Experimental Section

All reagents were purchased from Sigma-Aldrich (USA). Solvents were HPLC grade. All column chromatography were done on silica gel 60 Å, 70-240 Mesh (Merk), unless otherwise indicated. UV-vis spectra were recorded on a CARY-3 spectrophotometer, ^{1}H- and ^{19}F-NMR spectra were run on a Bruker Avance™ 300 MHz. Intermediate and dye spectra were measured in DMSO-d_6 , other solvents are stated between parenthesis; the chemical shifts were measured in ppm downfield from TMS, used as an internal standard; for ^{19}F-NMR they were measured upfield from $CFCl_3$; all coupling constants were measured in Hz. ESI-MS spectra were taken in a Finnigan ESI/APCI Ion Trap Mass Spectrometer on positive mode.

Dye stock solutions. Dye stock solutions were prepared in DMSO. Solutions were stable for at least 2 month in the dark and at room temperature; their concentrations were determined in methanol and were almost unchanged over the time. The extinction coefficients were determined in methanol as TO: ε_{502} = 63,000 $M^{-1}cm^{-1}$; TO-1F: ε_{500} = 64,000 $M^{-1}cm^{-1}$; TOp2F: ε_{489} = 65,000 $M^{-1}cm^{-1}$; TO-4F: ε_{485} = 55,200 $M^{-1}cm^{-1}$; TO-CF_3: ε_{516} = 65,000 $M^{-1}cm^{-1}$.

DNA solutions. DNAs were purchased from GE-Biosciences (former Amersham Bioscience), calf thymus DNA (CT-DNA) was used as received, while $[Poly(dA\text{-}dT)]_2$ was dissolved in 10 mM sodium phosphate buffer (pH 7) and 100 mM sodium chloride; its concentration was determined in buffer at 263 nm using the manufacturer extinction coefficient (ε_{263} = 13,200 $M^{-1}cm^{-1}$).

Spectroscopy

Absorbance and fluorescence emission spectra. All of the absorbance spectra were performed in solutions of 10 mM sodium phosphate buffer (pH 7) containing 100 mM sodium chloride, with or without 100 μM CT-DNA and at room temperature. The dye aggregation studies were done by measuring the absorbance of individual dye solutions containing increasing dye

concentrations in buffer. The DNA binding studies were performed in buffer containing 100 μM CT-DNA and the dyes were added in 5 μM concentrations. Their absorbance and emission fluorescence spectra were recorded after 5 min incubation. The melting temperatures were measured in complexes of 10 μM dye and 20 μM $[Poly(dA\text{-}dT)]_2$, in a buffer as above but without sodium chloride. Samples were stabilized for 5 min. The denaturation of the complexes was monitored by the absorbance changes at 263 nm and the T_ms were calculated as the second derivative of the melting curves. Fluorescence emission spectra were recorded by adding 1 μM dyes in buffer with and without CT-DNA, and all samples were excited at a wavelength such as they had the same absorbance. The fluorescence quantum yields (Φ_f) were calculated by the ratio of the slope of a plot Fluorescence Integral vs. Absorbance, obtained from five solutions of increasing concentration of the sample dye in relation to the slope of a similar graph obtained for fluorescein ($\Phi_f = 1$); the quantum yield for fluorescein was previously calculated using lucifer yellow as a reference ($\Phi_f = 0.22$). All standard and sample solutions had a concentration such as their maximum absorbance never exceeded 0.1 absorbance units using a 1 cm path cuvette. The excitation slits were set at 1 nm (2 nm bandwidth) and all samples were excited at 450 nm; all fluorescence spectra were corrected for their absorbance at the excitation wavelength. The ratio dye/CT-DNA was 1:35 to ensure complete binding of the dyes although the minimum ratio was determined to be ~1:20 by DNA titration into 1 μM dye solution.

