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Acridine–Psoralen Amines and Their Interaction with Deoxyribonucleic Acid†

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ABSTRACT: A series of novel compounds in which a 9-acridinyl nucleus is linked to a psoralen nucleus in the 5- or 8-position via polyamines was prepared and examined. Their reversible binding to DNA and their irreversible binding to DNA and DNA cross-linking upon irradiation with UV-A light were examined. It was found that they were all less efficiently photoreactive than 8-methoxypsoralen (8-MOP), both in cross-linking and photobinding to DNA, whereas the ratio between their photobinding and cross-linking was 40–400 times that of 8-MOP. Compounds in which the linker was attached to the 5-position in psoralen showed smaller cross-linking and photobinding efficiencies and larger ratios between photobinding and cross-linking than those of psoralens attached in the 8-position. This strongly indicates that the 9-substituents of the acridines are oriented toward the minor groove. Flow linear dichroism studies showed that the compounds were DNA intercalating with the acridine moiety, whereas the psoralen moiety in no case was clearly intercalating. This conclusion was further supported by viscometry which also strongly indicated monointercalation.

Psoralen derivatives are used clinically in the PUVA[1] treatments of dermatological diseases, e.g., psoriasis (Anderson & Voorhees, 1980), and as probes for the study of the secondary structure of nucleic acids (Song & Tapley, 1979). It is generally accepted that the photobiological effects of psoralens are due to their photoreactions with nucleic acids, which

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[1] Abbreviations: 8-MOP, 8-methoxypsoralen; PUVA, photochemotherapy with UV-A light (UV-A = 320–390 nm); LD, linear dichroism; IR, infrared; NMR, nuclear magnetic resonance; Me_2SO, dimethyl sulfoxide; SDS, sodium dodecyl sulfate; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetic acid.

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primarily lead to the formation of cyclobutane bridges with thymine and to a minor extent cytosine and maybe other pyrimidine and purine bases of the nucleic acids (Straub et al., 1981; Kanne et al., 1982a,b). Two types of photoreaction between the RNA/DNA and the psoralens are known, one resulting in monoattachment mainly through the 4′,5′-double bond of the psoralen (photobinding) and the other resulting in diattachment through the 3,4- and 4′,5′-double bonds of the psoralen (photo-cross-linking) (Song & Tapley, 1979; Straub et al., 1981; Kanne et al., 1982a,b).

The suspected carcinogenic side effects of the major clinically useful compound 8-methoxypsoralen (8-MOP) (Stern et al., 1979; Ashwood-Smith et al., 1980; Grekin & Epstein, 1981) have led to a widespread search for new psoralen derivatives. Simultaneously, the causes of photocarcinogenesis and phototoxicity of psoralens (Pathak, 1982; Ashwood-Smith et al., 1982; Forbes & Davies, 1981) and other compounds (Kaidbey & Kligman, 1978; Maguire & Kaidbey, 1982) are being intensely studied.

The biological effects of photobinding and photo-cross-linking are presumably different with regard to mutagenicity, lethality, and repair mechanism (Forbes & Davies, 1981), and possibly with regard to photocarcinogenesis. It is thus warranted to synthesize and examine new psoralen derivatives which are either exclusively photobinding or have very large cross-linking efficiencies and small photobinding efficiencies. Furthermore, psoralens with stronger DNA affinity and sequence specificity, as well as psoralens with improved quantum efficiency for DNA binding, could prove valuable both in phototherapy and as tools in molecular biology. In addition, such compounds could be very useful in the study of the reversible interaction between psoralens and nucleic acids.

An improved affinity for DNA could be obtained by supplying the reagents with basic groups, which are protonated under physiological conditions and which bind to the phosphate backbone of the DNA. A more important increase in the specific DNA affinity should be obtainable by supplying the psoralens with an auxiliary strongly intercalating group, e.g., a 9-aminoacridine moiety. This together with the alleged intercalation of the psoralen moiety could be expected to lead to reagents with very strong reversible DNA binding, possibly by bisintercalation. Furthermore, a previously demonstrated ability of energy transfer from a 9-acridinyl moiety on a linker to another linked group (Nielsen et al., 1983) might likewise make such acridine–psoralens photoactive at longer wavelengths than those corresponding to efficient psoralen absorption. This would be important in phototherapy.

We have prepared a series of psoralens attached to acridines with basic linkers and examined their reversible interaction with DNA, as well as their photobinding to DNA and their DNA photo-cross-linking properties.

The reversible DNA interaction was determined by measuring the flow linear dichroism (Nordén & Tjerneld, 1976, 1977, 1982) of the acridine and psoralen parts of the new reagents. This method can give information about the angular orientation of these groups relative to the DNA bases, and it is generally assumed that DNA-intercalated groups should be oriented exactly parallel to the planes of the DNA bases (Nordén & Tjerneld, 1976). Furthermore, the equilibrium constants (K_{eq}) between the reagents and DNA were examined by competition experiments with ethidium bromide (Cain et al., 1978). The interaction between DNA and the reagents was further studied by measuring the viscosity of sonicated DNA as a function of added reagents (Wright et al., 1980; King et al., 1982) and the increase in DNA melting point upon addition of reagents (Canellakis et al., 1976).

The stacking behavior of a set of selected reagents as a function of the rigidity of the linker was examined by comparing the UV-absorption characteristics of the acridine–psoralens with "fragment compounds", containing the linker and the psoralen or the acridine moiety. The excitation and emission spectra of the same set of compounds were measured in order to examine the energy transfer as a function of the linker rigidity, and thereby the stacking.

