.362(3) |
| C3 – C4 | 1.342(3) | C3 – O17 | 1.347(4) |
| C4 – C5 | 1.416(4) | C5 – N6 | 1.358(4) |
| C5- – C14 | 1.433(3) | N6 – C7 | 1.361(4) |
| C7 – C8 | 1.405(4) | C7 – C12 | 1.425(3) |
| C8 – C9 | 1.373(4) | C9 – C10 | 1.411(3) |
| C10 – C11 | 1.366(3) | C11 – C12 | 1.411(3) |
| C12 – C13 | 1.437(3) | C13 – C14 | 1.417(4) |
| C13 – N18 | 1.360(3) | O15 – C16 | 1.428(4) |
| C16 – O17 | 1.473(3) | N18 – C19 | 1.433(3) |
| C19 – C20 | 1.393(2) | C19 – C24 | 1.385(3) |
| C20 – C21 | 1.388(3) | C20 – O25 | 1.360(3) |
| C21 – C22 | 1.404(3) | C22 – C23 | 1.379(3) |
| C22 – N27 | 1.428(4) | C23 – C24 | 1.394(3) |
| O25 – C26 | 1.429(3) | N27 – S28 | 1.620(4) |
| S28 – O29 | 1.423(3) | S28 – O30 | 1.780(6) |
| S28 – C31 | 1.423(4) | O32 – C33 | 1.240(4) |

| Bond | Angle | Bond | Angle |
|---|---|---|---|
| C2 – C1 – C14 | 118.4(2) | C1 – C2 – C3 | 122.2(2) |
| C1 – C2 – O15 | 128.6(2) | C3 – C2 – O15 | 109.2(3) |
| C2 – C3 – C4 | 122.5(2) | C2 – C3 – O17 | 109.3(2) |
| C4 – C3 – O17 | 128.2(2) | C3 – C4 – C5 | 116.7(2) |
| C4 – C5 – N6 | 118.0(2) | C4 – C5 – C14 | 122.0(2) |
| N6 – C5 – C14 | 119.9(3) | C5 – N6 – C7 | 122.9(3) |
| N6 – C7 – C8 | 119.8(3) | N6 – C7 C12 | 120.0(2) |
| C8 – C7 – C12 | 120.2(2) | C7 – C8 – C9 | 120.4(3) |
| C8 – C9 – C10 | 120.1(2) | C9 – C10 – C11 | 120.0(2) |
| C10 – C11 – C12 | 121.9(2) | C7 – C12 – C11 | 117.4(2) |
| C7 – C12 – C13 | 118.0(2) | C11 – C12 – C13 | 124.6(2) |
| C12 – C13 – C14 | 119.6(2) | C12 – C13 – N18 | 117.9(2) |
| C14 – C13 – N18 | 122.4(2) | C1 – C14 – C5 | 118.0(2) |
| C1 – C14 – C13 | 123.9(2) | C5 – C14 – C13 | 118.2(2) |
| C2 – O15 – C16 | 108.0(2) | O15 – C16 – O17 | 105.7(2) |
| C3 – O17 – C16 | 107.3(2) | C13 – N18 – C19 | 126.3(2) |
| N18 – C19 – C20 | 120.9(2) | N18 – C19 – C24 | 119.2(2) |
| C20 – C19 – C24 | 119.6(2) | C19 – C20 – C21 | 120.1(2) |
| C19 – C20 – O25 | 116.3(2) | C21 – C20 – O25 | 123.6(2) |
| C20 – C21 – C22 | 119.3(2) | C21 – C22 – C23 | 120.9(2) |
| C21 – C22 – N27 | 115.4(2) | C23 – C22 – N27 | 123.7(2) |
| C22 – C23 – C24 | 118.8(2) | C19 – C24 – C23 | 121.1(2) |
| C20 – O25 – C26 | 116.8(2) | C22 – N27 – S28 | 126.2(3) |
| N27 – S28 – O29 | 109.4(2) | N27 – S28 – O30 | 106.1(3) |
| N27 – S28 – C31 | 105.4(2) | O29 – S28 – O30 | 108.1(3) |
| O29 – S28 – C31 | 119.3(3) | O30 – S28 – C31 | 107.9(2) |

