Synthesis and Antitumor Activity of Fused Quinoline Derivatives

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Some tetracyclic quinolines (9 and 14) with a [2-methoxy-4-[(methylsulfonyl)amino]phenyl]amino side chain were prepared and their deoxyribonucleic acid (DNA) intercalative properties, KB cytotoxicity, antitumor activity (P388 leukemia), and ability to induce topoisomerase II dependent DNA cleavage were investigated. The indoloquinoline derivative 9 exhibited the most potent activity (dose = 6.3 mg, T/C% = 300) in this series. The steric structural features of the chromophores of the compounds previously and newly synthesized were studied by a computer-associated molecular graphics technique. Relationships between the steric structural features of the chromophores and biological activities are also discussed.

Keywords synthesis; antitumor activity; intercalation; topoisomerase II; indoloquinoline; molecular graphics

Three-ring chromophores such as the acridine ring have generally been accepted as effective intercalating chromophores,[1,2] because they bind to deoxyribonucleic acid (DNA) in a conformation with the long axis in the chromophore parallel to the long axis of the base pairs such that maximum overlap occurs. However, we thought that variations in chromophore size, planarity, or linearity and electronics (inclusion of various hetero atoms) must cause variations in their intercalative and antitumor properties. On the basis of these considerations, we have previously designed and synthesized novel fused tri- (**1**—**3**) and tetracyclic (**4**—**8**) quinolines having [2-methoxy-4-[(methylsulfonyl)amino]phenyl]amino or [3-(*N*,*N*-dimethylamino)-propyl]amino side chains (the side chains of amsacrine (*m*-AMSA) and nitracrine, respectively).[3] Among them, the indenoquinoline derivative **4a** having the side chain of *m*-AMSA and is isosters, **7a** and **8a**, have been proved to intercalate DNA and to have remarkably potent activity comparable to that of *m*-AMSA against leukemia P388 *in vivo*. Moreover, extended study of **4a** has showed it to have a broad spectrum of activity against solid tumors *in vivo*.

These results suggested that four-ring chromophores such as **4a** may be effective as intercalating chromophores and may lead to compounds with a broad antitumor spectrum. This paper describes the syntheses, DNA-binding properties and antitumor activities of other classes of analogues with a four-ring chromophore. We also studied the steric structural feature of the chromophores of compounds in this series by a computer-associated molecular graphics technique. Relationships between the steric structural features of the chromophores and biological activities are discussed.