Experimental Procedures

Materials and Methods. UV spectra were recorded on a UNICAM SP800A or a Cary 219 spectrometer, LD spectra on a JASCO J-500A spectropolarimeter converted to LD mode (Davidsson & Nordén, 1976), IR spectra on a Perkin Elmer 157 spectrometer, and mass spectra on an AEI MS 902 instrument at 70 eV. NMR spectra were recorded on a JEOL FX90Q and emission spectra on a Perkin-Elmer Hitachi MPF-3 spectrometer. Melting points (uncorrected) were determined on a Büchi melting point apparatus. Elemental analyses were determined by Preben Hansen at the Microanalytical Laboratory, The H. C. Ørsted Institute, Copenhagen. All chemicals for synthesis and analysis were standard commercially available except 8-MOP which was a gift from Pharma Medica A/S, Copenhagen. Calf thymus DNA was obtained from Sigma (grade no. 1). For sonication the DNA was dissolved in buffer (4 mg/mL, 10 mM Tris-HCl and 10 μM EDTA, pH 7), filtered through a 0.6 μM Sartorius cellulose acetate membrane, and sonicated for 4 × 5 s at high energy levels at temperatures from 0 to 4 °C. The DNA concentrations were determined spectrophotometrically at 260 nm (ϵ_{260} = 6600 M^{-1} cm^{-1}).

Flow Linear Dichroism. The previously described method of flow dichroism with polarization modulation, for studying the interaction of molecules with DNA, was used (Nordén & Tjerneld, 1976, 1977, 1982). Solutions of DNA (E_{260} = 1–10) and reagent were run in Couette cells of Wada type (0.50 mm annular gaps) at a constant flow gradient of 1000 s^{-1}. The linear dichroism

$$LD = A_{\parallel} - A_{\perp} \quad (1)$$

with $A_{\parallel}$ and $A_{\perp}$ denoting absorbancies with the electric vector of light parallel ($A_{\parallel}$) and perpendicular ($A_{\perp}$) to the flow direction, was measured in the Davidsson–Nordén mode (Davidsson & Nordén, 1976). The DNA was used without any pretreatment. No shear degradation or denaturation was observed, and repeated scanning, over the region 200–600 nm, reproduced the LD spectrum on all essential points within 2%. From LD and absorbance, A_{iso}, for the isotropic solution, the reduced dichroism, $LD^r = LD/A_{iso}$, was formed, and interpreted in terms of the relation

$$LD^r = \frac{LD}{A_{iso}} = S\frac{3}{2}(3\langle\cos^2\alpha\rangle - 1) \quad (2)$$

where α is the angle between the active, absorbing transition moment and the DNA axis and S is a complicated function of flow geometry, gradient, and shape and flexibility of the DNA (Nordén & Tjerneld, 1977, 1982). For B-form DNA LD^r at 260 nm $\langle\cos^2\alpha\rangle$ = 0.005, which corresponds to an average α = 86° (Matsuoka & Nordén, 1983). The value of S can be determined from the measured dichroism at 260 nm, at a given stationary flow orientation (eq 2). This equation is subsequently used to obtain α values for the transitions of a DNA-associated molecule. The high accuracy of this method compared to, e.g., electric dichroism measurements, is due to its simultaneous measurements on the DNA bases and the

DNA-associated reagents of a sample.

Viscometry. This was performed with an "Ubbe-Lohde" viscometer having a 100 mm long × 0.5 mm wide capillary and a 1.5-mL bulb. The temperature was 20 ± 0.01 °C. The flow time for water was ca. 90 s. Sonicated DNA was diluted to ca. 5×10^{-4} M with buffer and filtered as described previously. The reagents were used in concentrations of ca. 7.5×10^{-4} M. DNA and reagent concentrations were measured by their UV absorptions.

To sonicated DNA (5.00 mL) were added reagent solutions in 50-μL portions giving [reagent]/[base pairs] < 0.15. The flow times were transformed to relative contour lengths as described by Wakelin et al. (1978) by using eq 3:

$$\frac{L}{L_0} = \left[\frac{t_c - t_0(v)}{t_d - t_0(v)}\right]^{1/3} \quad (3)$$

where L = the contour length of the reagents–DNA complex, L_0 = the contour length of free DNA, t_c = flow time for the complex, t_d = flow time for the DNA, and $t_0(v)$ = flow time for the buffer at a given total volume, v, in the viscometer. The L/L_0 values were plotted against [reagent]/[DNA]. The slope from this gives the viscosity index.

DNA Melting. Poly[d(AT)] was dissolved in Tris buffer as described above ($A_{260} \sim 1.0$), and solutions of reagents (as described above) were added. The reagent:poly[d(AT)] ratio was 1:10. Measurements were performed in a temperature regulated cell on the UNICAM SP800A spectrophotometer. The change in absorption as a function of temperature was measured, and the DNA melting point (T_m) was determined as the temperature corresponding to a 50% change in absorbancy.

Photobinding Yield. A 50-μg sample of the acridine–psoralen was mixed with 1 mg of sonicated calf thymus DNA in 1.2 mL of 10 mM Tris-HCl, pH 7.4. For determination of total dye, 2 × 50 μL was withdrawn and hydrolyzed to the acridone in 1.5 mL of 2% SDS for 10 min at 90 °C (Nielsen, 1982); 2 × 200 μL samples were subsequently taken as background, and the rest of the solution was irradiated for 120 min with light (1-cm acetone filter, cutoff ~ λ >325 nm, light intensity at the used distance from the lamp 15 cm was 1.1×10^{17} quanta s^{-1} cm^{-2}, determined with ferric oxalate actinometry) from an Osram SP 200 super pressure mercury lamp. The mixture was stirred and kept in ice during the irradiation; 3 × 200 μL samples were withdrawn. The dye–DNA complex was dissociated by the addition of 200 μL of Me_2SO, and the DNA was precipitated in 66% ethanol and 0.1 M NaOAc. The DNA was redissolved in 200 μL of H_2O and the above procedure repeated. Finally the DNA was washed with ethanol and dissolved in 1.5 mL of 2% SDS (10 min, 90 °C). The amount of DNA-associated acridine was estimated from the fluorescence of the SDS samples (λ_{ex} 410 nm, λ_{em} 460 nm, corresponding to the fluorescence of acridone produced from the hydrolysis of the acridine–psoralen compound by SDS) (Nielsen, 1982). From these results the yield of photobinding relative to the amount of added acridine–psoralen was calculated.

