Acridine Derivatives. V [1]. Synthesis and P388 Antitumor Activity of the Novel 9-Anilino-2,3-ethylenedioxyacridines

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A new class of deoxyribonucleic acid (DNA)-intercalating antitumor agents, novel 9-anilino-2,3-ethylenedioxyacridines (five compounds) have been synthesized and evaluated for activity against P388 leukemia *in vivo*. A few of them possessed the same potency of antitumor activity as amsacrine (*m*-AMSA) which is an important antitumor agent in clinical use.

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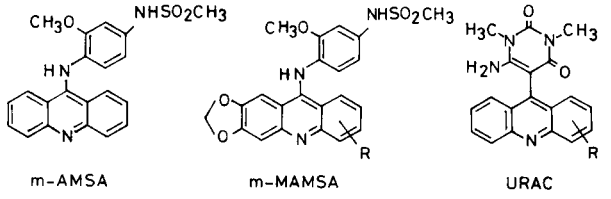
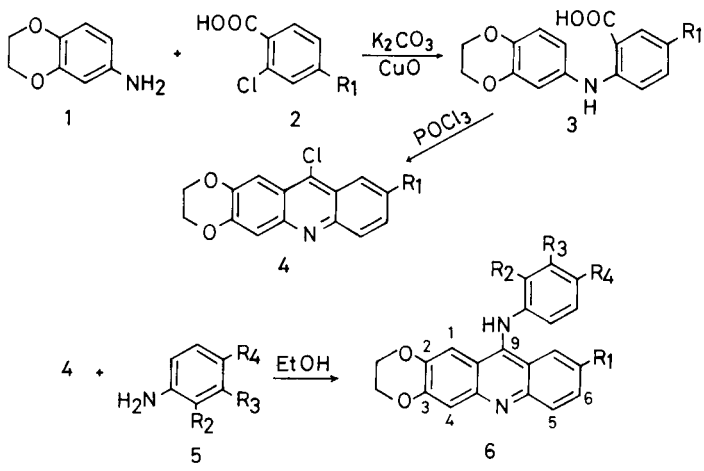
One of the important phenomenon of deoxyribonucleic acid (DNA) is their reversible binding ability to accept planar molecules (intercalator) which can be inserted between the base pairs of the double helix [2-5]. Many of these intercalators are mutagens or compounds that have other significant biological and physico-chemical properties [6-10]. We have been studying the properties of DNA-binding drugs possessing antitumor activities to develop potential antitumor agents [11,12]. It can be argued fairly that most aspects of the total cancer problem revolve around the abnormal behavior of DNA, and antitumor agents suppress it by intercalation with DNA.

The acridine derivatives known as DNA intercalators, such as acridine orange [9], quinacrine [13], terpyridine platinum [14], ethidium [15], 9-aminoacridine [16], proflavine [17], and ellipticine [18] are effective in chromosome binding studies in cytogenesis. Furthermore, the intercalation and the subsequent unwinding of the DNA double helix have been shown to be an important tool in the study of the superhelicity [19-23]. A series of 4'-(9-acridinylamino)methanesulfonyl-*m*-anisidide (amsacrine, *m*-AMSA) [24-26] analogues have been shown to exhibit excellent anticancer activity in clinical use as an intercalator within the DNA duplex. It is thus of great interest to find new types of acridine 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 previous papers [1,11,12], we have reported that novel acridinyl-substituted uracils and 9-anilino-2,3-methylenedioxyacridines (Scheme 1) were synthesized and their stereochemistry was investigated by X-ray diffraction studies. In addition, some of them had potent antitumor activities in the L1210 leukemia test.



Scheme 1. Substituent R of Products *m*-MAMSA and URAC is alkyl, alkoxy and choloride [1,11,12].

In this work, we have synthesized new types of acridines fused with an ethylenedioxy 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 P388 antitumor test (as shown in the biological section). We further synthesized 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 phenyl group at the 9-position of the acridine nucleus. The novel 9-anilino-2,3-ethylenedioxyacridines **6** were synthesized by the reaction process shown in Scheme 2. The 2-(3',4'-ethylenedioxyanilino)benzoic acids **3** were prepared by direct nucleophilic displacement (Ullman reaction) [27,28] of substituted 2-chlorobenzoic acids **2** with 3,4-ethylenedioxyaniline **1**. This synthetic method afforded 15-25% yields of the desired 2-(3',4'-ethylendiox)benzoic acids **3**. In addition, the 2-(3',4'-ethylenedioxyanilino)benzoic acids **3** were easily purified by steam distillation further purification was unnecessary for the next reaction step. The two ethylenedioxybenzoic acids, **3a** and **3b**, were synthesized by the synthetic method described in the Experimental. By using **3**, acridine ring formation was effected in many cases with phosohoryl chloride or thionyl chloride to provide a 2,3-ethylenedioxy-9-chloroacridine **4** directly without isolation of the acridines prepared as intermediates [29]. In many cases, this ring closure proceeded rapidly by refluxing in phosphoryl chloride solution during 2 or 3 hours changing to a green color to give the desired 9-chloro-2,3-ethylenedioxyacridines **4** in high yield. 9-Chloro-2,3-ethylenedioxyacridines **4** thus produced can be used for the next step without further purification. The 9-chloro-2,3-ethylenedioxyacridines **4** were then coupled with the appropriate arylamines **5** bear-



