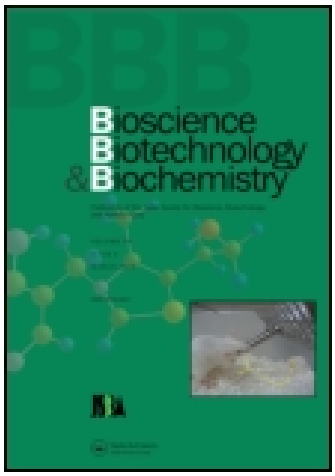

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The Effects of Substituents Introduced into 9-Aminoacridine on Frameshift Mutagenicity and DNA Binding Affinity

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Some derivatives of 9-aminoacridine (1) were synthesized, and their frameshift mutagenicity and DNA binding affinity were studied. The introduction of a methyl group into the acridine ring of 1 reduced the mutagenic activity and the intercalative DNA binding affinity, while the introduction of chlorine increased them. Halogenated derivatives of 1 showed higher toxicity against *Salmonella typhimurium* TA1537.

Key words: Ames test; mutagenicity; DNA intercalation; Scatchard analysis; acridine

A number of planar dyes including acridine compounds are known to cause frameshift mutations.[1] Acridine derivatives act as mutagens because they cause the insertion or the deletion of a base pair of DNA. A mechanism based on recombination errors has been proposed for the production of insertion and deletion mutations by DNA intercalation of acridine derivatives.[2] The mutagenic activity of 9-aminoacridine (**1**) has been characterized in the Ames test[3]: *Salmonella typhimurium* tester strain TA1537 with the histidine frameshift mutation *hisC3076* is reverted by **1**.

Derivatives of **1** have been studied as potential antitumor agents. The relationship between molecular structure and antitumor activity has been investigated for amsacrine and its analogues.[4] Quinacrine with chlorine in the acridine structure has been reported as an antimalarial drug and a DNA intercalator.[5] We have suggested that 9-amino-2,7-dibromoacridine induces two-molecule intercalation between neighboring base pairs of DNA.[6] We have also found that the introduction of a nitro group into **1** increases the frameshift mutagenicity.[7]

In this study, we have synthesized halogenated and methylated derivatives of **1** and investigated the substituent effects on their frameshift mutagenicity and DNA binding affinity. We now report the mutagenicity of the synthesized derivatives of **1** against strain TA1537 in the absence of rat liver S9,[8] and the intercalative binding affinity of those compounds to calf thymus DNA on the basis of UV-VIS absorption spectroscopy.[9,10]

Results

Synthesis of 9-aminoacridine derivatives

9-Amino-2-methylacridine (**2**) and 9-amino-2,7-dimethylacridine (**3**) were synthesized by the partly modified methods reported in the literature.[11,12] *N*-(4′-Methyl)-phenylanthranilic acid (**11**) was synthesized from anthranilic acid (**8**) and 4-bromotoluene (**10**). Treatment of **11** with phosphorus oxychloride followed by phenol and ammonium carbonate gave **2**. Using 5-methylanthranilic acid (**9**) as a starting material, **3** was synthesized from 5-methyl-*N*-(4′-methyl)phenylanthranilic acid (**12**) by the same procedure as that for **2**. Varying the reaction time did not improve the yields of **11** and **12**: their yields were low (3.9 and 5.6%) even at the optimum reaction time (24 h).

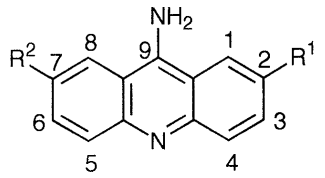
2-Chloroacridone (**14**) prepared from acridone (**13**) was used for the synthesis of 2,9-dichloroacridine (**15**) without the separation of **14** because the solubility of **14** in organic solvents was too low to develop on a silica gel column. Since 9-chloroacridine is unstable under neutral and acid conditions,[12] the purification of **15** was not attempted. Modifying the reported method,[12] compound **15** was treated with phenol to provide 2-chloro-9-phenoxyacridine (**16**), which was then converted to 9-amino-2-chloroacridine (**5**) by treatment with ammonium carbonate.

9-Amino-2-iodoacridine (**7**) was directly synthesized from 9-aminoacridine (**1**) by the reaction with iodine.