Photo-Cross-Linking Efficiency. A 100-μg sample of sonicated calf thymus DNA was mixed with 0.1–100 μg of the reagent in 200 μL of 10 mM Tris-HCl and 1 mM EDTA, pH 7.4. The mixture was irradiated for 60 min with a Philips TL 20W/09 fluorescent light tube (λ ~365 nm). Subsequently 200 μL of 4 M NaCl was added, and the DNA was denaturated at 100 °C (20 min). The high ionic strength and the long heating time were necessary due to the helix stabilizing effect exhibited by the acridine–psoralens. After rapid cooling in ice, the renaturable fraction of the DNA was determined by separating single- and double-stranded DNA on a hydroxylapatite column (1 × 6 cm) eluted with a linear gradient of 100 mL of 10–500 mM sodium phosphate, pH 7.0. The amount of reagent causing 50% cross-linking was determined by using a range of concentrations and the efficiency expressed relative to that of 8-methoxypsoralen. The values for nonirradiated samples were subtracted.

Equilibrium Constants. A total of 2 μg of calf thymus DNA and 2 μg of ethidium bromide (etBr) was mixed with 0–60 μg of the reagent in 1 mL of 10 mM Tris-HCl and 0.2 M NaCl, pH 7.4, in a test series. After equilibration at room temperature for 2 h the ethidium fluorescence (λ_{ex} 540 nm, λ_{em} 610 nm) was measured. The fluorescence intensity was plotted against the concentration of the acridine–psoralen, and from this the concentration causing 50% fluorescence "quenching", corresponding to replacement of 50% of the ethidium bromide in the DNA by the added acridine–psoralen, was found. Since the concentration of ethidium bromide is known, the equilibrium constant of the acridine–psoralen relative to ethidium bromide can be easily calculated:

$$K_{rel} = K_{etBr}[\text{etBr}][\text{acridine–psoralen at 50\% fluorescence}]^{-1}$$

where $K_{etBr} = 1.5 \times 10^5$ mol^{-1} L (Reinhardt & Krugh, 1978).

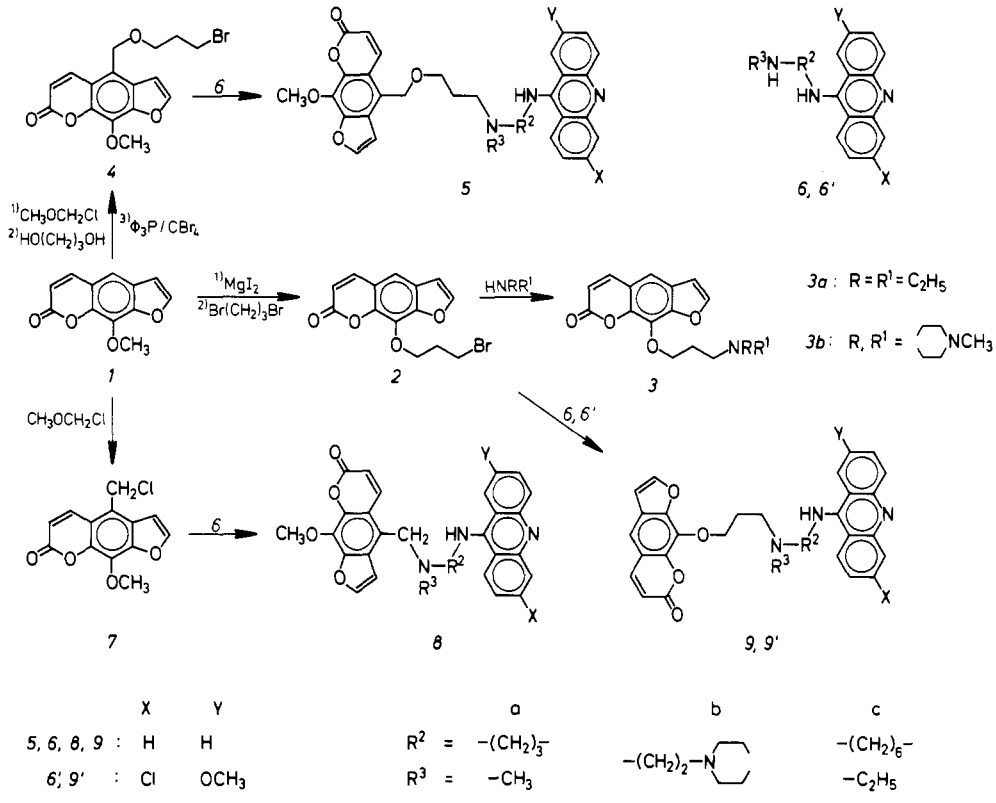
Furthermore, equilibrium constants at low ionic strengths (1 mM) were determined by using an aqueous two-phase system as previously reported (Nordén & Tjerneld, 1982).