Dye samples for photobleaching. Two dye samples were prepared in 1 cm path quartz cuvettes, by dissolving 5 μM of the respective dye in a buffer as above to give a total volume of 1 ml. An absorbance spectrum was measured for each sample immediately after preparation of the solutions (t = 0 min). One of the samples was stirred while irradiated with full intensity visible light for a 1 min period in a dark room. An absorbance spectrum was taken after the irradiation. This procedure was repeated until 10 minutes total irradiation was completed. A control sample was treated in the same way, but the beam of the lamp was blocked and only spurious light

reached the control cuvette. Samples containing DNA were prepared by adding 5 μM dye to 100 μM CT-DNA in a buffer solution as above so as to get a 1 ml total volume. They were incubated for 5 min before starting the experiment. The dye samples were irradiated by a 150 watt Xe-lamp using an Oriel lamp housing (model 66002) equipped with a liquid filter (Oriel #6117) filled with deionized water to avoid overheating of the irradiated sample. A long path filter (Oriel, filter #59484, cut on 455 nm) was placed in front of the light beam to absorb the ultraviolet radiation.

Synthesis[1,2]

Synthesis of quaternized heterocycles. Reagents 2-methylbenzothiazole, 5-fluoro-2-methylbenzothiazole and 4-methylquinoline, were quaternized by treatment with excess of methyl iodide by standard procedures to yield salts 2,3-dimethylbenzothiazolium iodide (**1**), 2,3-dimethyl-5-fluorobenzothiazolium iodide (**2**)**,** and 1,4-dimethylquinolinium iodide (**7**), respectively.

Synthesis of fluorinated 3-methyl-2-(methylthio)-benzothiazolium salts. General procedure. A mixture of the corresponding fluorinated aniline (20 mmol), sodium hydride (40 mmol) and carbon sulfide (20 mmol) in DMF (20 ml) is heated at 115 ^{o}C for one hour under nitrogen atmosphere. The solvent is then distilled under vacuum and glacial acetic acid is carefully added to react completely the excess of sodium hydride. Distilled water is added to precipitate the thione, which can be filtered off or alternatively, it can be extracted with DCM. In the latter case the solvent is dried with $MgSO_4$, filtered and evaporated to yield the crude thione. The product obtained is usually good enough to be used in the next step. The thione (5 mmol) is converted into its 2-(methylthio)-benzothiazole derivative by reacting it with methyl iodide (10 mmol) and K_2CO_3 (1 mmol) in DMF (5 ml) at room temperature, the mixture was stirred for 18 hs. The DMF is vacuum distilled and the residue is added with distil water and extracted with DCM as above. Quaternization of the nitrogen was achieved by treatment of the fluorinated 2-

(methylthio)-benzothiazole derivative (2.7 mmol) with neat methyl *p*-toluenesulfonate (2.7 mmol) at 150 ^{o}C for 1 h to yield the ammonium salt in variable yields depending on the degree of fluorination of the heterocycle ring. The tetrafluorinated derivative required longer time (see below). The identity and purity of all of the intermediate compounds were analyzed by TLC, ^{1}H- and ^{19}F-NMR.

1-Methyl-4-(methylthio)-quinolinium iodide (3). 4-Chloroquinoline (2.2 g, 12.2 mmol) was treated with sodium ethanethiolate (5.4 g, 61 mmol) in DMF (35 ml) under nitrogen atmosphere and at reflux for 7 hs. After the mixture was cooled at room temperature, the solvent was vacuum distilled and the residue was treated with methyl iodide (5 ml) overnight. The mixture was added with DCM (100 ml) and an off white precipitate was filtered off. The solvent was evaporated and the residue was added with ethyl ether to precipitate 4-methylthioquinoline which was subsequently quaternized by treatment with methyl iodide overnight at room temperature. The product was washed with DCM to yield the pure product (350 mg, 9%). ^{1}H-NMR (MeOD) δ: 8.97 (1H, d, *J* = 6.7); 8.53 (1H, br dd, *J* = 9.0, 1.0); 8.39 (1H, br d, *J* = 9.0); 8.23 (1H, ddd, *J* = 9.0, 7.0, 1.3); 7.99 (1H, ddd, *J* = 9.0, 7.0, 1.0); 7.81 (1H, d, *J* = 6.7); 4.52 (3H, s, NC$\underline{H}_3$); 2.90 (3H, s, SC$\underline{H}_3$). ESI-MS (M+), *m/z* 190.13 (calc. for $C_{11}H_{12}NS$: 190.28).