Scheme 2. Substituents R_1, R_2, R_3, and R_4 are described in Table 1.

ing CH_3O, $NHSO_2CH_3$ or CH_3 groups as side chains to provide the desired new type of 9-anilinoacridines **6**. After the coupling reaction, the novel compounds **6** were obtained in the crystalline state which were purified by recrystallization from methanol or ethanol by the addition of a small amount of water.

In the proton nuclear magnetic resonance (^{1}H-nmr) spectra measured at 200 MHz (in deuteriodimethyl sulfoxide), the multiplet at δ 4.2-4.7 are assignable to the ethylenedioxy group on the acridine ring, the sharp singlet signals of the methyoxy group at δ 3.0-3.5, and the multiplet signals at δ 6.0-8.5 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 around 1600 and 1500 cm^{-1}.

Biological Activity and Molecular Structure.

In the series of 9-anilino-2,3-ethylenedioxyacridines **6**, 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, five derivatives **6a-6e** with electron-donating or electron-withdrawing substituents on the phenyl ring as shown in Table 1 were prepared. The two compounds **6a** and **6b** with various substituents were tested for antitumor activity [30] by measuring the life extension assay with P388 and compared with *m*-AMSA, mitomycin C(MMC) and *m*-MAMSA (as shown in Table 2). Some variants provided only fair-potency or less active antitumor effects. However, the CH_3O and $NH_2SO_2CH_3$ groups at the *m*- or *p*-positions of the phenyl ring provided an agent with significant activity. It is clear from these biological activities that these variants have the combinations of *p*-methanesulfonamide, *o*-methoxy and 2,3-ethylenedioxy groups, and that less active agents result from other placement of the substituents.

Table 1

Formulae of Compounds **3**, **4**, and **6** in Scheme 2

Compounds **3**, **4**, and **6**	R_1	R_2	R_3	R_4
a	H	CH_3O	H	$NHSO_2CH_3$
b	Cl	H	H	$NHSO_2CH_3$
c	H	H	$NHSO_2CH_3$	H
d	H	H	CH_3	$NHSO_2CH_3$
e	H	H	H	H

Table 2

Antitumor Activity Against Murine P388 Leukemia

Compounds	Dose (mg/kg)	Mean survival days ± SD	ILS (%)	30 days 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	17.6 ± 1.1	83	0/5
	50	20.8 ± 2.9	117	0/5
	100	22.2 ± 9.4	131	0/5
MMC	2	17.6 ± 1.1	80	0/5
	4	14.8 ± 1.3	33	0/5
m-MAMSA	6.25	10.8 ± 0.8	13	0/5
	12.5	12.0 ± 1.4	25	0/5
	25	12.4 ± 0.5	29	0/5
	50	14.2 ± 1.3	48	0/5
	100	16.0 ± 2.0	67	0/5
6a	6.25	14.8 ± 0.8	54	0/5
	12.5	17.5 ± 2.0	82	0/5
	25	16.9 ± 1.2	76	0/5
	50	18.3 ± 2.4	91	0/5
	100	20.0 ± 3.0	108	0/5
6b	6.25	10.8 ± 0.9	13	0/5
	12.5	12.0 ± 1.4	25	0/5
	25	12.5 ± 1.5	30	0/5
	50	13.3 ± 1.5	39	0/5
	100	15.0 ± 2.4	56	0/5

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. 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 melting points were measured on a Yanagimoto micromelting point apparatus and were uncorrected.

General Procedure for the Reaction of 3,4-Ethylenedioxyaniline **1** with Benzoic Acids **2**.

2-(3',4'-Ethylenedioxyanilino)benzoic Acid (**3a**).

A mixture of 15 g (0.096 mole) of 2-chlorobenzoic acid, 25 g (0.165 mole) of 3,4-ethylenedioxyaniline, 15 g (0.11 mole) of anhydrous potassium carbonate, and 0.3 g of copper bronze was heated under reflux with stirring for 3 hours. The 35% potassium hydroxide solution (50 ml) was added, and the excess 3,4-ethylenedioxyaniline 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 15 g (58%), mp 196-198°; ir (nujol): ν 3350, 2725, 2640, 2566, 1662, 1573 cm^{-1}; ^{1}H-nmr (DMSO-d_6): 4.25 (s, 4H, -CH_2-CH_2-), 6.67-7.91 (m, 7H, aromatic protons), 9.44 (s, 1H, NH), 12.99 (br s, 1H, COOH).

Anal. Calcd. for $C_{15}H_{13}NO_4$: C, 66.40; H, 4.84; N, 5.16. Found: C, 66.54; H, 4.92; N, 5.27.