Table 6

Comparison of Molecular Geometry Between **9a** and *m*-AMSA

| | **9a** | *m*-AMSA [37] |
|---|---|---|
| Average C–C bond of ring A | 1.397Å | 1.398 Å |
| Average C–C bond of ring B | 1.399 | 1.399 |
| Average C–C bond of ring C | 1.391 | 1.349 |
| C9-N bond | 1.360 | 1.359 |
| Dihedral angle between ring A and B | 11.84° | 5.0° |
| Dihedral angle between acridine and phenyl rings | 109.3° | 132.0° |

standard deviations. The present studies have shown that the part of methylenedioxyacridine ring is completely planar. Using the structure of the compound **9a** elucidated by the X-ray diffraction method, the conformation was compared with those of *m*-AMSA (Table 6). As shown in Table 6, the average C-C distances of two benzene rings of the acridine nucleus are 1.397 and 1.399 Å, respectively, while that of the C-N distances of the acridine nucleus is 1.360 Å. On the other hand, the aniline part has average values of 1.391 Å in the C-C bond and 1.360 Å in the C-N bond. These values are normal, and the bond distances of the two benzene rings of the acridine nucleus are exactly equivalent within experimental errors. The derivatives from the least-squares planes through portions of the molecule are calculated, and the dihedral angle between the least-squares planes defined by the acridine ring and the aniline part is 109.3°. The calculated dihedral angle is 11.84° between the best planes through the outer rings (A and B in Figure 1) of the acridine ring system; these are compared with values of 5° for *m*-AMSA, 8° for quinacrine [1], 4.9° for ICR-191-OH [28], 7.5° for ICR-171-OH [29], 10.7° for ICR-170-OH [30], and 12.5° for ICR-449-OH [31]. The formulae of the above ICR compounds are shown in Figure 2. In these series of acridines, it seems that the more potent biological activities, such as antitumor and mutagenic activities, are caused by the flatter ring system and are better stacking ring system as intercalators to DNA. It is likely that compound **9a** and *m*-AMSA intercalates within the DNA duplex in a similar manner, and thus have similar interaction with the nucleic acid bases which are required for the antitumor activity.

## EXPERIMENTAL

### Spectroscopy.

The $^1$H-nmr spectra were measured in deuteriodimethyl sulfoxide solution in 5 mm tubes on a JEOL FX-200 spectrometer. Chemical shifts were recorded as units relative to tetramethylsilane (δ 0.0) as the internal standard. The ir spectra were measured on a JASCO A-3 spectrometer. The EI mass spectra were obtained with a Hitachi RMU-7MG mass spectrometer.

The melting points were measured on a Yanagimoto micro-melting point apparatus and are uncorrected.

### General Procedure for the Reaction of Benzoic Acids **1** and **5** with Anilines **2** and **4**.

### 2-Anilino-4,5-methylenedioxybenzoic Acid (**3a**).

A mixture of 16 g (0.08 mole) of 2-chloro-4,5-methylenedioxybenzoic acid [25], 9.3 g (0.1 mole) of freshly distilled aniline, 11.1 g (0.08 mole) of anhydrous potassium carbonate, and 0.4 g of copper bronze was heated under reflux with stirring for 6 hours. The 35% potassium hydroxide solution (50 ml) was added, and the excess aniline was removed by steam distillation. The residue was filtered and the filtrate was acidified with concentrated hydrochloric acid to give a purple solid, which was recrystallized from alcohol to yield 4.8 g (21%), mp 160-162°; ir (potassium bromide):

$\nu$ 3260 (OH), 1660 (C=O), 1630 $cm^{-1}$; $^1$H-nmr (DMSO-$d_6$): $\delta$ 5.95 (s, 2H, O-$CH_2$-O), 4.82 (s, 1H, NH), 6.65-7.95 (m, 7H, phenyl protons), 9.43 (br s, 1H, COOH).

*Anal.* Calcd. for $C_{14}H_{11}NO_4$: C, 65.36; H, 4.32; N, 5.45. Found: C, 65.12; H, 4.01; N, 5.23.

2-(2'-Methylanilino)-4,5-methylenedioxybenzoic Acid (**3c**).