The syntheses of 9-amino-2-nitroacridine (**4**) and 9-amino-2-bromoacridine (**6**) have already been reported.[7]

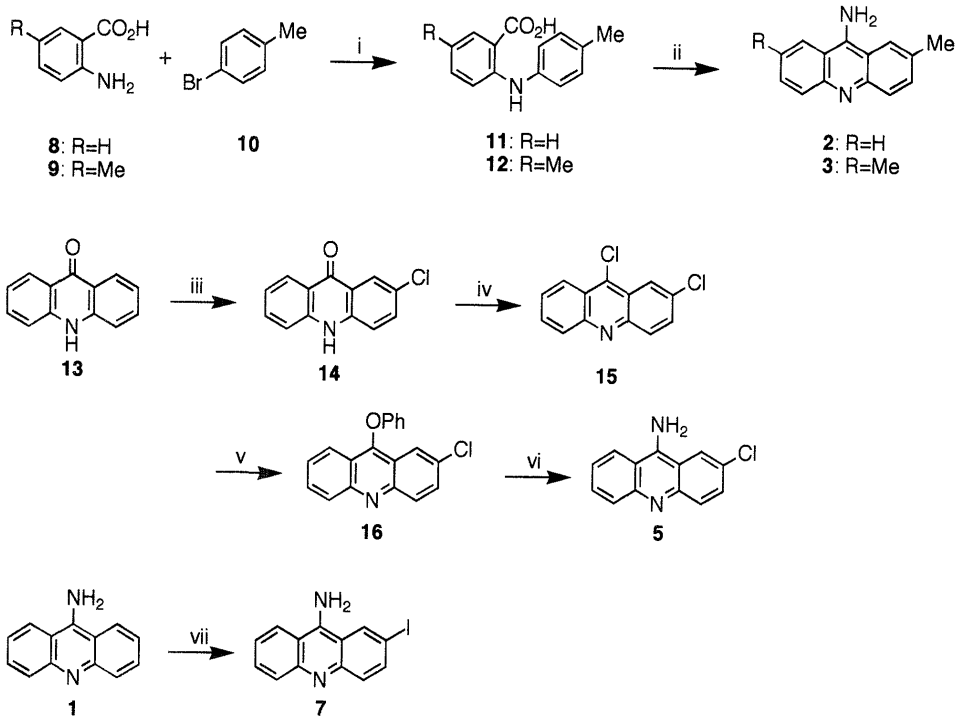
Frameshift mutagenicity and DNA binding affinity

The bioassay was done by means of the Ames test using *Salmonella typhimurium* TA1537.[8] The frameshift mutagenicity of tested compounds is expressed as the mutation rate (Fig. 1). On the whole, **2** had lower mutagenic activity than **1**. Compound **3** had little mutagenicity. The others, **4**, **5**, **6**, and **7**, showed higher mutagenic activity than **1** in that order. When a survival rate is less than 10%, the mutation rate is not presented in Fig. 1 because the toxicity is too



	R^1	R^2
9-Aminoacridine (**1**)	H	H
9-Amino-2-methylacridine (**2**)	Me	H
9-Amino-2,7-dimethylacridine (**3**)	Me	Me
9-Amino-2-nitroacridine (**4**)	NO_2	H
9-Amino-2-chloroacridine (**5**)	Cl	H
9-Amino-2-bromoacridine (**6**)	Br	H
9-Amino-2-iodoacridine (**7**)	I	H

† To whom correspondence should be addressed.



Scheme Synthesis of 9-Aminoacridine Derivatives **2**, **3**, **5**, and **7**.

i, Cu–Na_2CO_3–*n*-BuOH; ii, $POCl_3$, PhOH, and $(NH_4)_2CO_3$–PhOH; iii, SO_2Cl_2–AcOH; iv, $POCl_3$; v, PhOH; vi, $(NH_4)_2CO_3$–PhOH; vii, I_2–$HIO_4 \cdot 2H_2O$–H_2SO_4–AcOH.