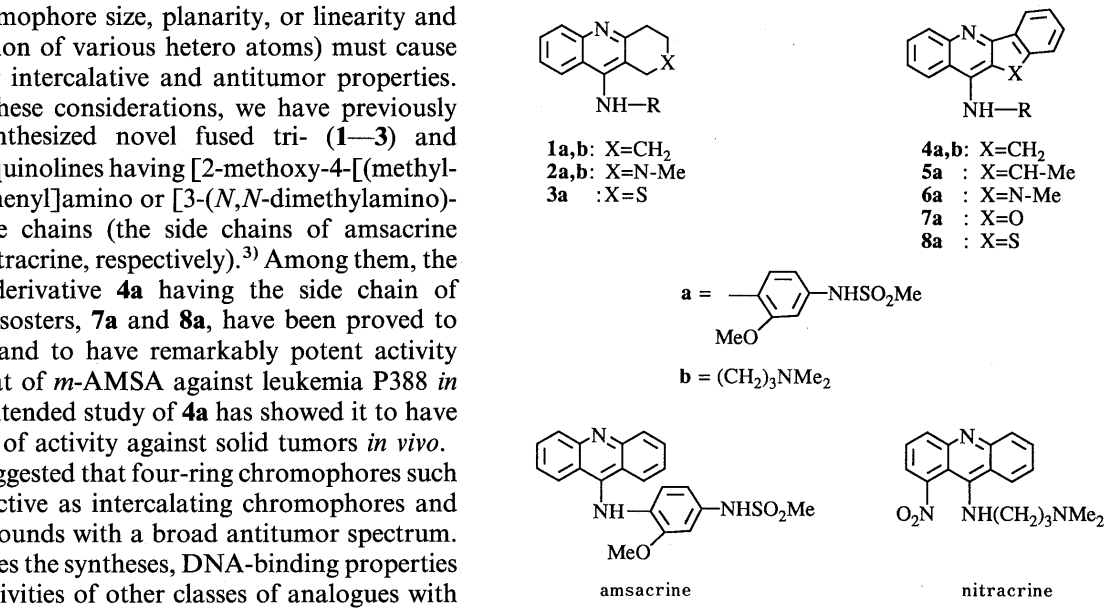


Fig. 1

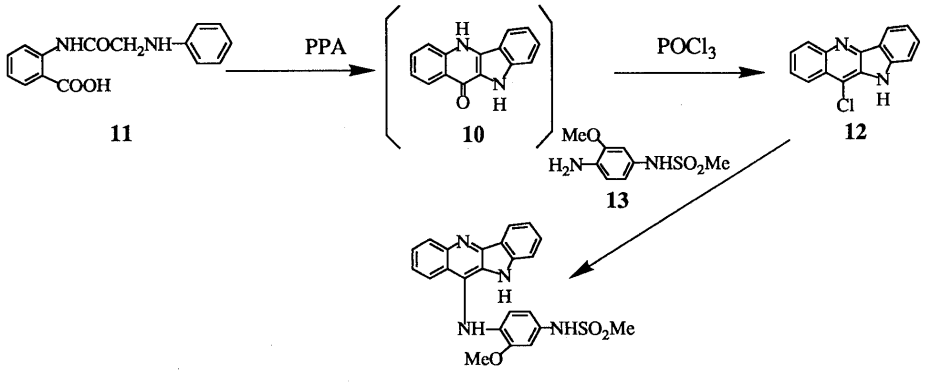


Chart 1

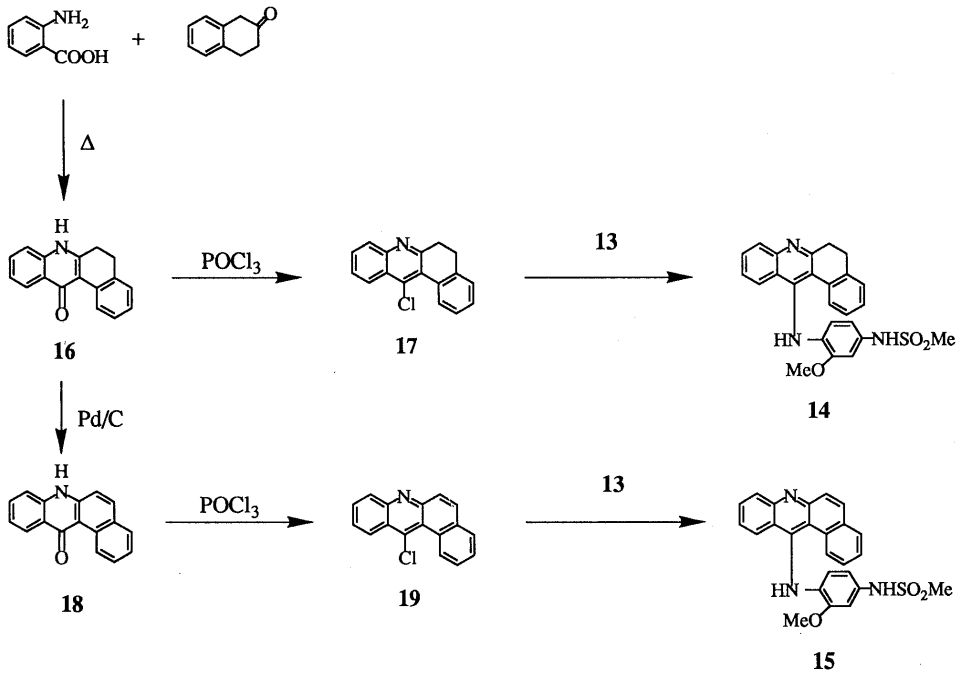


Chart 2

Synthesis The indoloquinoline derivative **9**, in which the methylene group of the C-ring in **4a** is replaced by an isosteric imino group, was prepared as shown in Chart 1. Heating of indoloquinolinone (**10**),[4] prepared from 2-[(*N*-phenylamino)acetamido]benzoic acid (**11**),[4] with phosphorus oxychloride at 80—90 °C afforded the indoloquinolyl chloride[4] **12** in a 63% yield. The reaction of **12** with 4-[(methylsulfonyl)amino]-2-methoxyaniline (**13**)[5] gave **9** in a 57% yield. The dihydrobenz[*a*]acridine derivative **14** was synthesized as shown in Chart 2. Compound **16**,[6] prepared by the condensation of anthranilic acid with 2-tetralone, was converted to the dihydrobenz[*a*]acridinyl chloride[6] **17** by treatment with phosphorus oxychloride. The reaction of **17** with **13** afforded **14** in a 60% yield. The benz[*a*]acridine derivative (**15**) was prepared from **16**. Dehydrogenation of **16** on Pd–carbon followed by chlorination afforded **19**,[7] which was reacted with **13** to afford **15** in a 58% yield. The structure of compound **15** was confirmed by the analytical data and by comparison with an authentic sample by its melting point.[8] Its regioisomers, benz[*b*]- (**20**) and benz[*c*]- (**21**) acridine derivatives, were prepared according to previously reported methods.[8]

Biological Properties Biological properties of **9**, **14**, **15**, **20**, and **21** were examined according to the previously reported methods. These compounds were tested for inhibitory activity against KB cells *in vitro*. Antitumor activity *in vivo* was evaluated by use of leukemia P388 in mice. DNA intercalative ability was determined by a fluorometric assay[9] based on the competition of the test compounds with ethidium bromide and by DNA unwinding assay.[10] Our previous study[3] showed that mammalian DNA topoisomerase II dependent DNA cleavage[11] is responsible for their antitumor activity *in vivo*. Therefore, we presently examined topoisomerase II dependent DNA cleavage *in vitro*. These biological results are listed in Table I.