This compound was obtained in a yield of 25%, mp 153-155°; ir (potassium bromide): $\nu$ 3300 (OH), 1670 (COOH), 1640 $cm^{-1}$; $^1$H-nmr (DMSO-$d_6$): $\delta$ 2.26 (s, 3H, $CH_3$), 2.34 (s, 1H, NH), 6.03 (s, 2H, O-$CH_2$-O), 6.60-7.95 (m, 6H, phenyl protons), 9.83 (br s, 1H, COOH).

*Anal.* Calcd. for $C_{15}H_{13}NO_4$: C, 66.40; H, 4.84; N, 5.16. Found: C, 66.30; H, 4.95; N, 5.32.

2-(3'-Methylanilino)-4,5-methylenedioxybenzoic Acid (**3d**).

This compound was obtained in a yield of 21%, mp 150-153°; ir (potassium bromide): $\nu$ 3300 (OH), 1670 (C=O), 1630 $cm^{-1}$; $^1$H-nmr (DMSO-$d_6$): $\delta$ 2.33 (s, 3H, $CH_3$), 4.85 (s, 1H, NH), 6.02 (s, 2H, O-$CH_2$-O), 6.30-7.80 (m, 6H, phenyl protons), 9.52 (br s, 1H, COOH).

*Anal.* Calcd. for $C_{15}H_{13}NO_4$: C, 66.40; H, 4.84; N, 5.16. Found: C, 66.61; H, 4.62; N, 5.22.

2-(4'-Methylanilino)-4,5-methylenedioxybenzoic Acid (**3e**).

This compound was obtained in a yield of 23%, mp 163-165°; ir (potassium bromide): $\nu$ 3250 (OH), 1660 (C=O), 1635 $cm^{-1}$; $^1$H-nmr (DMSO-$d_6$): $\delta$ 2.32 (s, 3H, $CH_3$), 4.90 (s, 1H, NH), 5.88 (s, 2H, O-$CH_2$-O), 6.65-8.10 (m, 6H, phenyl protons), 9.65 (br s, 1H, COOH).

*Anal.* Calcd. for $C_{15}H_{13}NO_4$: C, 66.40; H, 4.84; N, 5.16. Found: C, 66.71; H, 4.98; N, 5.30.

2-(4'-Chloroanilino)-4,5-methylenedioxybenzoic Acid (**3f**).

This compound was obtained in a yield of 20%, mp 125-128°; ir (potassium bromide): $\nu$ 3320 (OH), 1660 (C=O), 1640 $cm^{-1}$; $^1$H-nmr (DMSO-$d_6$): $\delta$ 5.02 (s, 1H, NH), 6.03 (s, 2H, O-$CH_2$-O), 6.50-7.52 (m, 6H, phenyl protons), 9.82 (br s, 1H, COOH).

*Anal.* Calcd. for $C_{14}H_{10}ClNO_4$: C, 57.64; H, 3.46; N, 4.80; Cl, 12.15. Found: C, 57.30; H, 3.63; N, 5.10; Cl, 12.31.

2-(2'-Methoxyanilino)-4,5-methylenedioxybenzoic Acid (**3h**).

This compound was obtained in a yield of 23%, mp 133-135°; ir (potassium bromide): $\nu$ 3300 (OH), 1670 (C=O), 1630 $cm^{-1}$; $^1$H-nmr (DMSO-$d_6$): $\delta$ 3.80 (s, 3H, $CH_3O$), 5.09 (s, 1H, NH), 6.05 (s, 2H, O-$CH_2$-O), 6.40-8.00 (m, 6H, phenyl protons), 9.83 (br s, 1H, COOH).

*Anal.* Calcd. for $C_{15}H_{13}NO_5$: C, 62.71; H, 4.57; N, 4.88. Found: C, 62.55; H, 4.36; N, 4.74.

2-(3'-Methoxyanilino)-4,5-methylenedioxybenzoic Acid (**3j**).

This compound was obtained in a yield of 23%, mp 136-139°; ir (potassium bromide): $\nu$ 3350 (OH), 1680 (C=O), 1650 $cm^{-1}$; $^1$H-nmr (DMSO-$d_6$): $\delta$ 3.92 (s, 3H, $CH_3O$), 4.83 (s, 1H, NH), 6.03 (s, 2H, O-$CH_2$-O), 6.60-8.00 (m, 6H, phenyl protons), 9.58 (br s, 1H, COOH).

