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 mutagenicity 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 test3): Salmonella typhimurium tester strain TA1537 with the histidine frameshift mutation hisC3076 is reverted by 1.

Derivatives of 1 have been studied as potential antimutator agents. The relationship between molecular structure and antimutator 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 in the absence 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)phenanthranilic acid (11) was synthesized from anthranilic acid (8) and 4-bromotoluene (10). Treatment of 11 with phosporus oxychloride followed by phenol and ammonium carbonate gave 2. Using 5-methylanthranilic acid (9) as a starting material, 3 was synthesized from 5-methyl-4-(4'-methyl)phenanthranilic 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-Chloroacridine (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,13) compound 15 was treated with phenol to provide 2-chloro-9-phenoxycacridine (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 bioassy was done by means of the Ames test using Salmonella typhimurium TA1537.9) 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...
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.¹,¹⁰ 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, 5, 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

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

i. Cu–Na₂CO₃–BuOH; ii. POCI₃, PhOH, and (NH₂)₂CO–PhOH; iii. SO₂Cl₂–AcOH; iv. POCI₃; v. PhOH; vi. (NH₂)₂CO–PhOH; vii. I₂–HI₉O₄–2H₂O–H₂SO₄–AcOH.

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

¹The 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.
Fig. 2. Toxicity* of Compound 1 and Its Derivatives against Salmonella typhimurium TA1537.

The toxicity is expressed as the survival rate of strain TA1537 (the number of survivors plated in the absence of the sample; 9.1 x 10³).
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

<table>
<thead>
<tr>
<th>Compound</th>
<th>$K$ (M⁻¹)</th>
<th>$n$ (bases)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>$1.6 \times 10^5$</td>
<td>2.2</td>
</tr>
<tr>
<td>2</td>
<td>$1.2 \times 10^5$</td>
<td>2.2</td>
</tr>
<tr>
<td>3</td>
<td>$9.2 \times 10^4$</td>
<td>2.1</td>
</tr>
<tr>
<td>4</td>
<td>$2.4 \times 10^4$</td>
<td>1.9</td>
</tr>
<tr>
<td>5</td>
<td>$2.3 \times 10^3$</td>
<td>1.9</td>
</tr>
<tr>
<td>6</td>
<td>$5.2 \times 10^2$</td>
<td>1.8</td>
</tr>
<tr>
<td>7</td>
<td>$8.8 \times 10^1$</td>
<td>0.96</td>
</tr>
</tbody>
</table>

* 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.

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

* Data for compounds 1 and 4 from ref. 6.

(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.1,5 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/ν value of 2.2, two-molecule intercalation has been proposed as a model of binding to DNA.  

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-aminomethylacridine (2), 9-aminomethacridine (3), 9-aminonitroacridine (4), and 9-aminomethacridine (5), the correlation between intercalative DNA binding affinity and frameshift mutagenicity 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-aminomethacridine, a rat liver S9 metabolite of 1, has lower frameshift mutagenicity and stronger DNA binding affinity than 1.  

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 substituents 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 1H-NMR spectra recorded here were taken in DMSO-d6, and coupling constant values, J, are given in Hz. A Varian Gemini-200H instrument was used to record 1H-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-Methylphenyl)anthranilic acid (11). A mixture of anthranilic acid (7.74 g, 20 mmol), 4-bromotoluene (3.7 ml, or 30 mmol), sodium carbonate (5.3g, 50 mmol), copper powder (0.25 g), and 1-butanol (50 ml) was refluxed for 24 h and then concentrated as the water azetrope from vacuo. Ethyl acetate (300 ml) and 6N HCl (100 ml) were added to the residue, and the organic layer was washed with 0.1 N HCl (100 ml x 2) and extracted with 0.5 N NH4, (200, 100, and 100 ml). The alkaline aqueous layers were combined, acidified with 6N HCl (100 ml), and then extracted with dichloromethane (200, 100, and 100 ml). The combined organic layers were dried with Na2SO4 and evaporated to dryness. The residue was developed on a silica gel column (30 x 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; δH 7.08-7.19 (5H, m, 3-2', 3', 5-2', and 6-3'), 7.34 (1H, dd, J1α, J1β 9.6, J2α 7.0, J6α 1.7, 4.7), 6.72 (1H, dd, J2β, J6β 1.1, J4α, J4β 7.0, J5, 8.0, 5-MH), 7.87 (1H, dd, J4α, J4β 1.7, J6α, J6β 8.0, 6-H), and 2.27 (3H, s, Me) (Found: M+, 227,0966. Calcd. for C19H14N2O3; M, 227,0946).