Results and Discussion

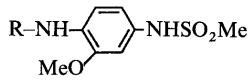
As shown in Table I, in all the compounds, a positive correlation was seen between the intercalative ability, antitumor activity *in vitro* and *in vivo*, and topoisomerase II dependent DNA cleavage activity.

The indoloquinoline derivative **9**, an aza isoster of the parent compound **4a**, exhibited the most potent antitumor activity against P388 in this series. It showed a T/C value at a low dose much higher than the parent compound **4a**. It was also found to intercalate DNA and to induce the topoisomerase II dependent DNA cleavage at a low dose.

The dihydrobenz[*a*]acridine derivative **14** was found to be inactive in all the assays, as presumed. The related benz[*a*]acridine derivative **15**, although it possesses a completely aromatized chromophore, did not intercalate and was inactive in the antitumor assays. Three regioisomers of **15**, **20**, and **21** were evaluated. The linear analogue **20** was judged to be active in the *in vitro* and *in vivo* assays, but the T/C values were very low at high doses. On the contrary, the benz[*c*]acridine **21** showed potent activities in all the assays. Denny and Baguley[8] have synthesized these three benzacridine derivatives (**15**, **20**, and **21**) and examined their DNA binding abilities and antitumor activities against P388. They reported that **15** had little effect on DNA binding but **20** and **21** strongly bound to DNA. They also described that **15** and **20** were inactive against P388 but **21** was only 3-fold less potent *in vitro* than *m*-AMSA and proved equally active as *m*-AMSA *in vitro* at a higher dose. In our screening, **20** exhibited the boundary line levels of the T/C value. However, in other points, there was not much difference between Denny's results and ours.