1-Methyl-2-(methylthio)-7-trifluoromethylquinolinium tosylate (4). 7-Trifluoromethyl-4-quinolinethiol (1.0 g, 4.4 mmol) was reacted with neat methyl iodide (3 ml) at room temperature and under nitrogen atmosphere for 30 min. An orange solid appeared; the reaction was kept stirring overnight and the solid turned yellow. A TLC showed complete conversion of the starting material. The resulting material was treated with methyl *p*-toluenesulfonate (812.0 mg, 13.2 mmol) at 110 ^{o}C for 18 hours under nitrogen atmosphere. The mixture was purified by column chromatography on silica gel with DCM to elute the unreacted material and with mixtures of DCM-MeOH (2%; 4%; 10% and 20%) to finally elute the pure salt (420 mg, 23%). ^{1}H-NMR δ: 9.26 (1H, d, *J* = 6.3); 8.74 (1H, s); 8.64 (1H, d, *J* = 8.7); 8.27 (1H, d, *J* = 6.0); 8.00 (1H, d, *J* =

6.6); 7.45 (2H, d, *J* = 8.2); 7.08 (2H, d, *J* = 8.2); 4.54 (3H, s, NC<u>H</u>$_3$); 2.91 (3H, s, SC<u>H</u>$_3$); 2.27 (3H, s, ArC<u>H</u>$_3$). ESI-MS (M+), *m/z* 258.13 (calc. for $C_{12}H_{11}F_3NS$: 258.16).

4,7-Difluoro-3-methyl-2-(methylthio)-benzothiazolium tosylate (5). 2,3,6-Trifluoroaniline (1.0 g, 6.8 mmol) was reacted with sodium hydride (326.0 mg, 13.6 mmol) and carbon disulfide (516.0 mg, 6.8 mmol) in DMF (5 ml) for one hour at 110 ^{o}C. After working up the reaction as described in the general procedure, spectroscopically pure 4,7-difluoro-2(3H)-benzothiazolethione was obtained. The thione was treated with neat methyl iodide to give 4,7-difluoro-2-(methylthio)-benzothiazole. Quaternization was achieved by treatment of the tertiary amine (433.0 mg, 2.0 mmol) with methyl *p*-toluenesulfonate (372.5 mg, 2.0 mmol) at 155-160 ^{o}C for one hour. The reaction mixture was washed with ethyl ether and finally added with DCM; the precipitate was filtered and washed again with ethyl ether to give pure 4,7-difluoro-3-methyl-2-(methylthio)-benzothiazolium tosylate (250.0 mg, yield 31%). ^{1}H-NMR δ: 7.82 (1H, ddd, *J* = 12.0, 9.0, 4.2); 7.70 (1H, td, *J* = 9.0, 3.3); 7.44 (2H, d, *J* = 8.0); 7.08 (2H, d, *J* = 8.0); 4.19 (3H, s, NC<u>H</u>$_3$); 3.18 (3H, s, SC<u>H</u>$_3$); 2.27 (3H, s, ArC<u>H</u>$_3$). ESI-MS (M+), *m/z* 232.07 (calc. for $C_9H_8F_2NS_2$: 232.29).

3-Methyl-2-(methylthio)-4,5,6,7-tetrafluorobenzothiazolium tosylate (6). To 2,3,4,5,6-pentafluoroaniline (3.7 g, 20 mmol) in DMF (20 ml), sodium hydride (1.0 g, 40 mmol) and carbon disulfide (1.2 ml, 20 mmol) were added and stirred for 1 h at 110-120 ^{o}C under nitrogen atmosphere. The mixture was treated as described in the general procedure to yield the pure 4,5,6,7-tetrafluoro-2(3H)-benzothiazolethione (3.4 g, 71% yield). A portion of it (1.2 g, 5 mmol) was stirred with methyl iodide (0.62 ml, 10 mmol) and K_2CO_3 (100 mg) in DMF (5 ml) at room temperature and under nitrogen atmosphere for 16 hs. After the usual work up, a brown clumpy powder was obtained (1.3 g). It was purified by column chromatography on acidic alumina using hexane-EtOAc (1:1) as the solvent. The product was eluted in the first fractions and after slow evaporation of the solvent the pure 2-(methylthio)-4,5,6,7-tetrafluorobenzothiazole crystallized as pale yellow needles [^{1}H-NMR ($CDCl_3$) δ: 2.84 ppm, s, S-C<u>H</u>$_3$]. The tertiary amine (256.0 mg,