*Anal.* Calcd. for $C_{15}H_{13}NO_5$: C, 62.71; H, 4.57; N, 4.88. Found: C, 62.88; H, 4.64; N, 5.02.

2-(3',4'-Methylenedioxyanilino)-4-chlorobenzoic Acid (**6b**).

This compound was obtained in a yield of 21%, mp 159-162°; ir (potassium bromide): $\nu$ 3300 (OH), 1700 (C=O), 1650 $cm^{-1}$; $^1$H-nmr (DMSO-$d_6$): $\delta$ 4.83 (s, 1H, NH), 5.99 (s, 2H, O-$CH_2$-O), 6.50-7.40 (m, 6H, phenyl protons), 9.58 (br s, 1H, COOH).

*Anal.* Calcd. for $C_{14}H_{10}ClNO_4$: C, 57.64; H, 3.46; N, 4.80; Cl, 12.15. Found: C, 57.80; H, 3.62; N, 4.57; Cl, 12.41.

2-(3',4'-Methylenedioxyanilino)-3-chlorobenzoic Acid (**6g**).

This compound was obtained in a yield of 20%, mp 163-165°; ir (potassium bromide): $\nu$ 3250 (OH), 1680 (C=O), 1630 $cm^{-1}$; $^1$H-nmr (DMSO-$d_6$): $\delta$ 4.93 (s, 1H, NH), 6.05 (s, 2H, O-$CH_2$-O), 6.65-7.90 (m, 5H, phenyl protons), 9.35 (br s, 1H, COOH).

*Anal.* Calcd. for $C_{14}H_{10}ClNO_4$: C, 57.64; H, 3.46; N, 4.80; Cl, 12.15. Found: C, 57.23; H, 3.17; N, 4.62; Cl, 12.02.

2-(3',4'-Methylenedioxyanilino)-5-methoxybenzoic Acid (**6i**).

This compound was obtained in a yield of 23%, mp 134-136°; ir (potassium bromide): $\nu$ 3300 (OH), 1700 (C=O), 1630 $cm^{-1}$; $^1$H-nmr (DMSO-$d_6$): $\delta$ 3.95 (s, 3H, $CH_3O$), 4.80 (s, 1H, NH), 6.05 (s, 2H, O-$CH_2$-O), 6.40-7.50 (m, 6H, phenyl protons), 9.90 (br s, 1H, COOH).

*Anal.* Calcd. for $C_{15}H_{13}NO_5$: C, 62.71; H, 4.57; N, 4.88. Found: C, 62.90; H, 4.82; N, 4.97.

General Procedure for the Preparation of Methylenedioxyacridines **9a-l** as shown in Scheme 1.

4'-[9-(2,3-Methylenedioxyacridinylamino)]methanesulfonyl-*m*-anisidine (**9a**).

A mixture of 2-anilino-4,5-methylenedioxybenzoic acid (**3a** (3.5 g, 0.012 mole) and 20 ml of phosphoryl chloride was heated under reflux for 3 hours in an oil bath, and the excess phosphoryl chloride was removed under reduced pressure. The reaction mixture was cooled to room temperature, and then the contents were slowly added to a large excess of ice-ammonium hydroxide. Care was taken to maintain alkalinity until all the remaining phosphoryl chloride had undergone hydrolysis with ammonium hydroxide. The resulting reaction product was extracted with chloroform, washed with dilute ammonium hydroxide, and promptly dried with calcium chloride. The chloroform layer was separated and concentrated to give 9-chloro-2,3-methylenedioxyacridine (**7a**). Other substituted 4'-amino-9-chloro-2,3-methylenedioxyacridines **7** were used for the next reaction without further purification.

A mixture of 9-chloro-2,3-methylenedioxyacridine (**7a**) (1.0 g, 0.0039 mole) and methanesulfonyl-*m*-anisidine (0.56 g, 0.0039 mole) in 40 ml of ethanol was stirred at room temperature for 20 hours. At the end of this period the resulting red solid was filtered off and recrystallized from methanol-water (5:1, w/v) to give yellow crystals of **9a**, 1.5 g in 85% yield, mp >300° dec; ir (potassium bromide): $\nu$ 3830, 3740, 3400, 1640, 1610, 1570 $cm^{-1}$; $^1$H-nmr (DMSO-$d_6$): $\delta$ 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, phenyl protons); ms: (m/e) 437 ($M^+$), 358 ($M^+$-$SO_2CH_3$).