In our previous and present studies, the slight structural modification of the chromophore moiety lead to dramatic change in the intercalative ability and antitumor activity. Thus, introduction of a methyl group on the C-ring of the potently active compounds **4a** or **9**, which lead to **5a** or **6a**, resulted in the loss of those biological activities. Moreover, the difference in the biological activities among the three benzacridine regioisomers **15**, **20**, and **21** presented an interesting question. We thought that these differences must

TABLE I. Biological Activities



Compd.	R	Intercalation act.		Topoisomerase II dependent DNA cleavage act.[c]	Inhibin. of KB cell growth, IC_{50} μg/ml	Antitumor act. P388 in mice	
		Fluorescence[a]	Unwinding[b]			Dose, mg/kg i.p.[d]	% (T/C)
1a	X=CH_2	93.30±0.95	−	−	41	Inactive[e]	
2a	X=NMe	104.30±1.12	NT[f]	NT[f]	>100	Inactive[e]	
3a	X=S	106.00±1.27	NT[f]	NT[f]	46	Inactive[e]	
4a	X=CH_2	67.60±0.81	+++	++	<0.3	25	240
5a	X=CHMe	95.27±0.30	−	−	15	Inactive[e]	
6a	X=NMe	93.63±0.97	±	±	3.6	Inactive[e]	
7a	X=O	81.40±0.51	++	++	<0.3	400	248
8a	X=S	79.33±0.93	+	++	<0.3	400	252
9	X=NH	63.57±0.64	+++	++	<0.3	12.5	203
						6.25	300
						3.12	177
14		92.17±0.61	±	−	18	Inactive[e]	
15		78.73±0.48	−	−	14.5	Inactive[e]	
20		74.80±0.93	±	++	1.2	200	130
						100	122
21		74.67±2.52	+	++	<0.3	200	204
						12.5	125
m-AMSA		63.73±0.97	++	+++	<0.3	40	223
						20	198
						10	174

a) The fluorescence is expressed as a percentage of the control fluorescence of the ethidium bromide–DNA complex. Data are the mean ± S.E. in three experiments. *b*) (−) unwinding was not observed at 100 μg/ml of drug; (+) unwinding was observed at 100 μg/ml, (++) at 25 μg/ml, or (+++) at 5 μg/ml. *c*) (−) inactive; (+) active. *d*) The dose listed was given once a day at days 1 and 5. *e*) Dose: 400 mg/kg. *f*) NT: not tested.

be due to the variation in planarity of the chromophore moiety. Therefore, we studied the steric structural features of the chromophores using the computer-associated molecular graphics technique.

Molecular Graphics We studied the energy profile of the chromophores of the compounds which were both previously and newly synthesized. All calculations were performed on an ACOS 1000 computer at the Okayama University Computation Center, using the MNDO[12,13] program in MOPAC.[14] The software for molecular display, data extraction, and providing starting geometries were internally developed. In the energy minimization of the chromophores, the side chain, namely the [2-methoxy-4-(methylsulfonyl)aminophenyl]amino group, was assumed to be an amino group for convenience of calculation.

The results from the conformational analysis showed that the minimized conformations of the chromophores of **4**, **7**—**9**, **20**, and **21** are coplanar, while those of **1**—**3**, **14**, and **15** are not coplanar. Thus, the completely aromatized chromophores, except for the benz[*a*]acridine **15**, adopt a coplanar ring system as the minimized conformation. As described, **15** was inactive in the assays of intercalation and antitumor activity. On the other hand, its regioisomers, the benz[*b*]- (**20**) and benz[*c*]- (**21**) acridine derivatives, were active in those assays. Interestingly, the minimized conformation of **15** was found to be not a coplanar one but a "butterfly" one. On the other hand, in the case of **20** or **21**, its coplanar conformation was the most stable. These results strongly suggest that **15** would adopt this butterfly conformation in solution, so that it could not intercalate DNA owing to the steric interference.

Compounds **5a** and **6a**, which have a methyl group on the C ring of the chromophore, did not retain intercalative ability. The conformational calculation shows that the methyl group is placed at a vertical position to the coplanar chromophore. The lack of activity in **5a** or **6a** might be attributed to its inability to intercalate owing to steric hindrance of the methyl group.