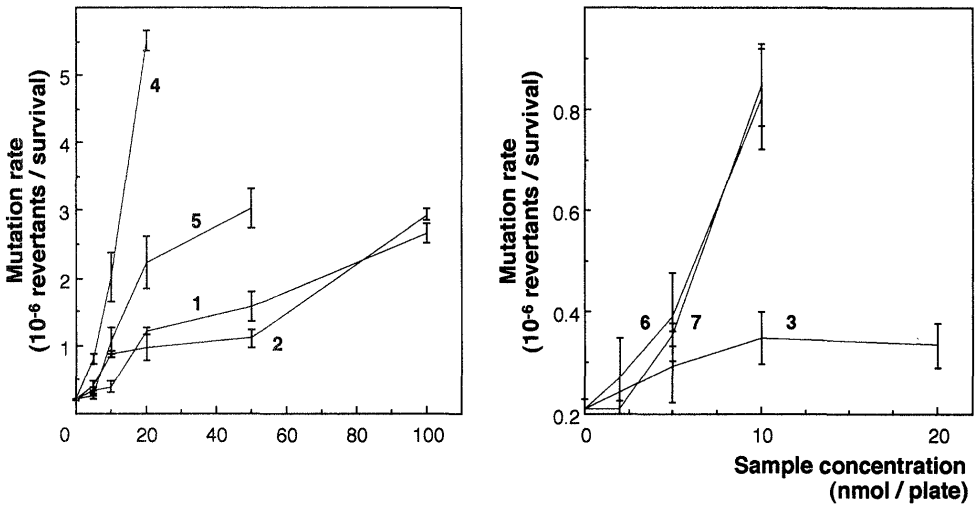


Fig. 1. Frameshift Mutagenicity[a] of Compound **1** and Its Derivatives with the Preincubation Assay Involving *Salmonella typhimurium* TA1537 without the S9 Mix.

[a] The mutagenicity is expressed as the mutation rate.
1, 9-aminoacridine; **2**, 9-amino-2-methylacridine; **3**, 9-amino-2,7-dimethylacridine; **4**, 9-amino-2-nitroacridine; **5**, 9-amino-2-chloroacridine; **6**, 9-amino-2-bromoacridine; **7**, 9-amino-2-iodoacridine.

high to evaluate the mutagenicity with exactness. Compounds **6** and **7** were exceedingly toxic to the test organism (Fig. 2).

To evaluate the abilities of **1** and its derivatives as DNA intercalators, the DNA binding constant (K) and the number of binding sites (n) were measured by the Scatchard analysis on the basis of UV-VIS absorption spectroscopy.[9,10] As shown in the Table, the intercalative binding affinities of **2** and **3** to calf thymus DNA were weaker than that of **1**, while those of **4**,[6] **5**, **6**,[6] and **7** were stronger.

Figure 3 shows that, except in the cases of **6** and **7**, the increase in the mutagenic activity parallels the increase of the K value, the mutagenicity expressed as the mutation rate per nanomole being obtained as the positive linear regression slope from Fig. 1. As shown in Fig. 4, an increase in the Hammett substituent constant accompanied the increase of the K value (**A**) and the introduction of a larger substituent caused a higher K value (**B**).

Discussion

The mutagenicity decreased on the introduction of a methyl group into the acridine ring of 9-aminoacridine (**1**), while it increased on the introduction of chlorine, bromine, iodine, or a nitro group. In the Ames test, the mutagenicity is usually expressed as the number of revertants. However, 9-amino-2-bromoacridine (**6**) and 9-amino-2-iodoacridine

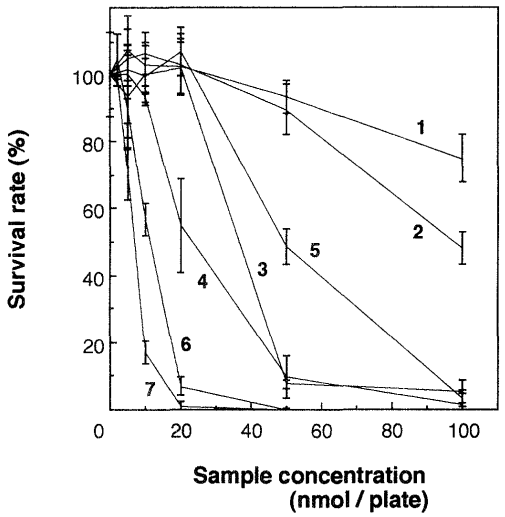


Fig. 2. Toxicity[a] of Compound **1** and Its Derivatives against *Salmonella typhimurium* TA1537.

[a] The toxicity is expressed as the survival rate of strain TA1537 (the number of survivals plated in the absence of the sample, 9.1×10^7).
1, 9-aminoacridine; **2**, 9-amino-2-methylacridine; **3**, 9-amino-2,7-dimethylacridine; **4**, 9-amino-2-nitroacridine; **5**, 9-amino-2-chloroacridine; **6**, 9-amino-2-bromoacridine; **7**, 9-amino-2-iodoacridine.