Synthesis (cf. Scheme I). 5-(Chloromethyl)-8-methoxypsoralen (**7**) was prepared by chloromethylation of 8-MOP (**1**) (Aboulezz et al., 1973), 8-[(3-bromopropyl)oxy]psoralen (**2**) by magnesium iodide assisted demethylation of 8-MOP (**1**) followed by treatment with 1,3-dibromopropane (Antonello et al., 1979), 8-[[(3-diethylamino)propyl]oxy]psoralen (**3a**) by treatment of **2** with diethylamine (Antonello et al., 1979), and the acridines **6a–c** and **6'b** by treatment of 9-chloroacridine or 2-methoxy-6,9-dichloroacridine with the relevant polyamines as previously described (Hansen et al., 1983). The psoralen–acridine heterodimers were prepared as described for compounds **9a**·2HCl and **9b**·2HCl. The elemental analyses and ^{1}H NMR and IR spectra of all the described compounds are in agreement with the assigned structures.

(A) 5-[[(3-Hydroxypropyl]oxy]methyl]-8-methoxypsoralen. 5-(Chloromethyl)-8-methoxypsoralen (**7**; 2.2 g, 8.3 mmol) was mixed with 1,3-propanediol (15 mL) and heated to 80–100 °C with stirring for 2.5 h, after which it was cooled to room temperature, water (50 mL) was added, and the resulting mixture was kept at 0–4 °C for 20 h. This caused precipitation of the title compound as white crystals which were isolated by filtration (1.9 g, 74%): mp 111–112 °C; mass spectrum, m/z 304 (M^+, 70), 229 [$M - O(CH_2)_3OH$, 100]. Anal. Calcd for $C_{16}H_{16}O_6$: C, 63.15; H, 5.30. Found: C, 63.02; H, 5.37.

(B) 5-[[(3-Bromopropyl)oxy]methyl]-8-methoxypsoralen (**4**). 5-[[(3-Hydroxypropyl)oxy]methyl]-8-methoxypsoralen (1.5 g, 4.9 mmol) was added to a stirred mixture of triphenylphosphine (2.7 g, 10.3 mmol) and carbon tetrabromide (3.3 g, 10.0 mmol) in dimethylformamide (30 mL) which caused an exothermic reaction that lasted 10–15 min. Upon completion of the reaction, ice–water (100 mL) was added, and the generated oily suspension was extracted with chloroform. The chloroform extract was dried with magnesium sulfate, and the solvent was removed, in vacuo. The resulting noncrystalline material was dissolved in ethyl acetate and subjected to column chromatography (silica gel) which gave compound **4** (0.80 g, 44%): mp 63–64 °C; mass spectrum m/z

Scheme I



368/366 (M^+, 43), 229 [M – $O(CH_2)_3Br$, 100]. Anal. Calcd for $C_{16}H_{15}O_5Br$: C, 52.34; H, 4.12. Found: C, 52.94; H, 4.06.

(*C*) *N-Methyl-N'-[3-(8-psoralenyloxy)propyl]piperazine Dihydrochloride* (**3b**). 8-[(3-Bromopropyl)oxy]psoralen (**2**; 365 mg, 1.15 mmol), *N*-methylpiperazine (112 mg, 1.10 mmol), and anhydrous potassium carbonate (0.5 g) in acetone (20 mL) were refluxed for 20 h and filtered, and the filtrate was concentrated, in vacuo. The resulting oily materials was dissolved in ethanolic HCl, and the hydrochloride of **3b** was precipitated by addition of ether (325 mg, 70%) and recrystallized from ethanol containing a little ether: mp >260 °C. Anal. Calcd for $C_{19}H_{24}N_2O_4 \cdot 2HCl \cdot 2H_2O$: C, 52.66; H, 6.05; N, 6.46. Found: C, 52.77; H, 6.04; N, 6.46.

(*D*) *N-Methyl-N-[3-(8-psoralenyloxy)propyl]-N'-(9-acridinyl)-1,3-propanediamine Dihydrochloride* (**9a**). 8-[(3-Bromopropyl)oxy]psoralen (**2**; 200 mg, 0.64 mmol), 9-[[3-(methylamino)propyl]amino]acridine (**6a**; 117 mg, 0.44 mmol), and anhydrous potassium carbonate (200 mg) in acetone (25 mL) were refluxed for 40 h. The reaction mixture was filtered while hot, the filtrate was concentrated, in vacuo, and the product was dissolved in a mixture of 1 N HCl (10 mL) and chloroform (25 mL). The aqueous phase was washed with chloroform (25 mL), adjusted to pH >10 with concentrated aqueous sodium hydroxide and extracted with chloroform (3 × 25 mL). The chloroform solution was subsequently dried by washing once with saturated sodium chloride solution (25 mL), dried over anhydrous magnesium sulfate, and concentrated, in vacuo. The product from this was dissolved in ethanolic HCl, and the hydrochloride of **9a** was precipitated by addition of ether (200 mg, 74%): mp 149–151 °C. Anal. Calcd for $C_{31}H_{29}N_3O_4 \cdot 2HCl \cdot 2H_2O$: C, 60.39; H, 5.78; N, 6.82; Cl, 11.50. Found: C, 60.33; H, 5.34; N, 6.94; Cl, 11.86.