1.0 mmol) was quaternized by reacting it with methyl *p*-toluenesulfonate (182.0 mg, 1.0 mmol) at 140-150 ^{o}C overnight with stirring and under nitrogen atmosphere. The ammonium tosylated salt was obtained after the usual work up (119.0 mg, 27% yield). ^{1}H-NMR δ: 7.44 (2H, d, *J* = 8.0); 7.09 (2H, d, *J* = 8.0); 4.19 (3H, d, *J* = 2.1, NC$\underline{H}_3$); 3.18 (3H, s, SC$\underline{H}_3$); 2.27 (3H, s, ArC$\underline{H}_3$). ^{19}F-NMR (MeOD) δ: 138.8 (ddd, *J* = 23.0, 12.4, 2.2); 151.9 (br dd, *J* = 21.5, 12.4); 152.4 (td, *J* = 21.5, 2.2); 155.6 (td, *J* = 23.0, 1.6).

1-Methyl-4-[(3-methyl-2(3H)-benzothiazolinylidene)methyl]-quinolinium iodide or thiazole orange, TO (8). The condensation of 2,3-dimethylbenzothiazolium iodide **1** (25.0 mg, 0.085 mmol) and 1-methyl-4-methylthioquinolinium iodide **3** (25.0 mg, 0.085 mmol) was achieved in methanol (1 ml) containing 5 meq of triethylamine at room temperature. Dark red crystals appeared few minutes after mixing. The reaction mixture was kept overnight and the final product was filtered and washed with ethyl ether (33.0 mg, 91% yield). ^{1}H-NMR δ: 8.79 (1H, d, *J* = 8.1); 8.60 (1H, d, *J* = 7.2); 8.08-7.97 (3H, m); 7.81-7.73 (2H, m); 7.60 (1H, td, *J* = 7.1, 1.2); 7.42 (1H, dd, *J* = 7.7, 1.0); 7.36 (1H, d, *J* = 7.1); 6.93 (1H, s, Hα); 4.17 (3H, s, N-C$\underline{H}_3$); 4.00 (3H, s, N-C$\underline{H}_3$); ESI-MS (M+), *m/z* 305.13 (calc. for $C_{19}H_{17}N_2S$: 305.42).

1-Methyl-4-[(5-fluoro-3-methyl-2(3H)-benzothiazolinylidene)methyl]-quinolinium iodide, TO-1F (9). The mixture of the iodide salts of 5-fluoro-2,3-dimethylbenzothiazolium **2** (104.5 mg, 0.34 mmol) and **3** (104.6 mg, 0.34 mmol) was treated with triethylamine in methanol (1 ml) overnight. After the work up as above, spectroscopically pure dye was obtained (108.5 mg, 24% yield). ^{1}H-NMR δ: 8.80 (1H, d, *J* = 8.3); 8.67 (1H, d, *J* = 7.1); 8.12-7.99 (3H, m); 7.79 (1H, ddd, *J* = 8.0, 6,7, 1.4); 7.75 (1H, dd, *J* = 10.1, 2.4); 7.37 (1H, d, *J* = 7.1); 7.26 (1H, td, *J* = 8.9, 2.3); 6.92 (1H, s, Hα); 4.19 (3H, s, N-C$\underline{H}_3$); 3.96 (3H, s, N-C$\underline{H}_3$); ESI-MS (M+), *m/z* 323.13 (calc. for $C_{19}H_{16}FN_2S$: 323.41).