The present theoretical study showed that there is a good correlation between the calculated conformational feature

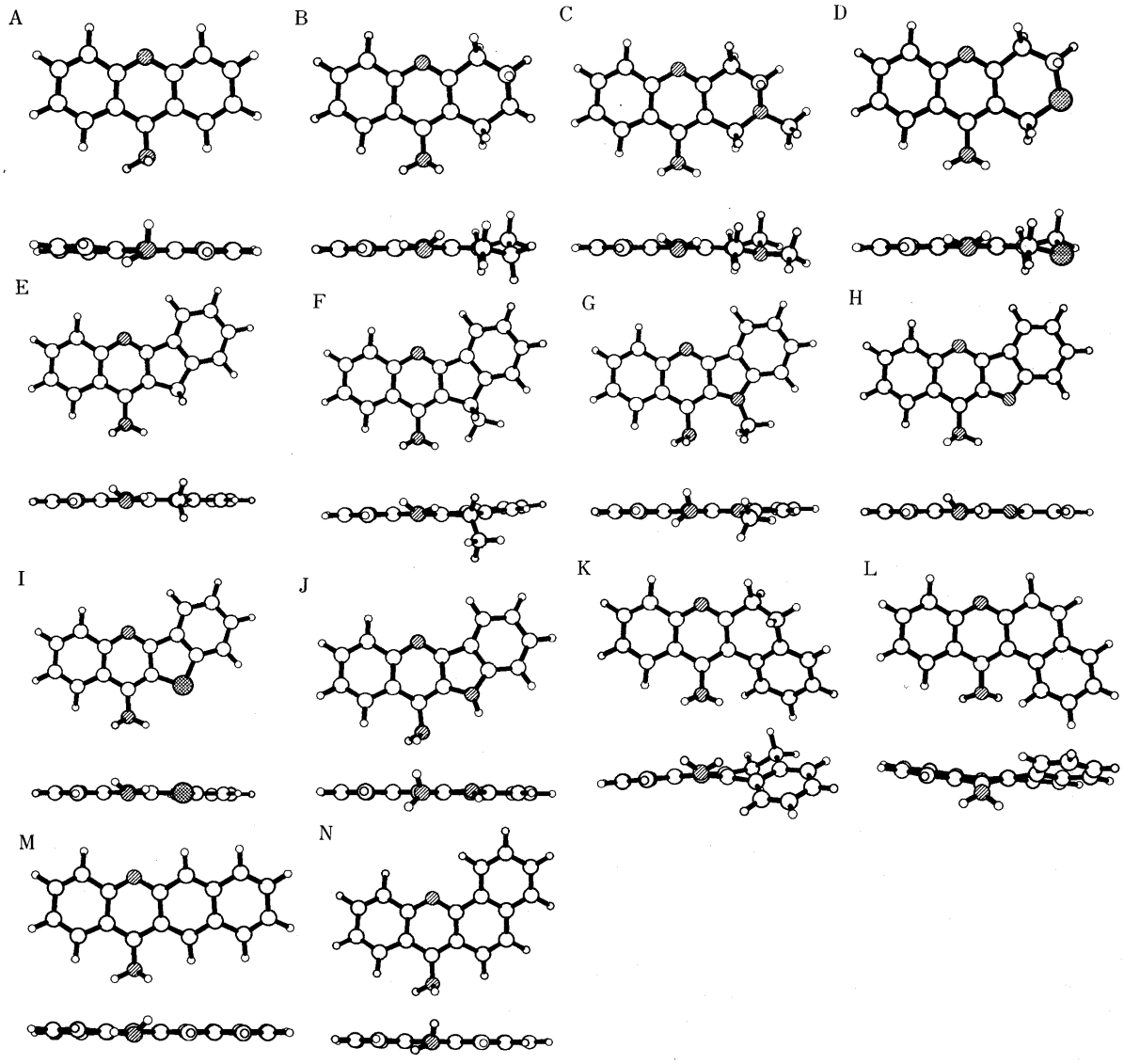


Fig. 2. Calculated Structures of Chromophore Moiety of Tested Compounds for Antitumor Activity

Both xy and yz plane orientations displayed: A, *m*-AMSA; B, **1a**; C, **2a**; D, **3a**; E, **4a**; F, **5a**; G, **6a**; H, **7a**; I, **8a**; J, **9**; K, **14**; L, **15**; M, **20**; N, **21**.

of the chromophores, intercalative ability, and antitumor activity. Potent antitumor-active compounds **4a** and **9** were selected and their pharmacological properties and structure–activity relationships are being studied.

