Steric and Conformational Effects on the Photophysical and DNA Binding Properties of Novel Viologen Linked Tolylacridines

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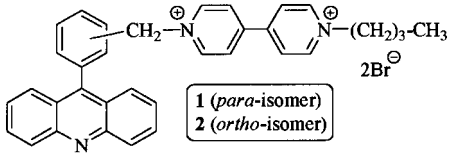
Two novel water soluble tolylacridines having viologen at the *para* and *ortho* to the acridine chromophore have been synthesized. Photophysical and DNA binding studies have revealed that the *para*-isomer exists in the extended conformation and binds to DNA by partial intercalation whereas the *ortho*-isomer exists both in folded and extended conformations and fails to interact with DNA.

There is widespread interest in the study of interactions of small molecules including drugs, dyes and toxic compounds with DNA and also in compounds that can bind and cleave DNA in unique and controllable ways.[1] These studies not only lead to the development of molecular probes for the recognition of DNA but also provides the chemical basis for carcinogenicity and serve as models for DNA–protein interactions. Recently increasing interest has been focused on the design of DNA probes based on intercalators, groove binding agents and also hybrid molecules consisting of both intercalative and groove binding moieties.[2]

With a view to designing molecular probes for DNA, we synthesized two viologen linked tolylacridines **1** and **2** and examined their photophysical and DNA binding properties. The acridine chromophore in these molecules is expected to undergo intercalation and upon excitation transfer an electron to the viologen moiety. The radical cation of acridine once formed can oxidize the DNA bases at the site of binding leading to the charge separated species and ultimately DNA cleavage. Considering the interactions of acridines with DNA, the study of effects of substituents present on the acridine ring on DNA binding characteristics has received less attention.[3] In this paper, we report that the tolyl group in **1** and **2** constitutes an interesting variation and it can be used to probe the steric and conformational effects on photophysical and DNA binding properties of acridine derivatives.



The synthesis of **1** and **2** was achieved in moderate yields through the S_N2 reaction of the corresponding bromomethylphenylacridine with 1-butyl-4,4'-bipyridinium bromide.[4,5] Absorption properties of **1** and **2** were examined in water, DMSO and alcohols. The absorption spectra of **1** resemble closely to that of the model compound *p*-tolylacridine in all solvents, whereas **2** in DMSO and alcoholic solvents exhibited a broad and structureless absorption at around 580 nm, in addition to the absorption due to the acridine chromophore at 376 nm. The broad band at 580 nm decreased with increasing temperature and solvent viscosity and was not observed in acidic methanol solutions. We assign this absorption due to the existence of ground state charge transfer interaction between the viologen and acridine moieties in **2**. The pK_a values were determined from the pH dependence of the absorbance at 376 nm and were found to be 4.9 and 3.5 for **1** and **2** respectively. Comparison of pK_a values indicates that **2** is more acidic than **1** and further supports the presence of effective interactions between the interacting units in **2**.

Fluorescence properties of **1** and **2** were examined in different solvents and the results in methanol and water are described in Table 1. Compounds **1** and **2** exhibited much lower quantum yields, when compared to tolylacridines, indicating thereby that the fluorescence of the acridine unit is efficiently quenched by the viologen moiety in these systems and the mechanism of such quenching could be by electron transfer from the excited acridine to viologen. The fluorescence quantum yields of **2** both in water and methanol were nearly 6 times lower than that of **1**, indicating an efficient quenching in the former case because of close proximity of the interacting units. Time-resolved fluorescence studies show that **1** exhibits single exponential decay both in methanol and water with a lifetime of 1.7 and 5.4 ns, respectively (Table 1). In contrast, compound **2** exhibited biexponential fluorescence decay both in methanol and water.