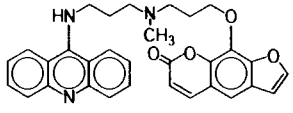
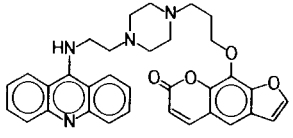
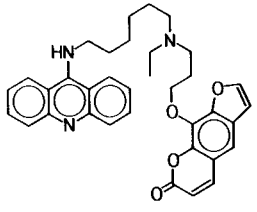
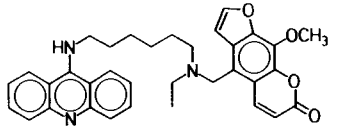
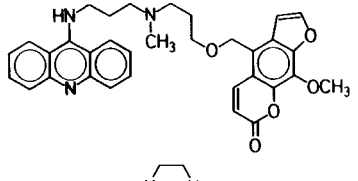
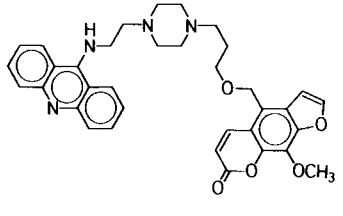
(*E*) *N-[3-(8-Psoralenyloxy)propyl]-N'-[2-(9-acridinylamino)ethyl]piperazine Trihydrochloride* (**9b**). *N*-[2-(9-Acridinylamino)ethyl]piperazine (**6b**; 200 mg, 0.65 mmol), 8-[(3-bromopropyl)oxy]psoralen (**2**; 300 mg, 0.92 mmol), and anhydrous potassium carbonate (200 mg) in acetone (25 mL) were refluxed for 40 h, filtered, and concentrated, in vacuo. The resulting oily material was treated with ice-cold 1 N HCl (5 mL), which resulted in precipitation of crystalline material. Upon recrystallization from methanolic HCl pure title compound was obtained (241 mg, 53%): mp >230 °C dec. Anal. Calcd for $C_{33}H_{32}N_4O_4 \cdot 3HCl \cdot 2H_2O$: C, 57.11; H, 5.65; N, 8.07; Cl, 15.32. Found: C, 56.84; H, 5.25; N, 7.97; Cl, 15.40.

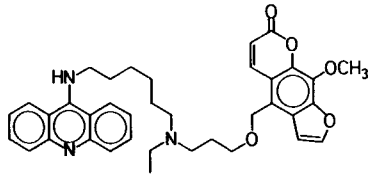
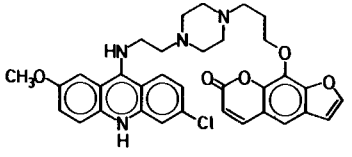
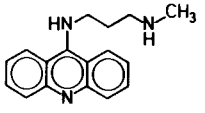
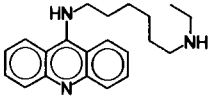
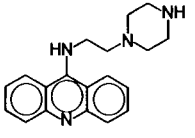
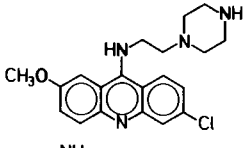
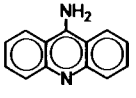
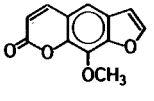
Results

Chemistry (*Scheme I*). The monoacridine derivatives (**6** and **6'**) and the monopsoralen derivatives (**3**) (J. B. Hansen et al., unpublished results) were prepared by previously described methods. Psoralens linked in their 8-position were prepared by demethylation of 8-MOP (**1**), treatment of the resulting hydroxy compound with 1,3-dibromopropane to give **2**, and reaction of **2** with compounds $(R^1)_2NH$ or **6** and **6'** to give compounds **3** or **9** and **9'**. The linking of the psoralens via the 5-position was done by (i) treating 8-MOP (**1**) with chloromethyl methyl ether to give **7** followed by treatment with compounds **6** to give compounds **8** and (ii) treatment of **7** with 1,3-propanediol to give the corresponding monoether, which was converted to the bromo derivative **4** by treatment with triphenylphosphine–carbon tetrabromide. Compound **4** was subsequently treated with compounds **6** to give the acridine–psoralen **5**.

Equilibrium Analysis and ΔT_m *Measurements* (*Table I*). The equilibrium constants were estimated by competition experiments (Cain et al., 1978) with ethidium bromide at high ionic strength (Table I). The binding constants of all the new compounds are of the same order of magnitude as that of 9-aminoacridine, thus displaying no major increase in DNA affinity due to the introduction of the psoralen moiety. Similarly, the T_m values are all of the same magnitude and corresponding to that of 9-aminoacridine. These clearly show that all of the new compounds stabilize the DNA double helix

Table I: Acridine–Psoralen Amines, 9-Aminoacridine, and 8-Methoxypsoralen: Preparative Yields, Physical and Biological Data, and Photochemical Results

compound		yield (%)	mp (°C)	$K_{eq} \times 10^{-4}$ (mol^{-1} L)[b]	viscosity index[c]	DNA X-linking[d]	photo-binding[e]	ratios between photobinding and X-linking[h]	ΔT_m (°C)	linear dichroism angle (deg) with DNA axis			
										S	acridine, 450 nm	psoralen 320 nm	psoralen 350 nm
	*9a*	74	149–151	2	1.5	0.08	0.8	10	26.0	0.075	90	66	73
	*9b*	53	>230 dec	15	1.2	0.34	8	24	36.5	0.075	90	66	63
	*9c*	74	>210 dec	6	2.8	2.0	20	10	31.0	0.081	90	71	64
	*8c*	84	>190 dec	19	1.6	0.04	4	100	26.0	0.080	90		
	*5a*	62	*a*	9	ND[f]	0.04	3	75	ND		ND		
	*5b*	71	>180 dec	10	1.4	0.02	1	50	35.0	0.092	90		

	*5c*	55	*a*	18	1.2	0.01	0.5	50	20.0	0.087	90	
	*9'b*	52	>205 dec	30	1.8	0.28	10	36	31.0	0.093	90	73	70
	*6a*	89	230–232	6	1.4				32.5			
	*6c*	60	214–215	10	1.7				36.0			
	*6b*	92	>260 dec	9	1.3				36.0			
	*6'b*	87	>275 dec	40	1.0				34.0			
				3.2	1.5				15.0	0.088	90	
				0.04[g]		100	27					

[a] The compounds were hydroscopic and crystals were not obtainable. [b] Competition experiment with ethidium bromide, $K_{etBr} = 1.5 \times 10^5$ mol^{-1} L (Reinhardt & Krugh, 1978). [c] $dL/L_o/d$[intercalator]/[base pair] measured with two preparations of sonicated DNA. [d] Relative to 8-MOP = 100. [e] Percent yield relative to amount of added reagent. [f] ND = not determined. [g] Isaacs et al. (1977). [h] The value is *not* the absolute ratio between monoadducts and cross-links.

