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DNA-Binding and DNA-Photodamaging Properties of Indolo[2,3-*b*]-Quinolizinium Bromide

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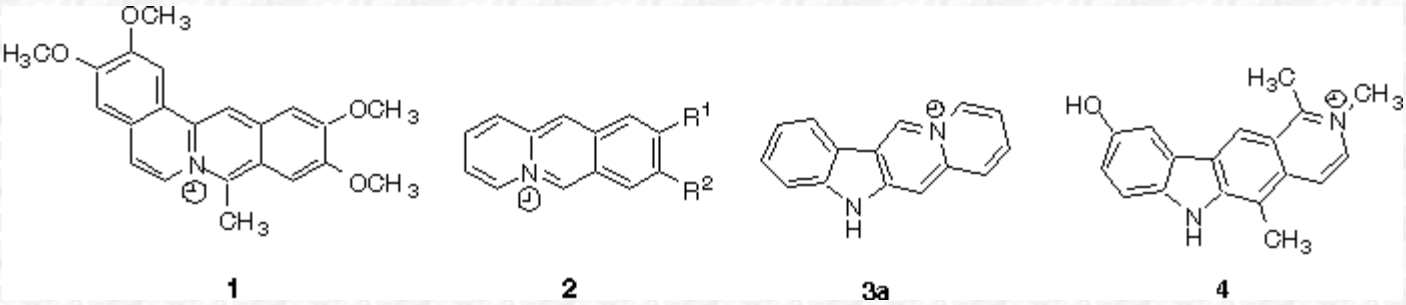
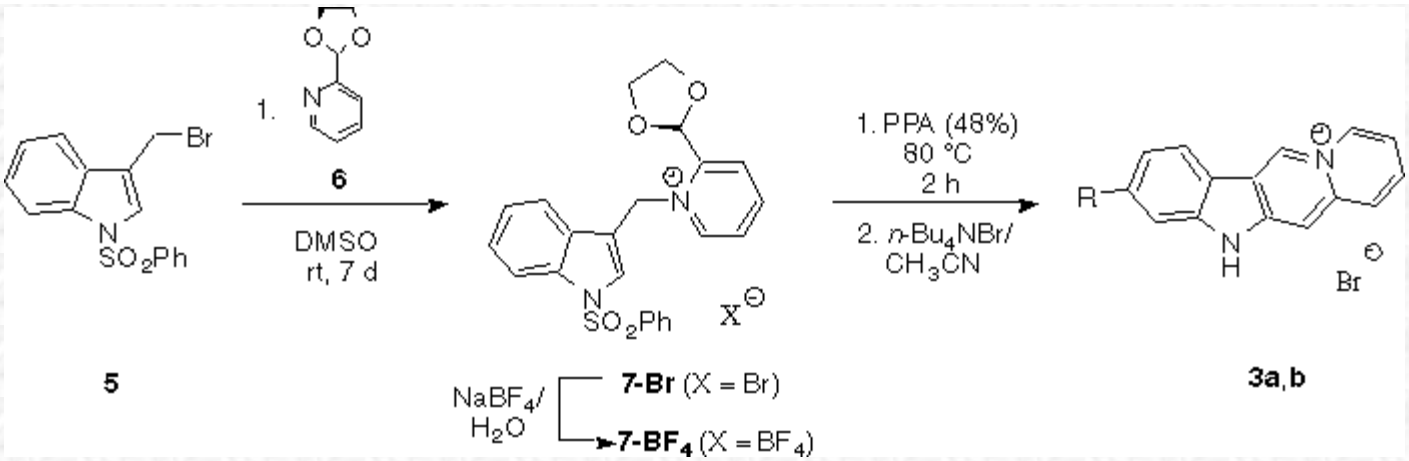
Cationic dyes have been shown to bind efficiently to DNA [1] and lead to photoinduced DNA damage [2]. Such photobiological features may be used to detect or characterize the nucleic acid [3]. Furthermore the intentional photo-induced damage of DNA, e. g. in tumor cells, may be applied in phototherapy [4,5]. Among the cationic aromatic compounds that have been investigated along these lines are coralyne (**1**) [6] and related quinolizinium salts [7]. During our studies on photobiological properties of aromatic heterocycles we showed that readily available benzo[*b*]-quinolizinium salts (acridizinium salts) **2** exhibit pronounced DNA-binding and DNA-photodamaging properties [8]. To further investigate the photobiological features of quinolizinium derivatives we synthesized indolo[2,3-*b*]-quinolizinium salt (**3a**) [9]. The interactions of this aromatic dye with DNA seemed to be of special interest since it resembles closely the structure of the known anti-tumor compound Céliptium® **4**. Herein, we present preliminary investigations of the interactions of quinolizinium dye **3a** with DNA.