Table 1. Fluorescence quantum yields (Φ_f), lifetimes (τ) and DNA association constants (K_{DNA}) of viologen linked tolylacridines **1** and **2**[a]

Compound	Φ_f[b] × 10^2		τ,[c] / ns (%)[d]		K_{DNA}[e]
	CH_3OH	Water	CH_3OH	Water	/ M^{-1}
1	11	0.60	1.7 (100)	5.4 (100)	1.1 x 10^5
2	1.8	0.08	0.4 (36) 1.2 (64)	0.4 (13) 10.6 (87)	[f]

[a]Average of more than two experiments. [b]Calculated using 9-aminoacridine as standard (Φ_f = 0.99),[9] error ca. ±5%. [c]Measured using Tsunami Spectraphysics picosecond single photon counting system and the data analyzed by IBH software, chi square values 1±0.1, error ca. ±8%. [d]Relative amplitude. [e]Calculated based on fluorimetric titration of the probe against calf thymus DNA in buffer containing 2 mM NaCl and 1 mM EDTA (pH 8.05), $K_{DNA} = 4.9 \times 10^4$ M^{-1} at 100 mM NaCl. [f]Binding is negligible.

These observations together with the absorption characteristics establish that **1** exists as a single conformer, while **2** exists in two conformations in which the viologen moiety has different orientations with respect to the acridine plane. In one of the conformations, the viologen lies above the acridine plane (folded), where considerable spatial interactions exist between the two interacting units, while in the other the viologen is away from the acridine moiety (extended). In support of this view, we have carried out minimum energy calculations based on AM1 program.[6] These results confirm that **1** exists only in the extended conformation (Figure 1) in which the distance between the two interacting units is around 9.8 Å. On the other

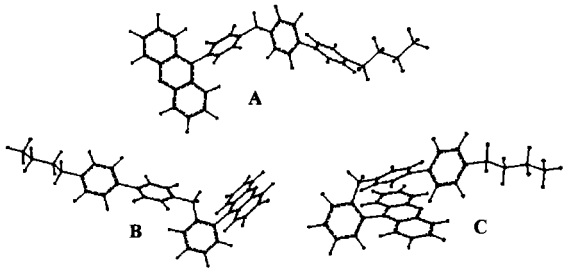


Figure 1. Minimum energy conformations of the viologen linked tolylacridines, obtained by the AM1 calculations: (A) extended form of **1**, (B) extended form of **2** and (C) folded form of **2**.

hand, compound **2** exists both in extended and folded conformations (Figure 1), where the extended conformer is slightly more stable than the folded one by 0.18 kcal mol^{-1}.

Figure 2 shows the change in absorption spectra of **1** obtained by the gradual addition of calf thymus DNA. Addition of DNA resulted in a strong decrease in absorption of the acridine chromophore (50% at the ligand to DNA ratio of 1:6), along with a small bathochromic shift of around 5 nm. Interestingly, the absorption spectra of **2** showed negligible changes with the addition of DNA.

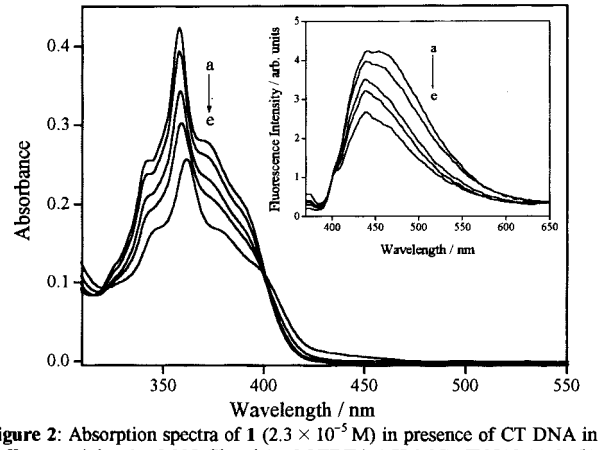


Figure 2: Absorption spectra of **1** (2.3×10^{-5} M) in presence of CT DNA in buffer containing 2 mM NaCl and 1 mM EDTA (pH 8.05). [DNA] (a) 0, (b) 0.0082, (c) 0.032, (d) 0.059 and (e) 0.128 mM. Inset shows the fluorescence spectra of **1** in presence of CT DNA under similar conditions. [DNA] (a) 0, (b) 0.08, (c) 0.16, (d) 0.2 and (e) 0.42 mM. Excitation wavelength, 355 nm.