*Anal.* Calcd. for $C_{22}H_{19}N_3O_5S$: C, 60.39; H, 4.39; N, 9.61; S, 7.33. Found: C, 60.12; H, 4.23; N, 9.43; S, 7.11.

4'-[9-(2,3-Methylenedioxy-6-chloroacridinylamino)]methanesulfonyl-*m*-anisidine (**9b**).

This compound was obtained in a yield of 75%, mp >300° dec; ir (potassium bromide): $\nu$ 3820, 3730, 3450, 1650, 1620, 1580 $cm^{-1}$; $^1$H-nmr (DMSO-$d_6$): $\delta$ 3.10 (s, 3H, $SO_2CH_3$), 3.48 (s, 3H, $CH_3O$), 6.32 (s, 2H, O-$CH_2$-O), 6.90-7.90 (m, 8H, phenyl 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, 56.20; H, 3.63; Cl, 7.30; N, 8.73; S, 6.55.

4'-[9-(2,3-Methylenedioxy-5-methylacridinylamino)]methanesulfonyl-*m*-anisidine (**9c**).

This compound was in a yield of 85%, mp >300° dec; ir (potassium bromide): ν 3850, 3740, 3450, 1650, 1630, 1600 $cm^{-1}$; $^1H$ nmr (DMSO-$d_6$): δ 2.51 (s, 3H, $CH_3$), 3.09 (s, 3H, $SO_2CH_3$), 3.51 (s, 3H, $CH_3O$), 6.30 (s, 2H, O-$CH_2$-O), 6.95-8.00 (m, 8H, phenyl protons).

*Anal.* Calcd. for $C_{23}H_{21}N_3O_5S$: C, 61.18; H, 4.70; N, 9.31; S, 7.10. Found: C, 61.40; H, 4.61; N, 9.15; S, 6.89.

4'-[9-(2,3-Methylenedioxy-6-methylacrininylamino)]methanesulfonyl-*m*-anisidine (**9d**).

This compound was obtained in a yield of 80%, mp >300° dec; ir (potassium bromide): ν 3850, 3740, 3430, 1700, 1640, 1610 $cm^{-1}$; $^1H$-nmr (DMSO-$d_6$): δ 3.04 (s, 3H, $CH_3$), 3.09 (s, 3H, $SO_2CH_3$), 3.39 (s, 3H, $CH_3O$), 6.29 (s, 2H, O-$CH_2$-O), 6.80-8.00 (m, 8H, pheny protons).

*Anal.* Calcd. for $C_{23}H_{21}N_3O_5S$: C, 61.18; H, 4.70; N, 9.31; S, 7.10. Found: C, 61.02; H, 4.39; N, 9.11; S, 6.98.

4'-[9-(2,3-Methylenedioxy-7-methylacridinylamino)]methanesulfonyl-*m*-anisidine (**9e**).

This compound was obtained in a yield of 85%, mp >300° dec; ir (potassium bromide): ν 3850, 3740, 3430, 1650, 1640, 1610 $cm^{-1}$; $^1H$-nmr (DMSO-$d_6$): δ 2.33 (s, 3H, $CH_3$), 3.08 (s, 3H, $SO_2CH_3$), 3.48 (s, 3H, $CH_3O$), 6.28 (s, 2H, O-$CH_2$-O), 7.02-7.98 (m, 8H, phenyl protons).

*Anal.* Calcd. for $C_{23}H_{21}N_3O_5S$: C, 61.18; H, 4.70; N, 9.31; S, 7.10. Found: C, 61.01; H, 4.52; N, 9.18; S, 6.90.

4'-[9-(2,3-Methylenedioxy-7-chloroacridinylamino)]methanesulfonyl-*m*-anisidine (**9f**).