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

This compound was obtained in a yield of 35% mp 207-209°; ir (nujol): ν 3346, 2617, 2550, 2459, 1659, 1574 cm^{-1}; ^{1}H-nmr (DMSO-d_6): 4.25 (s, 4H, -CH_2-CH_2-), 6.68-7.80 (m, 6H, aromatic protons), 9.37 (s, 1H, NH).

Anal. Calcd. for $C_{15}H_{12}ClNO_4$: C, 58.92; H, 3.96; Cl, 11.60; N, 4.48. Found: C, 58.75; H, 3.78; Cl, 11.83; N, 4.65.

General Procedure for the Preparation of Ethylenedioxyacridines **6a-6e** as shown in Scheme 2.

4'-[9-(2,3-Ethylenedioxyacridinylamino)]methanesulfonyl-*m*-anisidide (**6a**).

A mixture of 2-(3',4'-ethylenedioxyanilino)benzoic acid **3a** (3.0 g, 0.111 mole) and 9 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-ethylenedioxyacridine (**4a**). Another substituted derivative, 9-chloro-2,3-ethylenedioxy-7-chloroacridine **4b** was prepared by the same procedure and both **4a** and **4b** were used for the next reaction without further purification.

A mixture of 9-chloro-2,3-ethylenedioxyacridine (**4a**) (1.0 g, 0.0037 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 **6a**, 1.5 g in 85% yield, mp 300° dec; ir (potassium bromide): ν 3840, 3750, 3400, 1640, 1610 cm^{-1}; ^{1}H-nmr (DMSO-d_6): 3.10 (s, 3H, CH_3), 3.56 (s, 3H, CH_3O), 4.43-4.60 (m, 4H, -CH_2-CH_2-), 6.87-8.23 (m, 9H, aromatic protons), 14.55 (br s, 1H, NH).

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, 4.58; N, 9.15; S, 6.93.

4'-[9-(2,3-Ethylenedioxy-6-chloroacridinylamino)]methanesulfonylanilide (**6b**).

The compound was obtained in a yield of 85%, mp 300° dec; ir (potassium bromide): ν 3835, 3750, 3400, 1650, 1610 cm^{-1}; ^{1}H-nmr (DMSO-d_6): 3.15 (s, 3H, CH_3), 4.40-4.65 (m, 4H, -CH_2-CH_2), 6.95-8.50 (m, 9H, aromatic protons), 14.50 (br s, 1H, NH).

Anal. Calcd. for $C_{22}H_{18}ClN_3O_4S$: C, 57.95; H, 3.99; Cl, 7.78; N, 9.22; S, 7.03. Found: C, 58.03; H, 4.12; Cl, 7.91; N, 9.19; S, 7.15.

3'-[9(2,3-Ethylenedioxyacridinylamino)]methanesulfonylanilide (**6c**).

This compound was in a yield of 85%, mp 300° dec; ir (potassium bromide): ν 3900, 3810, 3450, 1630, 1610 cm^{-1}; ^{1}H-nmr (DMSO-d_6): 3.10 (s, 3H, CH_3), 4.37-4.50 (m, 4H, -CH_2-CH_2-), 7.03-8.26 (m, 10H, aromatic protons), 14.97 (br s, 1H, NH).

Anal. Calcd. for $C_{22}H_{19}N_3O_4S$: C, 62.69; H, 4.55; N, 9.97; S, 7.61. Found: C, 62.57; H, 4.43; N, 9.87; S, 7.52.

4'-[9-(2,3-Ethylenedioxyacridinylamino)]methanesulfonyl-*o*-toluidide (**6d**).

This compound was obtained in a yield of 80%, mp 300° dec; ir (potassium bromide): ν 3800, 3750, 3400, 1660, 1590 cm^{-1}; ^{1}H-nmr (DMSO-d_6): 2.32 (s, 3H, CH_3), 3.03 (s, 3H, SO_2CH_3), 4.36-4.60 (m, 4H, -CH_2-CH_2-), 7.11-9.28 (m, 9H, aromatic protons), 14.93 (br s, 1H, NH).

Anal. Calcd. for $C_{23}H_{21}N_3O_4S$: C, 63.42; H, 4.87; N, 9.65; S, 7.36. Found: C, 63.30; H, 4.78; N, 9.51; S, 7.45.

4'-[9-(2,3-Ethylenedioxyacridinylamino)]anilide (**6e**).

This compound was obtained in a yield of 90%, mp 300° dec; ir (potassium bromide): ν 3900, 3820, 3350, 1650, 1570 cm^{-1}; ^{1}H-nmr (DMSO-d_6); 4.35-4.50 (m, 4H, -CH_2-CH_2), 7.14-8.23 (m, 11H, aromatic protons), 14.90 (br s, 1H, NH).

Anal. Calcd. for $C_{21}H_{16}N_2O_2$: C, 76.80; H, 4.92; N, 8.53. Found: C, 76.93; H, 4.98; N, 8.64.

Antitumor Activity.

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) was 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 to the control group.

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