Table Intercalative Binding Affinity of Compound **1** and Its Derivatives to Calf Thymus DNA[a]

Compound	K (M^{-1})[b]	n (bases)[b]
1[c]	1.6×10^5	2.2
2	1.2×10^5	2.2
3	9.2×10^4	2.1
4[c]	2.4×10^5	1.9
5	2.3×10^5	1.9
6[c]	5.2×10^5	1.8
7	8.8×10^5	0.96

[a] Based on measurement of the UV-VIS absorption spectrum in a 1% (v/v) DMSO aqueous solution buffered with HEPES (5 mM) at pH 7.2.
[b] K, DNA binding constant; n, number of binding sites. The K and n values were calculated by the Scatchard analysis.
[c] Data from ref. 6.

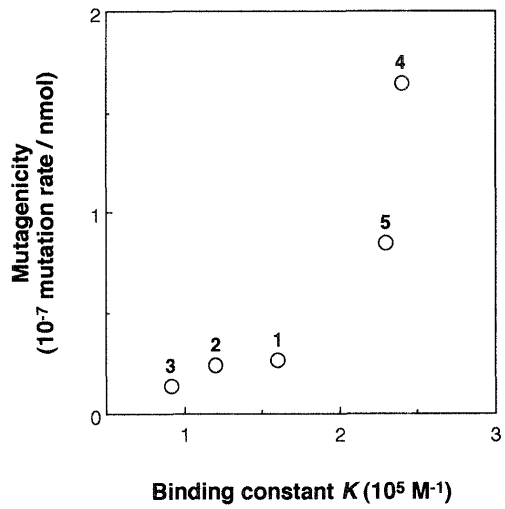


Fig. 3. Correlation between the DNA Binding Constant[a] and Frameshift Mutagenicity[b] of Compound **1** and Its Derivatives.

[a] Data for compounds **1** and **4** from ref. 6.
[b] The mutagenicity expressed as the mutation rate per nanomole is obtained as the positive linear regression slope from Fig. 1.
1, 9-aminoacridine; **2**, 9-amino-2-methylacridine; **3**, 9-amino-2,7-dimethylacridine; **4**, 9-amino-2-nitroacridine; **5**, 9-amino-2-chloroacridine.

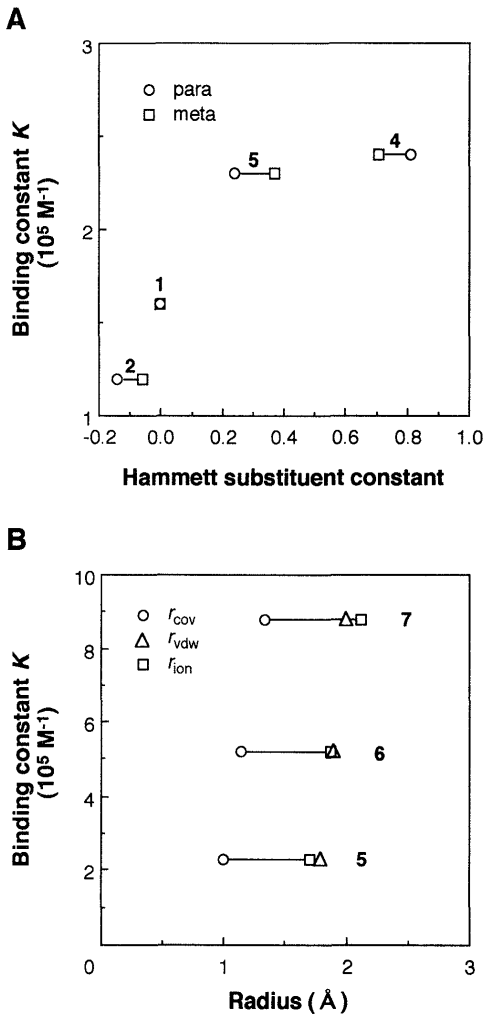


Fig. 4. Substituent Effects on the DNA Binding Constant of Compound **1** and Its Derivatives.

A, influence of the Hammett substituent constant[13] on binding constants.
B, correlation between substituents' radii[14] and binding constants: r_{cov}, covalent bond radius; r_{vdw}, van der Waals radius; r_{ion}, ionic radius.
1, 9-aminoacridine; **2**, 9-amino-2-methylacridine; **4**, 9-amino-2-nitroacridine; **5**, 9-amino-2-chloroacridine; **6**, 9-amino-2-bromoacridine; **7**, 9-amino-2-iodoacridine.

(**7**) were highly toxic to *Salmonella typhimurium* TA1537, and the number of revertants was not enough to evaluate the mutagenicity. Thus, in this study, the mutagenicity was expressed as the mutation rate obtained from both the numbers of revertants and survivals.

