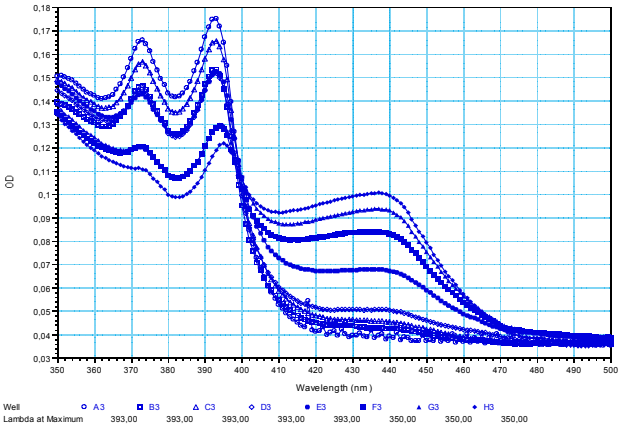
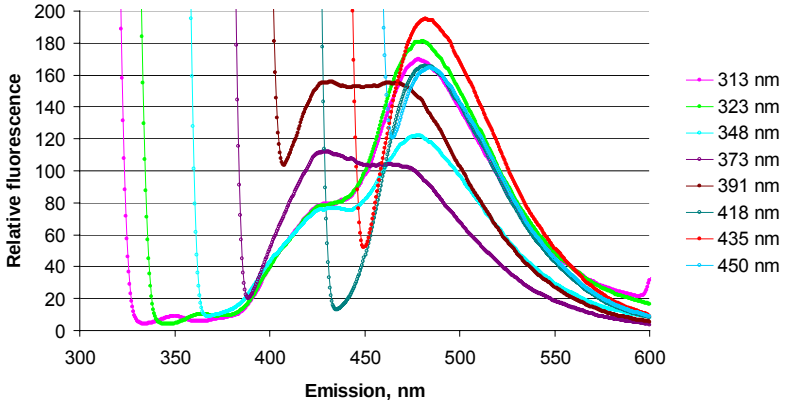
Supplementary Material

A. **Titration of BENA435 with plasmid DNA reveals an isosbestic point at 398 nm.**

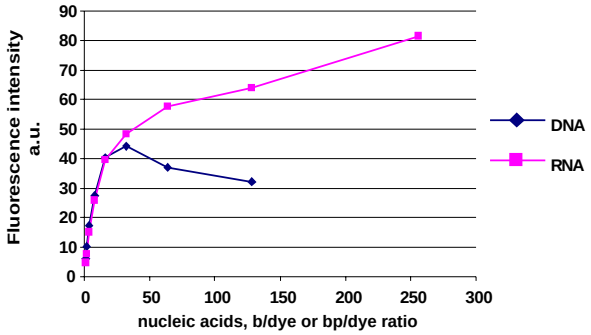


B. **Emission spectra of BENA435/DNA excited at different wavelengths**



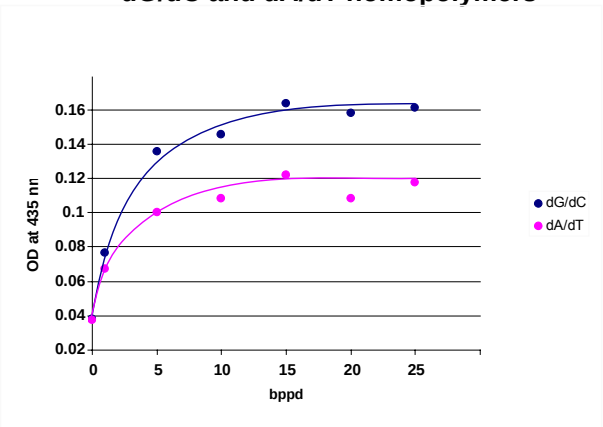
C.

Titration of 5 μM BENA435 with DNA and RNA
Emission at 484 nm



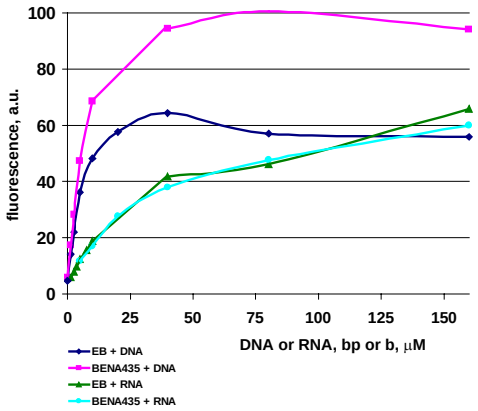
D.

