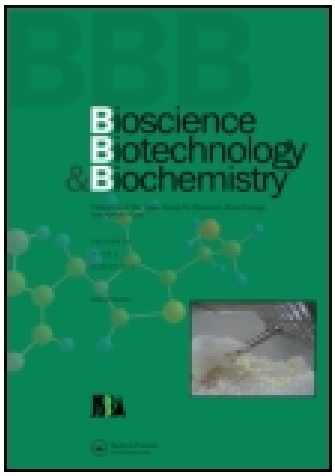

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Enhancement of Mutagenic Activity of 9-Aminoacridine by Introducing a Nitro Group into the Molecule

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Enhancement of Mutagenic Activity of 9-Aminoacridine by Introducing a Nitro Group into the Molecule

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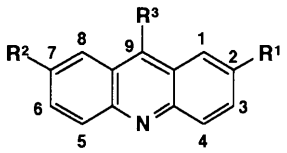
Mutagenic activity and DNA intercalation were examined for 9-aminoacridine (9-AA) and its derivatives. Introduction of a nitro group into the 9-AA molecule was found to enhance the activity enormously as was detected by the Ames test. Acetylation of amino group at 9-position of acridine ring inhibited the intercalation, the frameshift activity disappearing. Rat liver S9 converted 9-AA metabolically to 9-amino-2-hydroxyacridine.

Biological activities of acridine and its derivatives have been widely studied: activities against microorganisms were first reported in 1913[1] and antitumor activites in 1972.[2] Amsacrine, synthesized in 1974,[3] has been clinically used in the treatment of acute leukaemia since 1989.[4] Although 9-aminoacridine (**1**) is well known as a DNA intercalator and a frameshift mutagen, the poor solubility in water as well as in organic solvents caused difficulty in derivatization of this compound. We prepared several derivatives of **1** to investigate the effects of substituted groups on the intercalation and mutagenic activities.

The tested compounds were: compound **1**, acridine (**2**), 9-amino-2-bromoacridine (**3**), 9-amino-2,7-dibromoacridine (**4**), 9-acetylaminoacridine (**5**), 9-acetylamino-2-bromoacridine (**6**), 9-amino-2-nitroacridine (**7**), and 9-amino-2,7-dinitroacridine (**8**).

DNA intercalation was evaluated on the basis of the alteration of the UV-VIS absorption spectrum of each compound in the presence of calf thymus DNA, varying its concentration.[5,6] Mutagenic activity was detected by the Ames test using *Salmonella typhimurium* TA1537, known as a test organism for frameshift mutagenicity, in the absence of rat liver S9.[7]



		R1	R2	R3
(1)	9-Aminoacridine	H	H	NH_2
(2)	Acridine	H	H	H
(3)	9-Amino-2-bromoacridine	Br	H	NH_2
(4)	9-Amino-2,7-dibromoacridine	Br	Br	NH_2
(5)	9-Acetylaminoacridine	H	H	NHAc
(6)	9-Acetylamino-2-bromoacridine	Br	H	NHAc
(7)	9-Amino-2-nitroacridine	NO_2	H	NH_2
(8)	9-Amino-2,7-dinitroacridine	NO_2	NO_2	NH_2

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It is well established that the Ames test is done in the presence and absence of S9, considering the possibility of the metabolic conversion of tested compounds.[7] The rat liver metabolite of **1** was isolated and the structure was analyzed.

Results

Mutagenic activity

The introduction of a nitro group into the 9-aminoacridine (**1**) molecule enhanced the mutagenic activity. The activity of 9-amino-2-nitroacridine (**7**) was twice as high as that of **1**, and 9-amino-2,7-dinitroacridine (**8**) had very intense activity (Fig. 1). Acridine (**2**), 9-acetylaminoacridine (**5**), and 9-acetylamino-2-bromoacridine (**6**) did not show any activity. 9-Amino-2-bromoacridine (**3**) and 9-amino-2,7-dibromoacridine (**4**) were highly toxic to the test organism and mutagenic activity measurement was impossible at low concentrations.