The K values of halogenated and nitrated derivatives were higher than that of the reference compound **1** in contrast with those of methylated derivatives. Though a bulky bromine substituent had been expected to interfere with DNA intercalation, the K value of the brominated derivative **6** was very high in those of tested compounds. Moreover, compound **7**, with a bulkier iodine substituent, had a stronger affinity to DNA. The result that **6** and **7** intercalate into DNA raises the question how they are located between the base pairs of DNA. It is known that one molecule of **1** is located between nucleic acid base pairs.[15] The n value (0.96) of **7** clearly differs from those of the other tested compounds, the $2/n$ value being considered to be the number of molecules between neighboring base pairs of DNA, and the $2/n$ value (2.1) of **7** suggests that two molecules are situated between the DNA base pairs. In the case of 9-amino-2,7-dibromoacridine having

the 2/*n* value of 2.2, two-molecule intercalation has been proposed as a model of binding to DNA.[6] Compound **7** probably intercalates into DNA in the same mode as the dibrominated compound. In the intercalation of the compounds having bulky substituents in their planar rings, it is presumed that the bulky substituents are unavoidably placed outside the space between DNA base pairs.

In the cases of **1**, 9-amino-2-methylacridine (**2**), 9-amino-2,7-dimethylacridine (**3**), 9-amino-2-nitroacridine (**4**), and 9-amino-2-chloroacridine (**5**), the correlation between intercalative DNA binding affinity and frameshift mutatgenicity was found: *i.e.*, the increase of the *K* value accompanied an increase in the mutagenicity. However, halogenated derivatives **6** and **7** had lower mutagenicity than the chlorinated compound **5** in spite of their higher *K* values. The increase in the toxicity, except in the cases of methylated derivatives **2** and **3**, parallels the increase of the *K* value, the toxicity presumably resulting from the intercalative attack to DNA. Compound **2** and **3** having lower *K* values are more toxic than **1**, the toxicity of them seeming to come about in other mechanisms. The binding affinity to DNA increased on the introduction of a substituent having a higher Hammett substituent constant. This result can mean that the introduction of an electron-withdrawing group into the planar ring induces the increase in the binding affinity to DNA of the intercalator. Recently, we have reported that 9-amino-2-hydroxyacridine, a rat liver S9 metabolite of **1**, has lower frameshift mutagenicity and stronger DNA binding affinity than **1**.[16] The *K* value of the hydroxylated compound is between those of **5** and **6**, irrespective of its lower Hammett substituent constant. It may bind to DNA in an unknown intercalation mode. In addition, the large differences in the *K* values between halogenated compounds **5**, **6**, and **7** cannot be explained by the electron-withdrawing ability of substituents, their Hammett substituent constants being similar. On the contrary, their *K* values increase in the size order of subsitituents and seem to have influence on the difference in the mutagenicity. Thus, the substituents of tested compounds significantly affect both the mutagenicity and the binding affinity.

In this work, the effects of the substituents introduced into the reference compound **1** on DNA binding affinity and frameshift mutagenicity were studied. At present, although it is difficult to rationalize the increase in the binding affinity on the introduction of a bulky substituent, the characteristic substituent effects described above are interesting and arouse further studies on the relation between DNA binding affinity and frameshift mutagenicity.

Experimental

General methods. All the ^{1}H-NMR spectra recorded here were taken in DMSO-d_6, and coupling constant values, *J*, are given in Hz. A Varian Gemini-200H instrument was used to record ^{1}H-NMR spectra. HREI-MS spectra were obtained with a JEOL JMS-AX505WA instrument, and UV-VIS spectra with a Hitachi U-2000 spectrophotometer.