Experimental Section

Melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were taken on a Hitachi R-24 spectrometer at 60 MHz with Me_4Si as an internal standard. Mass spectra (MS) were recorded on a Shimadzu LKB-9000 spectrometer and infrared (IR) absorption spectra on a JASCO A-102 spectrometer.

11-Chloro-10*H*-indolo[3,2-*b*]quinoline (12) A mixture of 2-[(*N*-phenylamino)acetamido]benzoic acid[4] (**11**; 1.40 g, 4.00 mmol) and polyphosphoric acid (PPA, 40 g) was heated with mechanical stirring at 120—130 °C for 2 h. Ice water was added dropwise to the reaction mixture and the solution was basified with a saturated $KHCO_3$ solution. The resulting precipitates were collected, washed with water, and dried to give 0.72 g (70%) of 10*H*-indolo[3,2-*b*]quinolin-11-one (**10**).

A mixture of **10** (0.61 g, 2.80 mmol) and $POCl_3$ (7 ml) was heated at reflux for 2 h. The excess $POCl_3$ was removed and the residue was basified with a saturated $KHCO_3$ solution and extracted with $CHCl_3$. The organic layer was washed with a saturated NaCl solution and dried over $MgSO_4$. Removal of the solvent gave 450 mg (63%) of **12** as crystals, mp 220—224 °C (lit.[4] 225—228 °C).

***N*-[4-(10*H*-Indolo[3,2-*b*]quinolin-11-yl)amino-3-methoxyphenyl]methanesulfonamide (9)** A mixture of **12** (760 mg, 3.00 mmol), 4-[(methylsulfonyl)amino]-2-methoxyaniline (**13**; 720 mg, 3.30 mmol) was heated at reflux in 2-ethoxyethanol (15 ml) for 4 h. The resulting precipitates were collected and recrystallized from MeOH to give 800 mg (57%) of the hydrochloride of **9**. Free base **9** as crystals, mp 223—225 °C. IR (Nujol): 3460, 3350 cm^{-1}. ^{1}H-NMR (Me_2SO-d_6) δ: 3.10 (3H, s, SO_2CH_3), 3.58 (3H, s, OCH_3), 6.8—8.2 (10H, m), 8.40 (1H, dd, J=6.0, 1.2 Hz), 8.60—9.12 (2H, br, NH), 9.99 (1H, br, NH). MS *m/z*: 432 (M^+). *Anal.* Calcd for $C_{23}H_{20}N_4O_3S$: C, 63.89; H, 4.63; N, 12.96. Found: C, 63.90; H, 4.84; N, 13.18.

5,6-Dihydro-7*H*-benz[*a*]acridin-12-one (16) A mixture of anthranilic acid (1.41 g, 10.0 mmol) and 2-tetralone (1.50 g, 10.0 mmol) was heated at 120 °C for 1.5 h. The resulting precipitates were collected and recrystallized from a mixture of tetrahydrofuran (THF) and MeOH to give 1.56 g (62%) of **16**: mp > 300 °C (lit.[6] 336—337 °C).