DNA intercalation

Compound **1** showed a characteristic red shift and

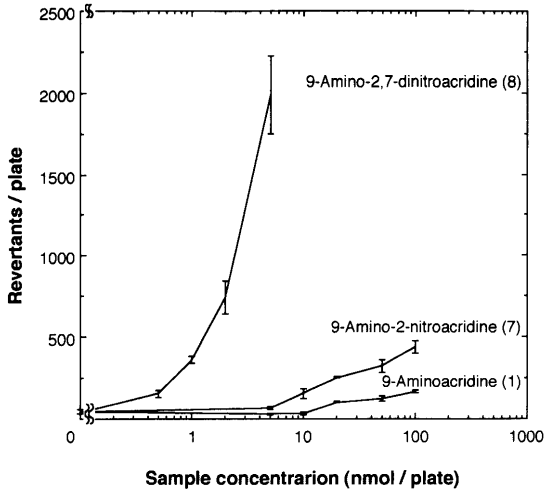


Fig. 1. Mutagenic Activity of Acridine Compounds.

The activity is expressed as numbers of revertant colonies in the Ames test using *Salmonella typhimurium* TA1537 in the absence of S9.

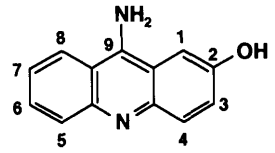
hypochromicity in the visible spectra upon binding to DNA (Fig. 2). The spectral alteration was observed in **3**, **4**, and **7**. The red shift was caused by DNA intercalation.[5] Figure 3 shows the binding ratio profiles for the intercalation. Acetylated compounds **5** and **6**, as well as non-aminated compound **2**, did not show the alteration in the presence of DNA. Dinitro compound **8** did not have enough solubility under the test conditions, and the intercalation experiment could not be done.

Structure of an S9 metabolite

One compound was given as the metabolite detected by UV irradiation (at 254 and 365 nm). The ^{1}H-NMR spectrum (Table) showed the signals of seven aromatic protons at δ 7.55–8.64. One aromatic proton signal at δ 7.55 was coupled with there protons at δ 7.90, 7.95, and 8.64. A double doublet at δ 7.69 was found to couple with two protons at δ 7.88 and 7.89. The other signals at δ 9.58, 9.76, and 10.36 disappeared on addition of D_2O, suggesting that these protons were bound to hetero atoms.

The S9 metabolite was acetylated using acetic anhydride and pyridine to obtain a compound having a molecular formula of $C_{15}H_{12}N_2O_2$ as was found by HREI-MS. Under the same reaction conditions, **1** was not acetylated, showing that the S9 metabolite is a hydroxylated compound of **1**.

The position of the hydroxyl group was identified by comparing the ^{1}H-NMR chemical shift of each proton: two protons corresponding to the 1- and 3-positions shifted to higher magnetic field. Thus the structure was 9-amino-2-hydroxyacridine.



The S9 metabolite
(9-amino-2-hydroxyacridine)

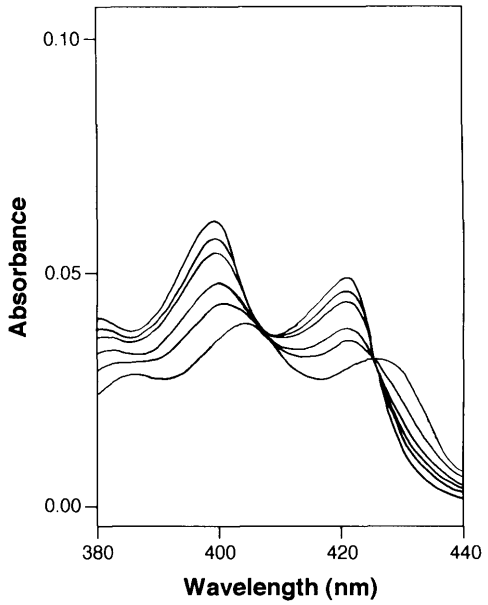


Fig. 2. Visible Absorption Spectra of 9-Aminoacridine (0.01 mM) in the presence and absence of Calf Thymus DNA.

Degradation of the absorbance is induced by increasing the DNA concentration (from 0 to 0.0741 mM).

Discussion

The frameshift mutagen 9-aminoacridine (**1**) and the toxin 9-amino-2-bromoacridine (**3**) were acetylated to 9-acetylaminoacridine (**5**) and 9-acetylamino-2-bromoacridine (**6**) respectively, the biological activity disappearing. As the result of the Ames test in the absence of S9, acetylation of the amino group seemed to cause the loss of the activity.