9-Aminoacridine (12). 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 NH4 (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 CaCl2 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 6N NaOH (30 ml x 2), dried over Na2SO4, and then evaporated to dryness. Recrystallization of product 9 from ethanol-n-hexane (63.9%) mp 216°C (decomp.); δH 6.85 (1H, s, 1-H), 7.49 (1H, d, J4α, J4β 8.8, 3.3-H), 7.72 (1H, d, J4α, J4β 8.8, 4-H), 7.77 (1H, d, J4α, J4β 8.2, 5-H), 5.79 (1H, dd, J6α, J6β 8.8, 6-H, 6-H), 7.27 (1H, dd, J6α, J6β 8.8, 7-H), 8.34 (1H, d, J6α, J6β 8-H), and 2.47 (3H, s, Me) (Found: M', 208,100. Calcd. for C19H13N2O2; M, 208,100).

5-Methyl-N-(4-methylphenyl)anthranilic 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; δH 7.04-7.21 (6H, m, 3-2', 3', 5-2', and 6-3'); 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 C19H17N2O2; M, 241,1105).

9-Amino-2,7-dimethacridine (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.); δH 8.11 (2H, br, s, 1- and 8-H), 7.44 (2H, dd, J1α, J1β 1.6, J6α, J6β 8.8, 3- and 5-H), 7.70 (2H, d, J4α, J4β 8.8, 4- and 5-H), and 2.46 (6H, s, Me x 2) (Found: M', 222,1183. Calcd. for C19H17N2O2; M, 222,1187).

2-Chloroacridine (14). A mixture of acridone (975 mg, 5 mmol), sulfuric chloride (0.6 ml), and glacial acetic acid (100 ml) was refluxed for 3 h. Into the reaction mixture, sat. Na2O2 (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 x Na2O2 (100 ml) and water (200 ml x 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 NH4 (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 CaCl2 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 6N NaOH (200 ml x 2), dried with Na2SO4, and then evaporated to dryness. The residue was developed on a silica gel column (30 x 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; δH 8.01 (1H, d, J1,3 2.3, 1-H), 7.87 (1H, dd, J1,3 2.3, J2,4 2.3, 3-H), 5.26 (1H, d, J3,4 2.3, 4-H), 8.24 (1H, br. d, J1,9 8.5, 5-H), 7.90 (1H, ddd, J2,4 8.5, J6,8 1.4, 6-H), 7.61 (1H, ddd, J2,4 1.1, J3,7 1.1, J6,7 1.1, 7.67, J1,9 8.6, 6-H), 8.00 (1H, br. d, J3,4 8.6, 8-H), 6.90 (2H, br. d, J2,3 and 5,6 8.0, 2-H and 6-H), 7.33 (2H, dd, J2,3 and 5,6 8.0, J7,8 and 8,9 7.4, 3-H and 5-H), and 7.10 (1H, t, JJ,5 1.1, J2,3 and 5,6 7.4, 3- and 5-H) (Found: M⁺, 319.9790. Calcd. for C13H12N2OCl: M⁺, 319.0607).

Bioassay. The standard preincubation assay 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
9) A. Blake and A. R. Peacocke, Biopolymers, 6, 1225–1226 (1967).