N-(4′-Methyl)phenylanthranilic acid (**11**). A mixture of anthranilic acid (2.74 g, 20 mmol), 4-bromotoluene (3.7 ml, *ca.* 30 mmol), sodium carbonate (5.30 g, 50 mmol), copper powder (0.25 g), and 1-butanol (50 ml) was refluxed for 24 h and then concentrated as the water azeotrope *in vacuo*. Ethyl acetate (300 ml) and 6 N HCl (100 ml) were added to the residue, and the organic layer was washed with 0.1 N HCl (100 ml × 2) and extracted with 0.5 N NH_3 (200, 100, and 100 ml). The alkaline aqueous layers were combined, acidified with 6 N HCl (100 ml), and then extracted with dichloromethane (200, 100, and 100 ml). The combined organic layers were dried with Na_2SO_4 and evaporated to dryness. The residue was developed on a silica gel column (30 × 180 mm) using dichloromethane and dichloromethane–ethyl acetate (5 : 1–1 : 1). Concentration of the collected fractions containing **11** gave the crude product (176 mg, 3.9%), which was used in the next step without further purification. Recrystallization from dichloromethane gave the analytical sample as faintly yellow crystals: mp 166–167°C; δ_H 7.08–7.19 (5H, m, 3-, 2′-, 3′-, 5′-, and 6′-H), 7.34 (1H, ddd, $J_{3,4}$ 9.6, $J_{4,5}$ 7.0, $J_{4,6}$ 1.7, 4-H), 6.72 (1H, ddd, $J_{3,5}$ 1.1, $J_{4,5}$ 7.0, $J_{5,6}$ 8.0, 5-H), 7.87 (1H, dd, $J_{4,6}$ 1.7, $J_{5,6}$ 8.0, 6-H), and 2.27 (3H, s, Me) (Found: M^+, 227.0966. Calcd. for $C_{14}H_{13}NO_2$: M, 227.0946).

9-Amino-2-methylacridine (**2**). Compound **11** (159 mg, 0.7 mmol), in phosphorus oxychloride (3 ml), was heated slowly to 90°C for 15 min, cooled at room temperature for 10 min, and then heated under reflux for 2 h. The reaction solution was cooled on an ice bath, and then poured carefully into a mixture of 15 N NH_3 (30 ml) and dichloromethane (30 ml) with ice cooling. After the aqueous layer had been extracted with dichloromethane (15 ml), the combined organic layers were dried with $CaCl_2$ and then evaporated to dryness. The residue dissolved in phenol (1.5 ml) was heated to 70°C for 5 min. Ammonium carbonate (100 mg) was added to the phenol solution, followed by heating at 120°C for 45 min. A mixture of 6 N NaOH (30 ml) and chloroform (30 ml) was added to the ammonia solution. The alkaline solution was extracted with chloroform (20 ml). The combined chloroform layers were washed with 6 N NaOH (30 ml × 2), dried over Na_2SO_4, and then evaporated to dryness. Recrystallization of product **2** (93.1 mg, 63.9%) from ethanol–*n*-hexane gave orange red needles: mp 216°C (decomp.); δ_H 8.15 (1H, s, 1-H), 7.49 (1H, d, $J_{3,4}$ 8.8, 3-H), 7.72 (1H, d, $J_{3,4}$ 8.8, 4-H), 7.77 (1H, d, $J_{5,6}$ 8.2, 5-H), 7.59 (1H, dd, $J_{5,6}$ 8.8, $J_{6,7}$ 6.8, 6-H), 7.27 (1H, dd, $J_{6,7}$ 6.8, $J_{7,8}$ 8.7, 7-H), 8.34 (1H, d, $J_{7,8}$ 8.7, 8-H), and 2.47 (3H, s, Me) (Found: M^+, 208.1001. Calcd. for $C_{14}H_{12}N_2$: M, 208.1000).

5-Methyl-N-(4′-methyl)phenylanthranilic acid (**12**). Compound **12** was synthesized using 5-methylanthranilic acid as a starting material with 4-bromotoluene by the same procedure as that for **11**. The crude **12** (271 mg, 5.6% on the basis of the starting acid) was used in the next step without further purification. Recrystallization from dichloromethane gave the analytical sample as greenish yellow crystals: mp 174–175°C; δ_H 7.04–7.21 (6H, m, 3-, 4-, 2′-, 3′-, 5′-, and 6′-H), 7.67 (1H, br. s, 5-H), 2.20 (3H, s, 5-Me), and 2.26 (3H, s, 4′-Me) (Found: M^+, 241.1105. Calcd. for $C_{15}H_{15}NO_2$: M, 241.1103).

9-Amino-2,7-dimethylacridine (**3**). Compound **3** was synthesized using **12** as a starting material by the same procedure as that for **2**. Recrystallization of product **3** (101 mg, 64.9%) from ethanol–*n*-hexane gave reddish brown crystals: mp 222°C (decomp.); δ_H 8.11 (2H, br. s, 1- and 8-H), 7.44 (2H, dd, $J_{1,3\text{ and }6,8}$ 1.6, $J_{3,4\text{ and }5,6}$ 8.8, 3- and 6-H), 7.70 (2H, d, $J_{3,4\text{ and }5,6}$ 8.8, 4- and 5-H), and 2.46 (6H, s, Me × 2) (Found: M^+, 222.1183. Calcd. for $C_{15}H_{14}N_2$: M, 222.1157).