1 **2** **3a** **4**

Indoloquinolizinium salt **3a** was synthesized from the pyridinium derivative **7**, which was obtained by quarternization of 2-(1,3-dioxolan-2-yl)pyridine (**6**) [10] with 3-bromomethylindole (**5**) [11]. Surprisingly, the cyclization of bromide salt **3-Br** with hydrobromic acid (48%) or polyphosphoric acid (PPA, 84%) led to the formation of bromo-substituted indoloquinolizinium salt **3b** in ow yield along with the parent compound **3a**. The latter could not be isolated from the reaction mixture. However, when the tetrafluoroborate salt **3-BF_4** was cylized with PPA, the quinolizinium salt **3a** was obtained in 41% yield by crystallization from methanol. Counterion exchange was achieved according to the procedure of Hünig *et al.* [12].



Scheme 1. Synthesis of quinolizinium salt **3a**

The structural assignment of the pyridinium salt **2** and the quinolizinium salts **3a** and **3b** is based on their 1D- and 2D-NMR and ESI-mass spectrometrical data and elemental analysis [13]. Both salts are yellow solids and exhibit an absorption maximum in methanol at l = 354 nm (**3a**: e = 11728 $M^{-1}cm^{-1}$) along with a broad absorption band with a zero onset at l = 420 nm. Moreover, a broad fluorescence band at l = 452 nm was observed in methanol. With an increasing donor number [14] of the solvent a slight shift of the emission maximum was detected (CH_3CN: 450 nm; DMF: 455 nm; DMSO: 456 nm). Thus, the quinolizinium salt **3a** exhibits ideal spectroscopic proerties which should enable the spectrometric observation of dye-DNA interactions in the visible region, where DNA does not absorb.

The addition of *calf thymus* DNA to a buffered solution of salt **3a** was monitored by absorption and emission spectroscopy (Figure 1). With an increasing amount of DNA concentration a decrease of the absorbance was observed. Moreover, the absorption maximum was red-shifted by 4 nm along with a broadening of the linewidth. Two isobestic points were observed which indicates that one quinolizinium-DNA complex is formed almost exclusively. The fluorescence intensity of quinolizinium salt **3a** is also significantly quenched by addition of DNA and the emission maximum exhibits a bathochromic shift of 4 nm. Such perturbations of the electronic spectra on DNA addition are usually observed for DNA-binding cationic dyes [1] and it may be concluded from these results that the novel quinolizinium salt **3a** also binds to DNA.

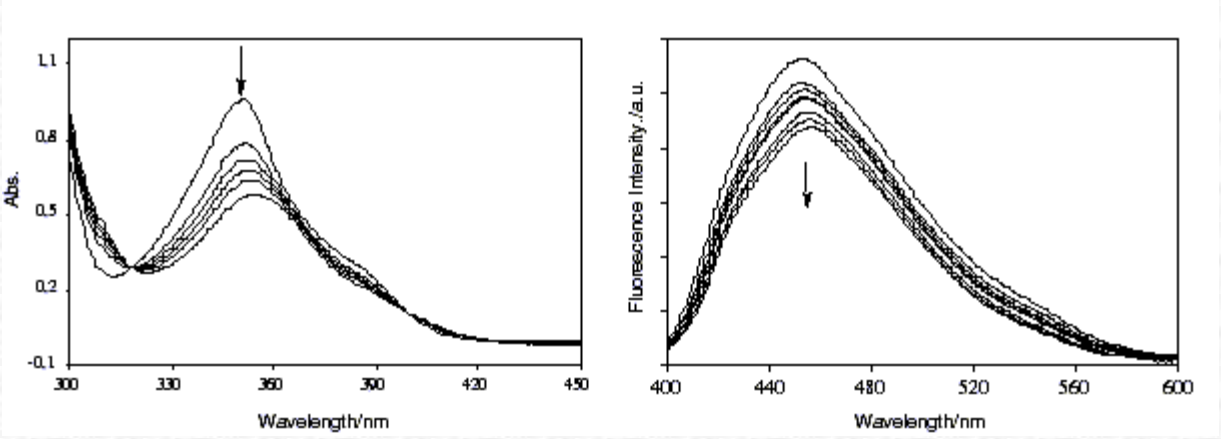
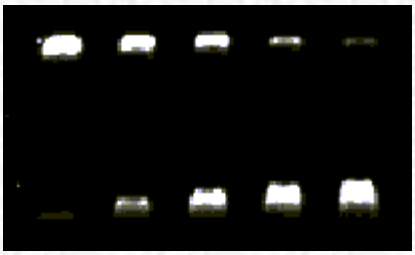


Figure 1. UV/VIS and fluorescence spectral changes on addition of *calf thymus* DNA to quinolizinium salt **3a** (in phosphate buffer; UV: c = 10^{-4} M; emission: c = 10^{-5} M, l_{ex} = 370 nm)

Since a binding interaction between the quinolizinium salt **3a** and DNA has been demonstrated, it was of special interest whether the complex formation may be used for photo-induced DNA damage, because efficient DNA photocleavage is required for potential phototherapeutic applications. Irradiation of supercoiled DNA (pBR 322) in the

presence of the quinolizinium salt **3a** at l = 350 nm under aerobic conditions yielded frank strand breaks (Figure 2) [15]. After 1 h, DNA damage of 80% was detected, showing that the quinolizinium salt **3a** has a high potentail as a photonuclease. After further irradiation (2 h), even linear DNA fragments were detected.