12-Chloro-5,6-dihydrobenz[*a*]acridine (17) A mixture of **16** (1.45 g, 6 mmol) and $POCl_3$ (15 ml) was heated at reflux for 10 min. The excess $POCl_3$ was removed, the residue was basified with a saturated $KHCO_3$ solution and then extracted with $CHCl_3$. The organic layer was washed with a saturated NaCl solution and dried over $MgSO_4$. Removal of the solvent gave 935 mg (60%) of **17** as crystals, mp 116 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.72—3.42 (4H, m, CH_2CH_2), 7.3—8.2 (6H, m), 8.2—8.6 (2H, m). *Anal.* Calcd for $C_{17}H_{12}ClN$: C, 76.83; H, 4.55; N, 5.27. Found: C, 76.61; H, 4.42; N, 5.44.

***N*-[4-(5,6-Dihydrobenz[*a*]acridin-12-yl)amino-3-methoxyphenyl]methanesulfonamide (14)** A mixture of **17** (831 mg, 3.13 mmol) and **13** (677 mg,

3.13 mmol) was heated at reflux in 2-ethoxyethanol (20 ml) for 3 h. The mixture was poured into a saturated $KHCO_3$ solution and extracted with $CHCl_3$. The organic layer was washed with a saturated NaCl solution and dried over $MgSO_4$. After removal of the solvent, the residue was chromatographed on alumina with CH_2Cl_2 to give 779 mg (60%) of **14** as crystals, mp 122—124 °C. IR (Nujol): 3260 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 2.91 (3H, s, CH_3SO_2), 2.72—3.39 (4H, m, CH_2CH_2), 4.00 (3H, s, CH_3O), 6.3—6.5 (2H, m), 6.79 (1H, br, NH), 7.0—8.2 (2H, m). MS *m/z*: 445 (M^+). *Anal.* Calcd for $C_{25}H_{23}N_3O_3S$: C, 67.39; H, 5.20; N, 9.43. Found: C, 67.17; H, 5.47; N, 9.15.

7*H*-Benz[*a*]acridin-12-one (18) A solution of **16** (290 mg, 1.20 mmol) in *p*-cymene was heated at reflux for 8 h in the presence of 5% Pd–carbon (290 mg). THF was added to the reaction mixture, then the catalyst was filtered off. Removal of the solvent from the filtrate gave 238 mg (83%) of **16**: mp > 300 °C.

12-Chlorobenz[*a*]acridine (19) A mixture of **18** (3.1 g, 13 mmol) and $POCl_3$ (30 ml) was heated at reflux for 2 h. The excess $POCl_3$ was removed and the residue was made basic with a saturated $KHCO_3$ solution and extracted with $CHCl_3$. The organic layer was washed with a saturated NaCl solution and dried over $MgSO_4$. Removal of the solvent gave 1.90 g (57%) of **19** as crystals, mp 156—158 °C (lit.[7] 159—160 °C).

***N*-[4-(Benz[*a*]acridin-12-yl)amino-3-methoxyphenyl]aminomethanesulfonamide (15)** A mixture of **19** (825 mg, 3.13 mmol) and **13** (667 mg, 3.13 mmol) was heated at reflux in 2-ethoxyethanol (20 ml) for 3 h. The resulting precipitates were collected and recrystallized from a mixture of MeOH and dimethylformamide (DMF) to give 798 mg (58%) of the hydrochloride **15** as crystals, mp 215—216 °C (lit.[8] 212—214 °C). ^{1}H-NMR (CF_3COOD) δ: 3.13 (3H, s, SO_2CH_3), 3.97 (3H, s, OCH_3), 6.6—7.3 (3H, m), 7.5—8.5 (12H, m). MS *m/z*: 443 (M^+). *Anal.* Calcd for $C_{25}H_{24}ClN_3O_3S$: C, 62.56; H, 4.62; N, 8.76. Found: C, 62.36; H, 4.55; N, 8.60.

Intercalation with DNA. Fluorometric Measurement The technique used was essentially the same as that reported by Cain *et al.*[9]

A Hitachi fluoresence spectrometer was used at maximum sensitivity. Excitation of the buffer solution was achieved by using a 564 nm filter. Fluoresence emission was measured at 595 nm. The buffer contained Hepes (2 mM), ethylenediaminetetraacetic aicd (EDTA) (10 mM), and NaCl (9.4 mM). The pH was adjusted to 7.0 with NaOH. Ethidium bromide (3 mM, Tokyo Kasei), a calf thymus DNA (20 mM, Sigma, high polymerized type I), and DMSO (10 mM) were dissolved in the buffer. The fluorescence of the DNA–ethidium bromide complex was measured in the presence (T) and in the absence (C) of drug (10 mM). Percentages in the fluorescence were calculated as (T/C) × 100.