2-Chloroacridone (**14**). A mixture of acridone (975 mg, 5 mmol), sulfuryl chloride (0.6 ml), and glacial acetic acid (100 ml) was refluxed for 3 h. Into the reaction mixture, sat. Na_2CO_3 (150 ml) was added slowly with stirring followed by cooling on an ice bath for 5 min. The crude **14** was precipitated, filtered off, washed with 2 N NaOH (100 ml) and water (200 ml × 2), and then dried *in vacuo*. The residue (1157 mg) as yellow solids was used in the next step without separation.

2-Chloro-9-phenoxyacridine (**16**). The crude compound **14** (1157 mg), in phosphorus oxychloride (20 ml), was heated slowly to 90°C for 15 min, cooled at room temperature for 10 min, and then refluxed for 2 h. The reaction solution was cooled on an ice bath, and then poured carefully into a mixture of 15 N NH_3 (200 ml) and dichloromethane (200 ml) with ice cooling. After the aqueous layer had been extracted with dichloromethane (100 ml), the combined organic layers were dried with $CaCl_2$ and evaporated to dryness. Phenol (10 ml) was added to the residue, and the solution was heated slowly to 70°C for 5 min and then at 90°C for 20 min. A mixture of 6 N NaOH (200 ml) and dichloromethane (200 ml) was added to the reaction solution, and then the aqueous layer was extracted with dichloromethane (100 ml). The combined organic layers were washed with 6 N NaOH (200 ml × 2), dried with Na_2SO_4, and then evaporated to dryness. The residue was developed on a silica gel column (30 × 750 mm) with dichloromethane. Fractions containing **16** were collected and evaporated to dryness. The crude **16** (208 mg, 13.6% on the basis of acridone)

was used in the next experiment without further purification.

An analytical sample of **16** was obtained as greenish yellow crystals on recrystallization from benzene–*n*-hexane: mp 148°C; δ_H 8.01 (1H, d, $J_{1,3}$ 2.3, 1-H), 7.87 (1H, dd, $J_{1,3}$ 2.3, $J_{3,4}$ 9.2, 3-H), 8.26 (1H, d, $J_{3,4}$ 9.2, 4-H), 8.24 (1H, br. d, $J_{5,6}$ 8.8, 5-H), 7.90 (1H, ddd, $J_{5,6}$ 8.8, $J_{6,8}$ 1.4, 6-H), 7.61 (1H, ddd, $J_{5,7}$ 1.1, $J_{6,7}$ 6.7, $J_{7,8}$ 8.6, 7-H), 8.00 (1H, br. d, $J_{7,8}$ 8.6, 8-H), 6.90 (2H, br. d, $J_{2',3' \text{ and } 5',6'}$ 8.0, 2'- and 6'-H), 7.33 (2H, dd, $J_{2',3' \text{ and } 5',6'}$ 8.0, $J_{3',4' \text{ and } 4',5'}$ 7.4, 3'- and 5'-H), and 7.10 (1H, tt, $J_{2',4' \text{ and } 4',6'}$ 1.1, $J_{3',4' \text{ and } 4',5'}$ 7.4 4'-H) (Found: M^+, 305.0581. Calcd. for $C_{19}H_{12}NOCl$: M, 305.0607).

9-Amino-2-chloroacridine (**5**). Ammonium carbonate (75 mg) was added to compound **16** (158 mg, 0.5 mmol) in phenol (1 ml), and then the solution was heated at 120°C for 45 min. A mixture of 6 N NaOH (30 ml) and dichloromethane (50 ml) was added to the reaction solution. The alkaline aqueous layer was extracted with dichloromethane (30 and 20 ml). These organic layers were collected, washed with sat. NaOH (30 ml × 2), dried over Na_2SO_4, and then evaporated to dryness. The residue was developed on a silica gel column (30 × 200 mm) with dichloromethane–methanol (20 : 1–4 : 1). Fractions containing **5** were collected and evaporated to dryness. Recrystallization of product **5** (107 mg, 93.9%) from ethanol–*n*-hexane gave yellow fluffy needles: mp 193°C (decomp.); δ_H 8.88 (1H, br. s, 1-H), 7.93–8.04 (4H, m, 3-, 4-, 5-, and 6-H), 7.57 (1H, ddd, $J_{5,7}$ 2.2, $J_{6,7}$ 5.9, $J_{7,8}$ 8.8, 7-H), and 8.69 (1H, br. d, $J_{7,8}$ 8.8, 8-H) (Found: M^+, 228.0491. Calcd. for $C_{13}H_9N_2Cl$: M, 228.0454).