This compound was obtained in a yield of 70%, mp >300° dec; ir (potassium bromide): ν 3850, 3740, 3450, 1650, 1610, 1560 $cm^{-1}$; $^1H$-nmr (DMSO-$d_6$): δ 3.09 (s, 3H, $SO_2CH_3$), 3.51 (s, 3H, $CH_3O$), 6.32 (s, 2H, O-$CH_2$-O), 7.00-8.20 (m, 8H, phenyl 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, 55.71; H, 3.54; Cl, 7.29; N, 8.65; S, 6.50.

4'-[9-(2,3-Methylenedioxy-5-chloroacridinylamino)]methanesulfonyl-*m*-anisidine (**9g**).

This compound was obtained in a yield of 65%, mp >300°; ir (potassium bromide): ν 3850, 3730, 3420, 1640, 1610, 1550 $cm^{-1}$; $^1H$-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, phenyl 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, 55.78; H, 3.78; Cl, 7.75; N, 8.79; S, 6.98.

4'-[9-(2,3-Methylenedioxy-5-methoxyacridinylamino)]methanesulfonyl-*m*-anisidine (**9h**).

This compounds was obtained in a yield of 63%, mp >300° dec; ir (potassium bromide): ν 3830, 3730, 3500, 1650, 1610, 1580 $cm^{-1}$; $^1H$-nmr (DMSO-$d_6$): δ 2.42 (s, 3H, $CH_3O$), 2.99 (s, 3H, $SO_2CH_3$), 3.40 (s, 3H, $CH_3O$), 6.23 (s, 2H, O-$CH_2$-O), 7.00-7.95 (m, 8H, phenyl protons).

*Anal.* Calcd. for $C_{23}H_{21}N_3O_6S$: C, 59.08; H, 4.54; N, 8.99; S, 6.86. Found: C, 58.89; H, 4.78; N, 9.21; S, 6.97.

4'-[9-(2,3-Methylenedioxy-7-methoxyacridinylamino)]methanesulfonyl-*m*-anisidine (**9i**).

This compound was obtained in a yield of 65%, mp >300° dec; ir (potassium bromide): ν 3850, 3740, 3520, 1650, 1620, 1550 $cm^{-1}$; $^1H$-nmr (DMSO-$d_6$): δ 2.52 (s, 3H, $CH_3O$), 2.99 (s, 3H, $SO_2CH_3$), 3.44 (s, 3H, $CH_3O$), 6.21 (s, 2H, O-$CH_2$-O), 6.80-8.10 (m, 8H, phenyl protons).

*Anal.* Calcd. for $C_{23}H_{21}N_3O_6S$: C, 59.08; H, 4.54; N, 8.99; S, 6.86. Found: C, 58.89; H, 4.73; N, 8.72; S, 6.66.

4'-[9-(2,3-Methylenedioxy-6-methoxyacridinylamino)]methanesulfonyl-*m*-anisidine (**9j**).

This compound was obtained in a yield of 60%, mp >300° dec; ir (potassium bromide): ν 3850, 3740, 3430, 1630, 1600, 1520 $cm^{-1}$; $^1H$-nmr (DMSO-$d_6$): δ 2.31 (s, 3H, $CH_3O$), 3.01 (s, 3H, $SO_2CH_3$), 3.43 (s, 3H, $CH_3O$), 6.30 (s, 2H, O-$CH_2$-O), 7.00-7.95 (m, 8H, phenyl protons).

*Anal.* Calcd. for $C_{23}H_{21}N_3O_6S$: C, 59.08; H, 4.54; N, 8.99; S, 6.86. Found: C, 59.35; H, 4.83; N, 9.03; S, 7.04.

9-(4-Methylanilino)-2,3-methylenedioxyacridine (**9k**).

This compound was in a yield of 90%, mp >300° dec; ir (potassium bromide): ν 3830, 3750, 3400, 1645, 1590, 1570 $cm^{-1}$; $^1H$-nmr (DMSO-$d_6$): δ 3.05 (s, 3H, $CH_3$), 6.30 (s, 2H, O-$CH_2$-O), 7.18-8.22 (m, 10H, phenyl protons).

*Anal.* Calcd. for $C_{21}H_{16}N_2O_2$: C, 76.80; H, 4.92; N, 8.53. Found: C, 76.66; H, 4.79; N, 8.26.

9-Anilino-2,3-methylenedioxyacridine (**9l**).