Unwinding Measurement DNA unwinding effects of intercalators were assayed according to the Chen method.[10]

Unwinding measurements were done in reaction mixtures (20 mM each) containing Tris (pH 7.5, 40 mM), KCl (100 mM), $MgCl_2$ (10 mM), $MgCl_2$ (10 mM), dithiothreitol (0.5 mM), EDTA (0.5 mM), bovine serum albumin (30 mg/ml), relaxed PBR322 DNA dimer (20 mg/ml), and calf thymus DNA topoisomerase I (200 ng/ml). The reaction mixture was incubated for 30 min at 37 °C at 5, 25, or 100 mg/ml drug concentration.

Agarose Gel Assay for Topoisomerase II Dependent DNA Cleavage Calf thymus DNA topoisomerases were purified according to Halligan's method.[11]

Proteinase K was from Sigma. Reaction mixtures (20 ml) containing Tris–HCl (pH 7.5, 50 mM), KCl (100 mM), $MgCl_2$ (10 mM), adenosine triphosphate (ATP) (1 mM), dithiothreitol (0.5 mM), EDTA (0.5 mM), bovine serum albumin (30 mg), PBR322 DNA (0.4 mg), and calf thymus DNA topoisomerase II were incubated at 37 °C. After 60 min, reactions were terminated by the addition of 2 ml of a solution containing 5% sodium dodecyl sulfate (SDS) and protenase K (2.5 mg/ml). Following an additional 60-min incubation at 37 °C, the samples were electrophoresed through a 1.2% agarose gel in Tris-borate (pH 8.3, 89 mM)–EDTA (2 mM) buffer containing 0.1% SDS. After electrophoresis, gels were stained with ethidium bromide and photographed.

Culture of KB Cells and Determination of ED_{50} of the Drugs A clonal KB cell line, established by Dr. M. Green, St. Louis University, and kindly supplied by Dr. K. Fujinaga, Sapporo Medicinal College, was grown in Eagle's minimal essential medium containing 10% calf serum (Grand Island Biological).[15] Cells were grown in plastic dishes (Lux Scientific) at 37 °C in 5% CO_2–95% air. The cells grew exponentially for at least 72 h under the experimental conditions, and the doubling time of the KB cell populations was about 20 h.

The cytotoxic activity of the drugs on cultured KB cells was measured by determining the IC_{50}.[15] KB cells were seeded in plastic dishes (diameter 60 mm; Lux Scientific) at a density of 2100 cells/cm^2 growth surface. At 24 h after inoculation, the medium was changed and the cells were treated with graded concentrations (0.3—100 mg/ml) of the drugs. Two dishes were used for each drug concentration. The cells were cultivated for 48 h in the presence of drugs. The medium was removed and the cell layer was washed with phosphate-buffered saline (PBS) and trypsinized with an aliquot of 0.25% trypsin–EDTA (Grand Island Biological). PBS containing 2% fatal calf serum was added to neutralize the trypsin. The cells were suspended by pipetting and enumerated with a Coulter counter. The IC_{50} of each drug was obtained by plotting the logarithm of the drug concentration *vs.* the growth rate (percentage of control) of the treated cells.

Antitumor Activity Mouse tumor used in the experiment was P388 leukemia kindly supplied by the National Cancer Institute (U.S.) P388 (10^6) cells were transplanted i.p. into $CD2F_1$ mice (six mice per group). The drugs were dissolved in 0.9% NaCl solution with an addition of one drop of Tween 80 and administered i.p. on days 1 and 5. Antitumor activity was evaluated by the median survival time (MST) of the mice and expressed as % (T/C), T and C being the MST of treated and control groups, respectively.

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