9-Amino-2-iodoacridine (**7**). After a solution of 9-aminoacridine (776 mg, 4 mmol), periodic acid dihydrate (200 mg), and iodine (500 mg) in a mixture of conc. sulfuric acid (0.2 ml), water (0.8 ml), and glacial acetic acid (5 ml) had been heated under reflux for 3 h, the reaction solution was stirred with sat. $Na_2S_2O_3$ (80 ml) at room temperature for 5 min. A mixture of 6 N NaOH (120 ml) and dichloromethane (200 ml) was added to the treated solution, and then the alkaline aqueous layer was extracted with dichloromethane (120 and 80 ml). The combined organic layers were washed with 6 N NaOH (120 ml × 2), dried with Na_2SO_4, and then evaporated to dryness. The residue was developed on a silica gel column (35 × 400 mm) using dichloromethane and dichloromethane–methanol (50 : 1–4 : 1). Fractions containing **7** were collected to give the crude product (291 mg, 22.7%). The analytical sample was obtained as yellow crystals on recrystallization from ethanol–*n*-hexane: mp 233°C (decomp.); δ_H 8.84 (1H, br. s, 1-H), 7.87 (1H, br. d, $J_{3,4}$ 9.1, 3-H), 7.59 (1H, d, $J_{3,4}$ 9.1, 4-H), 7.81 (1H, d, $J_{5,6}$ 8.8, 5-H), 7.69 (1H, dd, $J_{5,6}$ 8.8, $J_{6,7}$ 6.7, 6-H), 7.35 (1H, dd, $J_{6,7}$ 6.7, $J_{7,8}$ 8.8, 7-H), and 8.39 (1H, d, $J_{7,8}$ 8.8, 8-H) (Found: M^+, 319.9790. Calcd. for $C_{13}H_9N_2I$: M, 319.9811).

Bioassay. The standard preincubation assay[8] involving *Salmonella typhimurium* TA1537 was done in the absence of rat liver S9.

DNA intercalation. The DNA binding affinity of intercalators was measured on the basis of the UV-VIS absorption spectra of the intercalators in the absence and presence of calf thymus DNA, as previously described.[6,7]

References

1) S. Brenner, L. Barnett, F. H. C. Crick, and A. Orgel, *J. Mol. Biol.*, **3**, 121–124 (1961).
2) L. S. Lerman, *Proc. Natl. Acad. Sci. U.S.A.*, **49**, 94–102 (1963).
3) B. N. Ames, F. D. Lee, and W. E. Durston, *Proc. Natl. Acad. Sci. U.S.A.*, **70**, 782–786 (1973).
4) B. C. Baguley, W. A. Denny, G. J. Atwell, and B. F. Cain, *J. Med. Chem.*, **24**, 170–177 (1981).
5) M. W. Davidson, B. G. Griggs, D. W. Boykin, and W. D. Wilson, *J. Med. Chem.*, **20**, 1117–1122 (1977).
6) H. Tomosaka, K. Anzai, and S. Omata, *Biosci. Biotech. Biochem.*, **59**, 960–961 (1995).
7) H. Tomosaka, S. Omata, and K. Anzai, *Biosci. Biotech. Biochem.*, **58**, 1420–1423 (1994).
8) D. M. Maron and B. N. Ames, *Mutation Res.*, **113**, 173–215 (1983).
9) A. Blake and A. R. Peacocke, *Biopolymers*, **6**, 1225—1253 (1968).
10) W. D. Wilson and I. G. Lopp, *Biopolymers*, **18**, 3025–3041 (1979).
11) H. Jensen and F. Rethwisch, *J. Am. Chem. Soc.*, **50**, 1144–1150 (1928).
12) A. Albert and B. Ritchie, *Org. Synth., Coll. Vol. III*, 53–56.
13) J. March, "Advanced Organic Chemistry," 4th Ed., John Wiley, New York, 1992, p. 280.
14) F. A. Cotton, G. Wilkinson, and P. L. Gaus, "Basic Inorganic Chemistry," 2nd Ed., John Wiley, New York, 1987, p. 60.
15) T. D. Sakore, S. C. Jain, C. Tsai, and H. M. Sobell, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 188–192 (1977).
16) H. Tomosaka, K. Anzai, E. Hasegawa, T. Horigome, and S. Omata, *Biosci. Biotech. Biochem.*, **60**, 714–716 (1996).