Lane	1[a]	2	3	4	5
Irradiation time [min]	0	15	30	45	60
Strand breaks[b] [%]	10	25	40	70	80

[a] Blank sample; [b] error: ± 10%

Figure 2. Photocleavage of plasmid DNA pBR322 with quinolizinium salt **3a** (gel-electrophoresis pattern: top line = supercoiled DNA; bottom line = open-cirular DNA)

In summary, we have shown that the novel indolo-annelated quinolizinium salt **3a** binds to DNA and has a high potential to cleave DNA on irradiation. Further investigations on the distinct binding mode and photocleavage mechanism are presently performed.

Acknowledgements

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[13] **7-Br**: mp. 116-117 °C; ^{1}H NMR (200 MHz, d_6-DMSO): 4.20 (s, 4 H), 6.04 (s, 2 H), 6.43 (s, 1 H), 7.29-8.96 (m, 14 H); ^{13}C NMR (50 MHz, d_6-DMSO): 154.4, 148.5, 146.9, 139.1, 136.8, 136.0, 131.0, 130.3, 130.0, 129.8, 128.4, 127.6, 127.2, 125.6, 120.5, 115.3, 115.1; elemental analysis calcd (%) for $C_{23}H_{21}BrN_2O_4S\bullet H_2O$ (519.4): C 53.19, H 4.46, N 5.39, S 6.17; found C 53.63, H 4.26, N 5.50 S 6.27. **7-BF$_1$**: mp. 168–170 °C (dec.); elemental analysis calcd (%) for $C_{23}H_{21}B_1F_4N_2O_4S_1$ (508.3): C 54.35, H 4.16, N 5.51, S 6.31; found C 54.13, H 4.32, N 5.55 S 6.40. **3a**: mp. 290–295 °C (dec.); ^{1}H NMR (200 MHz, CD_3OD): 7.26 (ddd, *J* = 8 Hz, *J* = 7 Hz, *J* = 1 Hz, 1 H), 7.42 (dd, *J* = 8 Hz, *J* = 1 Hz, 1 H), 7.48–7.58 (m, 2 H), 7.80 (ddd, *J* = 9 Hz, *J* = 7 Hz, *J* = 1 Hz, 1 H), 8.03 (s, 1 H), 8.11–8.18 (m, 2 H), 8.89 (d, *J* = 7 Hz, 1 H), 9.88 (s, 1 H); ^{13}C NMR (50 MHz, d_6-DMSO): 104.7, 113.3, 120.3, 120.5, 123.6, 123.6, 123.7, 127.1, 131.5, 132.2, 132.4, 135.6, 141.7, 144.6, 145.0; MS (ESI): 299 (100) [M^+].); elemental analysis calcd (%) for $C_{15}H_{11}Br_1N_2\bullet 2H_2O$ (335.2): C 53.75, H 4.51, N 8.33; found C 54.38, H 4.32, N 8.33. **3b**: mp. 332-333 °C; ^{1}H NMR (200 MHz, d_6-DMSO): 7.79 (dd, *J* = 7 Hz, *J* = 7 Hz, 1 H), 7.82 (d, *J* = 9 Hz, 1 H), 7.99 (dd, *J* = 9 Hz, *J* = 2 Hz, 1 H), 8.07 (dd, *J* = 7 Hz, *J* = 9 Hz, 1 H), 8.52 (d, *J* = 9 Hz, 1 H), 8.54 (s, 1 H), 8.77 (d, *J* = 2 Hz, 1 H), 9.12 (d, *J* = 7 Hz, 1 H), 10.21 (s, 1 H), 13. 04 (s, 1 H). ^{13}C NMR (50 MHz, d_6-DMSO): 104.2, 113.9, 114.6, 119.2, 121.3, 125.3, 125.9, 131.5, 132.2, 135.0, 140.3, 142.2, 143.7; MS (ESI): 299 (100) [M^+].

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[15] Irradiations were performed with a Rayonet photoreactor at l = 350 nm at 0 °C. Strand breaks were determined by agarose gel electrophoresis with ethidium bromide as indicator. Spots were detected by exposure to a UV transilluminator (366 nm) and recorded with a Herolab EASY 429K camera. For comparison, a blank sample of the plasmid was simultaneoulsy irradiated under the same conditions without dye; and after 1 h of photolysis the amount of supercoiled DNA did not change within the error limit.