This compound was in a yield of 90%, mp >300° dec; ir (potassium bromide): ν 3825, 3750, 3390, 1640, 1600, 1570 $cm^{-1}$; $^1H$-nmr (DMSO-$d_6$): δ 6.30 (s, 2H, O-$CH_2$-O), 7.18-8.40 (m, 11H, phenyl protons).

*Anal.* Calcd. for $C_{20}H_{14}N_2O_2$: C, 76.41; H, 4.50; N, 8.91. Found: C, 76.38; H, 4.60; N, 8.79.

The physical data of 2-anilino-4,5-methylenedioxybenzoic acids **3a**, **3c-f**, **3h**, **3j**, **6b**, **6g**, **6i** and 9-anilino-2,3-methylenedioxy acridines **9a-l** are summarized in Table 1 and 2.

X-ray Crystallography.

The hydrochloride of the product **9a** was recrystallized by the slow evaporation of a methanol-water solution at room temperature. The crystal used for the X-ray measurement was a cleaved piece with approximate dimensions of 0.20 x 0.25 x 0.30 mm. The preliminary examination of the crystal which was carried out on a Rigaku automated four-circle diffractometer showed it to be triclinic with the space group $P\bar{1}$.

Crystal Data.

$C_{22}H_{19}N_3O_5S{\cdot}HCl$ (fw 473.96), triclinic $P\bar{1}$, a = 11.889(2), b = 11.701(2), c = 9.793(1) Å, α = 116.20(1), β = 76.27(1), γ = 107.89(1)°, V = 1155.03 $Å^3$. Dc = 1.36 $g/cm^3$ for Z = 2. The intensity data were collected at room temperature on a diffractometer utilizing nickel-filtered CuKα radiation (λ = 1.54178 Å). The θ-2θ scan mode was employed for data collection. The scan rate was 6° $min^{-1}$ in 2θ, and the scan range in θ was varied by 1.2° + 0.15° tan θ. Backgrounds were counted for 5s at both ends of the scan with an offset of 50% of the scan range from the calculated postion of the Kα peak. The total of 3516 unique reflections was measured (2θ < 125°), of which 3224 were observed. The standard deviations in the structure amplitudes, σ(Fo), were derived from the counting statistics. Lorentz and polarization corrections were applied, but on absorption correction was made.

Solution and Refinement of the Structure.

The structure was solved by the direct method (MULTAN76)

[32], determining the phases of the 250 reflections with $|Eo| > 1.85$. The structure was refined by the block-diagonal least-squares method. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$, where $w = 1$ for $0 < |Fo| \leq 20$ and $w = (20/|Fo|)^{-2}$ for $|Fo| > 20$. An initial reflection using individual isotropic temperature factors for the non-hydrogen atoms led to a conventional $R = (\Sigma ||F_o| - |F_c||)/\Sigma |F_o|$ of 0.117. After successive refinement with anisotropic temperature factors for the non-hydrogen atoms, all the hydrogen atoms except for that of hydrogen chloride were readily located in the difference Fourier map; these atoms were then included in the further refinement with isotropic temperature factors. After the refinement was completed, the final values of R and $Rw = (\Sigma w(|F_o| - |F_c|)^2/\Sigma w|F_o|^2)^{1/2}$ were 0.065 and 0.064 respectively. The final atomic positional and thermal parameters are given in Tables 4a-b. The atomic scattering factors for the S, O, and C atoms were taken from International Tables for X-Ray Crystallography (1974), while the factors for H were those of Stewart, Davidson and Simpson [33]. Computations were performed using the crystallographic program system UNICS [34], and program system made by one (M. K.) of the authors [35].

Biological Testing.

The biological test consisted of intraperitoneal inoculation of 2 x $10^5$ L1210 cells into 18-25 g DBA/2 (SLC) hybrid mice on day 1. Drug treatment was initiated 24 hours later and continued for 6 days; drugs were administered twice daily by the intraperitoneal route on days 2, 4, and 6 to evaluate activity against the intraperitoneally implanted tumor. All administrations (10 mg/kg) were given in 10% chromophore suspension. Groups of six animals per dose level were used with one control group for every five tests. Average survivals (M. S. D.) were calculated in the usual way. The new medical results (T/C) are ratios of survival times of treated mice over control mice, expressed as a percent. T/C values were compared with control groups, and the results are recorded in Table 3.

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