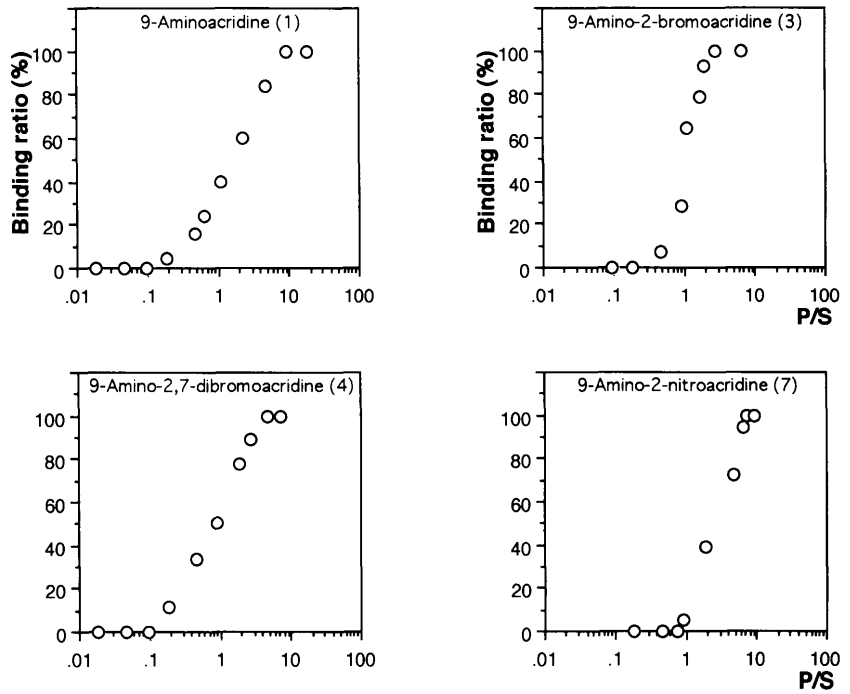


Fig. 3. Binding Ratio Profiles for DNA Intercalation of Acridine Compounds.

Reduction of UV-VIS absorption of a tested sample upon adding calf thymus DNA presents the basis of the quantitative estimation of DNA intercalation. P, concentration of phosphate residue in DNA; S, sample concentration (0.01 mM). Binding ratio (%): (DNA-binding sample concentration) × 100 ÷ S.

Table ^{1}H-NMR Data for the S9 Metabolite

Position	Chemical shift (ppm)	Coupling	Constant (Hz)
1	7.88	d	2.5
3	7.69	dd	2.5, 9.2
4	7.89	d	9.2
5	7.90	dd	1.5, 7.6
6	7.95	ddd	0.9, 6.7, 7.6
7	7.55	ddd	1.5, 6.7, 8.5
8	8.64	dd	0.9, 8.5
O^2	10.36	s	
N^9	9.58	br s	
N^9	9.76	br s	

Introducing a nitro group into the acridine ring greatly increased the mutagenic activity: increase in numbers of nitro groups seemed to enhance frameshift mutagenicity. A lower activity of 9-amino-2-nitroacridine (**7**) was reported by another group,[8] their data being in conflict with our result.

The distance between base-pairs of DNA and the covalent bond radius of a bromo group are about 3 and 1.14 Å respectively. Considering the distribution of π-electrons in the aromatic ring of DNA, the bulky bromo substituent was presumed to inhibit DNA intercalation sterically. Observation of the intercalation of brominated compounds **3** and 9-amino-2,7-dibromoacridine (**4**) raises the problem of how these compounds were located between the nucleic acid bases.

The loss of DNA intercalation by acetylating the amino group of **1** is coincident with the disappearance of the frameshift mutagenicity. The necessity of an amino group can be ascribed to the ionic attraction to the phosphate backbone of DNA.

Compound **1** has been studied as a DNA intercalator causing frameshift mutations without considering the metabolic conversion of **1** to a biologically active compound. This study shows that there is a possibility, based on the fact that 9-amino-2-hydroxyacridine was obtained as the S9 metabolite though with a low yield (1 mg from 80 mg of **1**), that **1** is metabolically converted to 9-aminoacridine-1,2-oxide to react with a nucleic acid base causing a base-substitution mutation.[9,10]

Experimental

General methods. ^{1}H-NMR and NOE spectra were recorded on a JEOL JNM-GSX500 or a JEOL JNM-EX270L instrument and taken in DMSO-d_6. EI- and HREI-MS spectra were obtained using a Hitachi M-80A instrument. UV-VIS spectra were given by a Hitachi model 200-20 spectrophotometer.