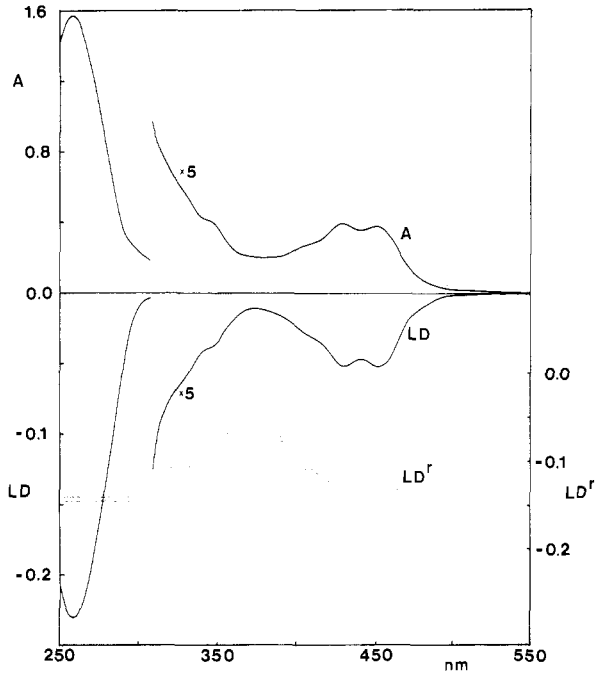


FIGURE 1: Absorption (A) and linear dichroism (LD) spectra of **9'b** plus DNA (r = 0.025; DNA, 0.239 mM; NaCl, 1 mM; EDTA, 1 mM).

(Canellakis et al., 1976). The melting curves were simple monophasic for both the acridine–psoralens and the aminoacridines.

In connection with the dichroism measurements some equilibrium analyses were carried out by using an aqueous two-phase partition system based on dextran and poly(ethylene glycol) (Nordén & Tjerneld, 1982). For the studied compounds (**9a–c** and **9'b**) a much higher binding constant was obtained ($\sim 10^7$ mol^{-1} L) at low ionic strength (1 mM), as expected if the binding is due partly to electrostatic interaction with the acridine group.

Viscosity Measurements (Table I). These determinations were performed with sonicated linear calf thymus DNA. Most of the values are comparable, within experimental error, to that of 9-aminoacridine, whereas compound **9c** gave a value which was twice as large.

Thus, most of the compounds gave values which are lower than theoretically expected even for monointercalators. Such an effect has previously been observed for other 9-aminoacridines (Wright et al., 1980; King et al., 1982). The effect is most pronounced for those compounds which have amino groups in the side chain or the linker. It is known that polyamines have a viscosity decreasing effect on DNA (Kapicak & Gabbay, 1975). Thus, we are observing a combination of two opposite effects. The large viscosity effect of compound **9c** indicates that this may have some bisintercalating character (Wakelin et al., 1978).

Linear Dichroism Measurements. The results are presented in Table I in terms of angles α (and S values) obtained by means of eq 2 from LD^r at different wavelengths. A typical spectrum is shown in Figure 1. The strong absorption band at 260 nm characterized by an almost constant LD^r is due to the transitions of the DNA bases and was used to determine S (by taking α to be 90°). The 9-aminoacridine moiety is known to have its whole long-wavelength absorption (400–500 nm) polarized exactly parallel to the short in-plane symmetry axis (Matsuoka & Nordén, 1982). A weak, long-axis polarized band at 330 nm is overlapped by an intense absorption of psoralen and cannot be used for determining the orientation of the acridine long axis. Results from linear dichroism of oriented psoralen and from MO calculations (Wittwer & Zanker, 1959) indicate that there are two transitions, at 310 and 360 nm, with moments that are directed essentially parallel and perpendicular to the long axis of psoralen (a third, long-axis polarized transition at 255 nm is hidden by the DNA absorption).

The unsymmetric methoxy and chloro substituents in **9'b** can be expected to significantly change the polarizations, however, only in form of rotations in the acridine plane. Still, as is seen from Figure 1, LD^r at 450 nm is approximately the same as for the DNA band (α = 90°), in consistency with intercalation. In **5c** the methoxy substitution of psoralen shifts the absorption into the DNA band and obviates discrimination.

The orientation of the acridine groups of compounds **5**, **8**, and **9** with their short axis perpendicular to the helix (α = 90°) and a similar orientation of the long-wavelength transition of **9'b** are strong pieces of evidence that the acridine moieties of the present acridine–psoralens are intercalated, just as in the DNA–9-aminoacridine complex. Contrary to this, the psoralen group appears not to be intercalated in any of the reagents, as judged from an angular deviation of 20–30° from coplanarity with the DNA bases. The apparent α values at 320 and 350 nm (which should correspond essentially to the long and short axis of psoralen) vary in the range 63–73° between the different complexes, which could indicate different binding geometries. Extensive overlap between the corresponding absorptions, however, makes it hard to estimate the significance of the variation.