1-Methyl-4-[(4,7-difluoro-3-methyl-2(3H)-benzothiazolinylidene)methyl]-quinolinium iodide, TO-p2F (10). 4,7-Difluoro-3-methyl-2-(methylthio)-benzothiazolium tosylate **5** (100.0

mg, 0.5 mmol) was reacted with 1,4-dimethylquinolinium iodide **7** (130.8 mg, 0.5 mmol) in pyridine (4 ml) overnight at room temperature. Crystal separated from the reaction mixture and they were purified by column chromatography using DCM-MeOH (95:5) as eluent. The dye (45.0 mg, 20% yield) showed to be pure by spectroscopic means. ^{1}H-NMR δ: 8.84 (1H, d, *J* = 8.2); 8.74 (1H, d, *J* = 7.1); 8.17 (1H, d, *J* = 8.7); 8.08 (1H, t, *J* = 8.5); 7.85 (1H, t, *J* = 7.4); 7.58 (1H, d, *J* = 7.0); 7.50 (1H, ddd, *J* = 12.5, 9.1, 4.6); 7.28 (1H, dt, *J* = 8.7, 2.7); 6.94 (1H, s, Hα); 4.28 (3H, s, N-C$\underline{H}_3$); 4.07 (3H, s, N-C$\underline{H}_3$); ESI-MS (M+), *m/z* 341.13 (calc. for $C_{19}H_{15}F_2N_2S$: 341.40).

1-Methyl-4-[(4,5,6,7-tetrafluoro-3-methyl-2(3H)-benzothiazolinylidene)methyl]-quinolinium iodide, TO-4F (11). The dye was synthesized by condensing 3-methyl-2-(methylthio)-4,5,6,7-tetrafluorobenzothiazolium tosylate **6** (48.9 mg, 0.11 mmol) and **7** (34.8 mg, 0.12 mmol) in pyridine (1 ml) at room temperature and reacting overnight. Pyridine was washed out by adding ethyl ether to the reaction mixture and decanting several times. The final gummy product was purified by column chromatography using DCM-EtOAc mixtures of increasing polarity to obtain pure **TO-4F** (5.4 mg, 9% yield). ^{1}H-NMR δ: 8.85 (1H, d, *J* = 8.1); 8.81 (1H, d, *J* = 6.9); 8.21 (1H, br d, *J* = 8.9); 8.10 (1H, td, *J* = 7.5, 1.1); 7.87 (1H, td, *J* = 6.9, 0.9); 7.62 (1H, d, *J* = 6.9); 6.97 (1H, s, Hα); 4.31 (3H, s, N-C$\underline{H}_3$); 4.04 (3H, s, N-C$\underline{H}_3$); ESI-MS (M+), *m/z* 377.13 (calc. for $C_{19}H_{13}F_4N_2S$: 377.38). ^{19}F-NMR δ: 141.6 (dd, *J* = 23.3, 11.2); 155.9 (t, *J* = 20.7); 156.6 (dd, *J* = 20.7, 11.2); 162.6 (t, *J* = 22).

1-Methyl-7-trifluoromethyl-4-[(3-methyl-2(3H)-benzothiazolinylidene)methyl]-quinolinium iodide, TO-CF_3 (12). To a portion of 1-methyl-2-(methylthio)-7-trifluoromethylquinolinium tosylate **4** (26.0 mg, 0.06 mmol) dissolved in pyridine, salt **1** (17.8, 0.06mmol) was added and reacted at room temperature overnight. The mixture was worked up as for compound **11** and purified by column chromatography with DCM and DCM-MeOH (95:5) as eluents. Pure dye was obtained (9 mg, 30% yield). ^{1}H-NMR δ: 9.00 (1H, d, *J* = 8.9); 8.59 (1H, d, *J* = 7.3); 8.29 (1H, s); 8.10 (1H, d, *J* = 8.0); 7.99 (1H, dd, *J* = 9.1, 1.3); 7.85 (1H, d, *J* = 8.1); 7.65

(1H, t, *J* = 7.8); 7.47 (1H, t, *J* = 8.0); 7.37 (1H, d, *J* = 7.3); 7.00 (1H, s, Hα); 4.19 (3H, s, N-C$\underline{H}_3$); 4.07 (3H, s, N-C$\underline{H}_3$); ESI-MS (M+), *m/z* 373.20 (calc. for $C_{20}H_{16}F_3N_2S$: 373.42).

References

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