9-Amino-2-bromoacridine (**3**) *and 9-amino-2,7-dibromoacridine* (**4**). A mixture of 9-aminoacridine hydrochloride monohydrate (500 mg, 2 mmol), bromine (1.3 g, 8 mmol), and chloroform (300 ml) was stirred at room temperature for 2 h. The mixture was concentrated to dryness *in vacuo*, and the residue was treated with a mixture of chloroform (200 ml) and 0.1 N NaOH (100 ml). After the aqueous layer was extracted with chloroform, the organic layers were combined, washed with water, and evaporated to dryness under reduced pressure. The residue (600 mg) was developed on a silica gel column (55 × 600 mm) with chloroform–methanol (30 : 1–4 : 1). Fractions of **3** and **4** were collected respectively and evaporated to dryness.

Recrystallization of **3** from hexane–ethanol gave sulfur-yellow needles (60 mg, in 11.1% yield): mp 223°C (decomp.). ^{1}H-NMR δ: 8.79 (1H, d, J=2.1 Hz, H-1), 7.88 (1H, dd, J=2.1, 8.8 Hz, H-3), 7.78 (1H, d, J=8.8 Hz, H-4), 7.84 (1H, d, J=3.2 Hz, H-5), 7.84 (1H, d, J=4.7 Hz, H-6), 7.46 (1H, ddd, J=3.2, 4.7, 8.6 Hz, H-7), 8.49 (1H, d, J=8.6 Hz, H-8). HREI-MS m/z: 271.9952 (M^+, $C_{13}H_9N_2Br_1$, calcd. 271.9949).

Compound **4** was recrystallized from hexane–methanol to give a gold powder (15 mg, in 2.4% yield): mp 271°C (decomp.). ^{1}H-NMR δ: 8.73 (2H, d, J=1.7 Hz, H-1 and H-8), 7.85 (2H, dd, J=1.7, 9.0 Hz, H-3 and H-6), 7.76 (2H, d, J=9.0 Hz, H-4 and H-5). HREI-MS m/z: 349.9062 (M^+, $C_{13}H_8N_2Br_2$, calcd. 349.9054).

9-Acetylaminoacridine (**5**). A mixture of 9-aminoacridine hydrochloride monohydrate (500 mg, 2 mmol), acetic anhydride (1 g, 10 mmol), and pyridine (10 ml) was stirred at around 70°C for 24 h. The mixture was evaporated as the toluene (10 ml) azeotrope to dryness *in vacuo*. The residue was put on a silica gel column (35 × 550 mm) and chromatographed, developed with chloroform–methanol (100 : 1–10 : 1). Fractions of **5** were collected and evaporated to dryness. The crude product of **5** was purified by recrystallization from hexane–ethanol to give fluffy yellow needles (320 mg, in 67.8% yield): mp 252°C (decomp.). The position of the acetyl group was confirmed by NOE spectra, the acetyl protons being shown in the vicinity of three protons at the 1-, 8- and N^9-positions. ^{1}H-NMR δ: 8.13 (2H, d, J=8.4 Hz, H-1 and H-8), 7.62 (2H, dd, J=8.4, 6.7 Hz, H-2 and H-7), 7.85 (2H, dd, J=6.7, 8.5 Hz, H-3 and H-6), 8.16 (2H, d, J=8.5 Hz, H-4 and H-5), 2.36 (3H, s, CH_3), 10.68 (1H, s, N*H*). EI-MS m/z: 236 (M^+).

9-Acetylamino-2-bromoacridine (**6**). The same procedure as that for the synthesis of **3** was used for the synthesis of **6** except that bromination of **5** was done on one-fifth scale. The mixture was developed on a silica gel column (35 × 550 mm) eluting with chloroform–methanol (80 : 1–5 : 1). Fractions of **6** were collected and evaporated to dryness. Recrystallization from hexane–ethanol gave **6** as fluffy dark-orange needles (34 mg, in 27.2% yield): mp 261°C (decomp.). ^{1}H-NMR δ: 8.33 (1H, d, J=2.1 Hz, H-1), 7.93 (1H, dd, J=2.1, 9.2 Hz, H-3), 8.15 (1H, d, J=9.2 Hz, H-4), 8.09 (1H, d, J=9.1 Hz, H-5), 7.88 (1H, dd, J=9.1, 7.8 Hz, H-6), 7.64 (1H, dd, J=7.8, 9.0 Hz, H-7), 8.12 (1H, d, J=9.0 Hz, H-8), 2.19 (3H, s, CH_3). HREI-MS m/z: 314.0053 (M^+, $C_{15}H_{11}N_2O_1Br_1$, calcd. 314.0054).