Photoreactions with DNA. The photo-cross-linking efficiency of the compounds was estimated by measuring the renaturable fraction of DNA samples which had been treated with long-wavelength ultraviolet light (365 nm) in the presence of the psoralens (Table I). The acridine–psoralens were 0.01–2% as efficient as 8-MOP in cross-linking DNA, in vitro, and a clear trend in efficiency was observed. Compounds connected through the 8-position of the psoralen (**9a–c** and **9'b**) gave higher values than those connected through the 5-position (**5a–c** and **8c**). The efficiency appeared to be increased for compounds connected through the 8-position with long, flexible linkers.

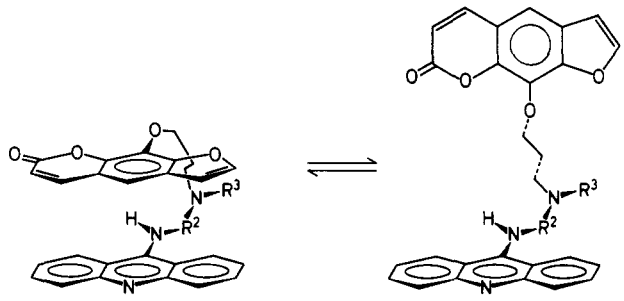
The photobinding was measured by irradiating mixtures of DNA and reagent after which the amount of photobound reagent was measured fluorometrically. The observed values showed less structural dependency than the cross-linking. They were all lower than the value obtained with 8-MOP (determined by using tritium labeled 8-MOP). However, the values for **9b**, **9c**, and **9'b** are of the same order of magnitude as that for 8-MOP (30–75%), while the values for the rest are from 2 to 15%. Also here the 5-substituted psoralens were less efficient than the 8-substituted except for compound **9a**, but more interestingly, the ratio between photobinding and photo-cross-linking was up to 10 times larger for the 5-substituted compounds than for the 8-substituted compounds.

Compound **9a** showed unexpectedly low values for photobinding, and especially for photo-cross-linking in comparison with the values of **9b,c** and **9'b**, which we attribute to its pronounced tendency for intramolecular stacking (see below).

UV Spectroscopy and Fluorescence Studies. In order to examine the relationship between behavior toward DNA and intramolecular stacking (Scheme II), the UV maxima of the acridine part of compounds **9a** and **9b** were measured, and the fluorescence characteristics were examined.

By comparison of the UV spectra of compounds **9a**, **6a**, 8-[[3-(diethylamino)propyl]oxy]psoralen (**3a**), and a mixture

Scheme II



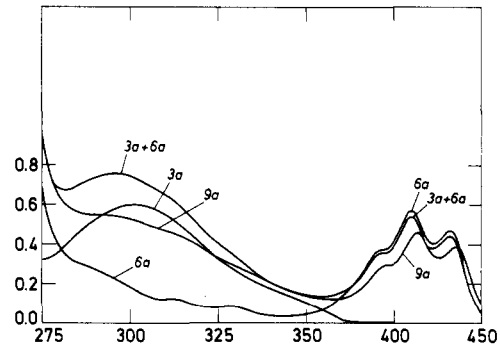


FIGURE 2: Absorption spectra of **9a**, **3a** + **6a**, **6a**, and **3a** ($\sim 5 \times 10^{-6}$ M in 10 mM Tris, pH 7.0).

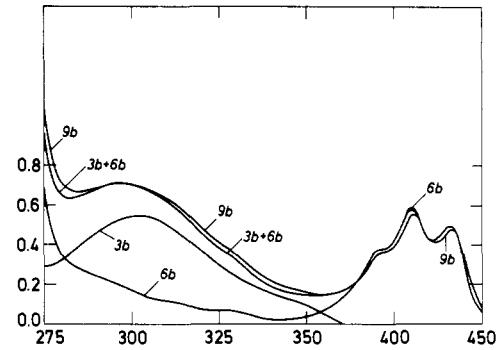


FIGURE 3: Absorption spectra of **9b**, **3b** + **6b**, and **3b** in 10 mM Tris buffer, pH 7.0. The concentration of the drugs was 5×10^{-5} M.

of **6a** and **3a**, it is clearly seen that the molecular absorbance as well as the λ_{max} values in **9a** are changed in a manner that is typical for intramolecular stacking (Leonard, 1979) (Figure 2). A similar UV analysis for compound **9b** showed the absence of intramolecular stacking (Figure 3). The long-wavelength UV maxima of compounds **5a**, **5c**, **8c**, and **9c** experienced shifts similar to that of **9c**, compared to those of compounds **6a** and **6b**, whereas this was not the case for compounds **5b** and **9'b**. The excitation spectra of **9a** and **9b** in the presence and absence of DNA were measured at 460 nm, which is the emission maximum for 9-aminoacridines, and at 540 nm, which is the optimal wavelength for distinguishing the psoralen emission. In the absence of DNA, the excitation spectrum of **9b** showed λ_{max} at 315 and 405 nm, corresponding to absorption mainly in the psoralen (315) and the acridine (405) part of the molecule. At observation wavelength 460, the ratio between the two λ_{max}'s was 1:3, while observation wavelength 540 showed the ratio as ca. 1:1. This indicates that there is a certain degree of electronic independence between the two chromophores. When DNA was added, the excitation efficiency at 320 nm was increased, indicating a better energy transfer due to the more fixed conformation. No such electronic independence between the two chromophores was observed for compound **9a**.