The fluorescence emission of **1** decreased rapidly with the increase in concentration of DNA (inset of Figure 2) and reached saturation at the ligand to DNA ratio of 1:17 with a net quenching of fluorescence yield by 60%. No, significant changes in the fluorescence spectra were observed when DNA was added to **2** in buffer. From the fluorescence data, the association constants of **1** with DNA were calculated according to McGhee and von Hippel[7] (Table 1). The values were found to be $1.1 \times 10^5\ M^{-1}$ and $4.9 \times 10^4\ M^{-1}$ at 2 and 100 mM NaCl, respectively, indicating the existence of some electrostatic interactions.[8] It is interesting to note that both the absorption and fluorescence studies in the presence of DNA reveals that the *para*-isomer **1** has considerable binding affinity towards DNA, while the *ortho*-isomer **2** fails to interact with DNA.

The structural analysis of viologen linked tolylacridines reveals that the acridine as well as the phenyl rings are out of plane to each other and the dihedral angle according to the minimum energy conformations is 86° and 76° for **1** and **2**, respectively. In order to achieve effective intercalation of the acridine chromophore, the phenyl ring has to undergo rotation during the process of intercalation and the feasibility of this rotation is the deciding factor in effective binding of these molecules to DNA. In the case of **2** where it exists predominantly in the extended conformation in buffer (Figure 1B), the steric effects offered by the *ortho*-substituted viologen group inhibits the rotation of the phenyl group and thereby the substituent as a whole acts like a block in preventing the intercalation of the acridine ring. No such steric factors exist in the case of **1** and hence the intercalation of the acridine ring was observed. However, the association constants of **1** with DNA (Table 1) under different conditions are found to be one order less than the values reported for acridines,[3] indicating thereby the possibility of only partial intercalation of the acridine chromophore in the case of **1**.

In conclusion, this paper demonstrates that the steric and conformational factors discriminate between **1** and **2** in their photophysical and DNA binding properties. Also, these studies indicate that unfavorable chain length and steric effects in **2** inhibit the electrostatic and minor groove binding abilities of the viologen moiety with the phosphate backbone of DNA. Since these molecules constitute an electron donor–acceptor system, we believe that these molecules and their derivatives can have potential applications in photoactivated DNA cleavage studies.

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4 **1**: mp 268–269 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.96 (3H, t, J = 2.89 Hz), 1.31–1.38 (2H, m), 1.90–1.95 (2H, m), 4.72 (2H, t, J = 2.80 Hz), 6.18 (2H, s), 7.57–7.65 (6H, m), 7.88–7.92 (4H, m), 8.25 (2H, d, J = 1.70 Hz), 8.83 (2H, d, J = 1.20 Hz), 8.88 (2H, d, J = 1.25 Hz), 9.43 (2H, d, J = 1.20 Hz), 9.69 (2H, d, J = 1.25 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 13.33, 18.77, 32.70, 60.64, 63.87, 124.23, 126.21, 126.38, 126.79, 127.26, 128.94, 129.36, 130.64, 131.09, 134.42, 136.21, 145.76, 146.00, 147.74, 148.36, 149.23; MS *m/z* 561, 563 (10) $[M+Br]^+$, 481 (100) $[M^+]$.

5 **2**: mp 224–225 °C; ^{1}H NMR (DMSO-d_6, 300 MHz) δ 0.95 (3H, t, J = 2.89 Hz), 1.31–1.39 (2H, m), 1.95–2.00 (2H, m), 4.73 (2H, t, J = 2.85 Hz), 5.56 (2H, s), 7.22 (2H, d, J = 1.70 Hz), 7.43 (2H, t, J = 3.0 Hz), 7.51 (2H, d, J = 1.40 Hz), 7.75–7.89 (4H, m), 8.20–8.23 (4H, m), 8.41 (2H, d, J = 1.25 Hz), 8.55 (2H, d, J = 1.22 Hz), 9.41 (2H, d, J = 1.26 Hz); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 13.35, 18.78, 32.68, 60,64, 62,02, 124.24, 125.34, 125.95, 126.49, 126.68, 127.08, 129.47, 129.98, 130.44, 130.55, 131.36, 131.90, 132.08, 135.64, 142.77, 145.27, 145.82, 147.80, 148.50; MS *m/z* 561, 563 (10) $[M+Br]^+$, 481 (100) $[M^+]$.

6 PC SPARTAN software from Wave function Inc.; 18401 Von Karman, Suite 370, Irvine, CA 92612.

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