9-Amino-2-nitroacridine (**7**) *and 9-amino-2,7-dinitroacridine* (**8**). After a mixture of sodium nitrate (500 mg, 6 mmol) and cold sulfuric acid (8 ml) was dropped into a solution of 9-aminoacridine hydrochloride monohydrate (500 mg, 2 mmol) in sulfuric acid (2 ml) with cooling on ice bath, the mixture was stirred under the same condition for 30 min, diluted with ice water (100 ml), and alkalified carefully with sodium hydrogen carbonate. The solution was extracted repeatedly with chloroform (each 200 ml). The combined chloroform layers were washed with water and evaporated to dryness under reduced pressure. The residue (590 mg) was developed on a silica gel column (55 × 600 mm) using chloroform–methanol (100 : 1–6 : 1) as the eluting solvent. Fractions of **7** and **8** were collected respectively and evaporated to dryness.

The crude sample of **7** was purified by recrystallization from hexane–ethanol to give a wine-red powder (46 mg, in 9.6% yield): mp 262°C (decomp.). ^{1}H-NMR δ: 9.58 (1H, d, J=2.6 Hz, H-1), 8.28 (1H, dd, J=2.6, 9.6 Hz, H-3), 8.85 (1H, d, J=9.6 Hz, H-4), 7.86 (1H, dd, J=1.7, 8.2 Hz, H-5), 7.43 (1H, ddd, J=8.2, 6.8, 1.8 Hz, H-6), 7.78 (1H, ddd, J=1.7, 6.8, 8.3 Hz, H-7), 8.45 (1H, dd, J=1.8, 8.3 Hz, H-8). EI-MS m/z: 239 (M^+).

Recrystallization of **8** from hexane–ethanol gave a dark-orange powder (85 mg, in 15.0% yield): mp 268°C (decomp.). ^{1}H-NMR δ: 9.60 (2H, d, J=2.3 Hz, H-1 and H-8), 8.38 (2H, dd, J=2.3, 8.7 Hz, H-3 and H-6), 7.90 (2H, d, J=8.7 Hz, H-4 and H-5). HREI-MS m/z: 284.0553 (M^+, $C_{13}H_8N_4O_4$, calcd. 284.0544).

Mutagenic activity. Frameshift mutagenicity of acridine compounds in the Ames test was examined using *Salmonella typhimurium* TA1537 in the absence of rat liver S9.[7]

DNA intercalation. Tested compounds were dissolved in DMSO and diluted to 1% (v/v) DMSO-aqueous solution for spectral measurement. The solutions containing calf thymus DNA were buffered at pH 7.2 by addition of HEPES. The absorbance of acridine compounds was measured for each solution consisting of DNA (varied concentration), HEPES (5 mM), and the tested compounds (0.01 mM). DNA concentrations were measured using an extinction coefficient of 6600 M^{-1} cm^{-1} at 260 nm and are expressed in terms of nucleotide equivalents per liter.[6] The binding ratio of the intercalator was calculated by using the hypochromic effect.[6]

S9 Metabolite from 9-aminoacridine. 9-Aminoacridine (80 mg) was added to 10% S9-mix[7] (160 ml) prepared from rat liver S9. The mixture was shaken at 37°C for 2 h, heated at 75°C for 5 min, and centrifuged at 1670 × *g* for 20 min. The supernatant was extracted repeatedly with butanol (each 20 ml). The combined organic layer was washed with water and evaporated as the water azeotrope to dryness *in vacuo*. The residue was developed on an alumina TLC plate with chloroform–methanol (10 : 1). The UV-positive target band was collected and eluted with chloroform–methanol (2 : 1). The eluate was chromatographed on a silica TLC plate with chloroform–methanol (5 : 1). The UV-positive target band was collected and eluted with chloroform–methanol (5 : 1). The eluate was evaporated to dryness to give the S9 metabolite (yield 1 mg).

A mixture of the isolated S9 metabolite, acetic anhydride (10 mg), and pyridine (1 ml) was stirred at room temperature for 3 h to obtain the acetylated compound. HREI-MS *m/z*: 252.0863 (M^+, $C_{15}H_{12}N_2O_2$, calcd. 252.0897).

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