Discussion

The attachment of a 9-acridinylamino moiety to a psoralen nucleus via different linkers gave rise to molecules **5**, **8**, and **9/9'**, which behaved toward DNA in an almost identical manner to that of the acridine linker "fragment molecules" (**6/6'**), and quite analogously to that of 9-aminoacridine, in the absence of UV-A light. Thus, the equilibrium constants are of the same order of magnitude for all the compounds [(2–40) $\times 10^4$ mol^{-1} L, in 0.2 M NaCl; Table I).

For primitive model considerations, based on a bisintercalative binding mode, it could have been expected that the present type of acridine–psoralens should have equilibrium constants $\sim K_{eq}$(acridine linker "fragment molecules") $\times$ K_{eq}(psoralens), *i.e.*, $\sim 10^5 \times 10^2$ mol^{-1} L. The observed constants do not correspond to such a model but do correspond very well to monointercalating 9-aminoacridines. With one exception, namely, compound **9c**, this is also confirmed by the viscosity measurements, and linear dichroism measurements (Table I) show that only the acridine part of the acridine–psoralens, including compound **9c**, is truly intercalated into the DNA. This similar behavior toward DNA is likewise reflected in the ΔT_m measurements. Consequently, no influence of the psoralen parts of the molecules is evidenced by any of these measurements.

However, upon irradiation with UV-A light, the psoralen part of the molecules reacts in the well-known manner, giving rise to both mono adducts and cross-linking adducts, which must require a position of the psoralen part of the molecules, corresponding to intercalation. The photobinding of the acridine–psoralens is less efficient than that of 8-MOP, but not drastically so, whereas the cross-linking is markedly decreased compared to that of 8-MOP.

This indicates that the dynamic behavior of the DNA–acridine–psoralen complexes results in enough flexibility to allow a fraction (<10%) of the psoralen moieties to be in an intercalated position.

The variation in photoattachment behavior between the 5- and 8-substituted psoralens is in correspondence with the recent structural studies of mono and bis photoadducts between psoralens and DNA (Straub et al., 1981; Kanne et al., 1982a,b), as well as with molecular model considerations. According to these, cross-linking requires an orientation with the 8-substituent in the minor groove of the DNA. Such an orientation is obtained easily by compounds like 8-MOP, 5-(aminomethyl)-8-methoxypsoralen (Hansen & Buchardt, 1981), and **3a**, and we have shown that these three compounds cross-link DNA with comparable efficiency (unpublished results). For all of the acridine–psoralens, such an orientation is more difficult to obtain than for the simpler psoralens, since the gross binding to DNA is obviously determined by the acridine part of the molecules. This leads to general poor photo-cross-linking. However, as the length of the linker is increased, the possibility for the psoralen part of the molecule to obtain the correct orientation for cross-linking is also increased. This is reflected in the results.

The psoralens linked to the acridines through the 5-positions generally show a much higher ratio between photobinding and cross-linking than those linked through the 8-position, and they are also overall less efficient in cross-linking DNA. This is interesting, and the plausible explanation is that the 9-aminoacridines preferably intercalate with the 9-position in the minor groove, thereby allowing the proper psoralen orientation for DNA cross-linking only for the 8-substituted compounds.

The overall decrease in photoreactivity toward DNA may also be due to the preferential 9-aminoacridine intercalation in GC-rich base sequences. Since psoralens preferably react with thymidine residues, the strong guidance exerted by the acridine part of the molecule may reduce such psoralen–thymidine interaction, thereby resulting in the observed effect.

The formation of mono adducts between the DNA and psoralens requires a less stringent orientation with regard to the DNA, possibly not even intercalation, which is reflected in the present findings as well.

Compound **9c** is distinguished by having by far the highest efficiency for both photobinding and cross-linking, and the viscosity measurements indicate that it may be bisintercalating to a certain extent. The quantum efficiencies for this photoreaction are quite small ($<10^{-3}$; O. Buchardt et al., unpublished results), and small differences in the dynamic behavior of the DNA–acridine–psoralen complexes may lead to large differences in the photochemical behavior.

The existing clinical use of psoralens in the treatment of dermatological disorders, e.g., psoriasis (Anderson & Voorhees, 1980), in the PUVA therapy is strongly suspected of being carcinogenic (Stern et al., 1979; Ashwood-Smith et al., 1980). This severe side effect is assumed to be caused by DNA cross-linking. Since the presently described compounds have only 0.01–2% of the cross-linking efficiencies of the most widely used PUVA drug, 8-MOP, they represent an interesting series of compounds for testing the influence of cross-binding vs. mono adduct formation on their therapeutical and other biological activities. Furthermore, since the orientation of the photobound psoralen part of such heterodimers is fixed with the 8-position toward the minor groove, other related heterodimers may be useful in investigations of other DNA-interacting compounds.

Acknowledgments

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Registry No. **1**, 298-81-7; **2**, 69150-32-9; **3a**, 85079-39-6; **3b**, 86863-15-2; **3b**·2HCl, 86863-16-3; **4**, 86863-17-4; **5a**, 86863-18-5; **5b**, 86863-19-6; **5c**, 86863-20-9; **6a**, 86863-21-0; **6b**, 86863-22-1; **6'b**, 86863-22-1; **6c**, 86863-24-3; **7**, 43111-03-1; **8c**, 86863-25-4; **9a**, 86863-26-5; **9a**·2HCl, 86863-27-6; **9b**, 86863-28-7; **9b**·3HCl, 86863-29-8; **9'b**, 86863-30-1; **9c**, 86863-31-2; 1,3-propanediol, 504-63-2; *N*-methylpiperazine, 109-01-3; 5-[[(3-hydroxypropyl)oxy]-methyl]-8-methoxypsoralen, 86863-32-3.

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