Absorption of BENA435 at 435 nm when titrated with dG/dC and dA/dT homopolymers

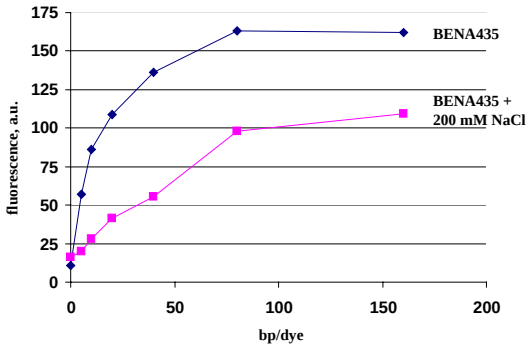


E.

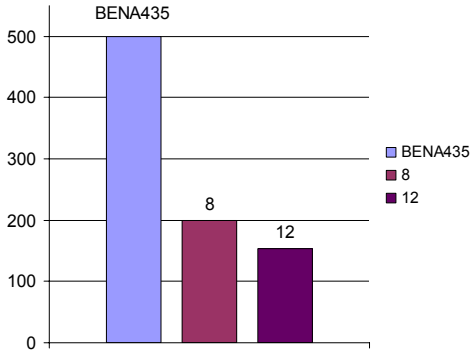
Titration of BENA435 and ethidium bromide with DNA or RNA



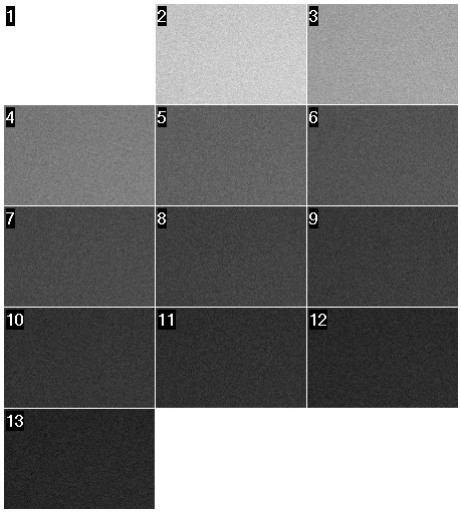
F. **Effect of salt on BENA435/DNA fluorescence**



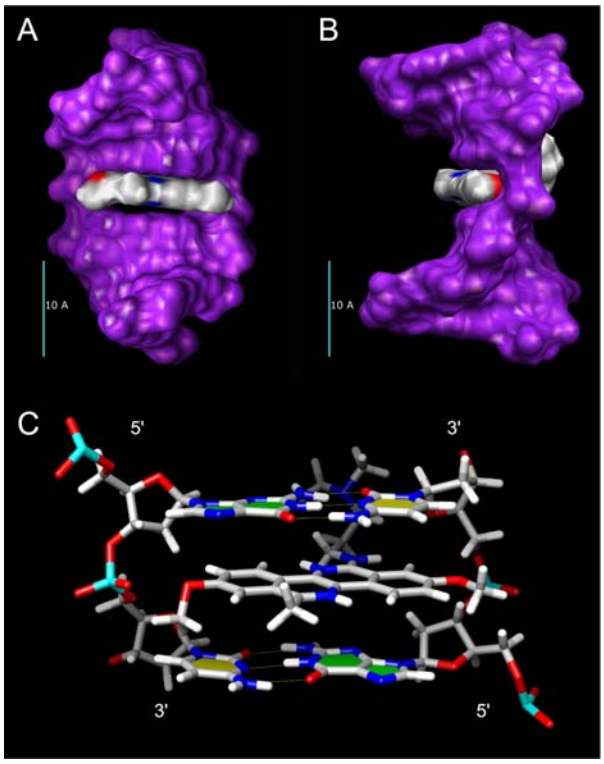
G. **Comparative fluorescence intensities at 484 nm of BENA435 and compounds 8 and 12**



H . **BENA453 does not show photoactivation when complexed with pure plasmid DNA**



I. **Model of BENA435 intercalation between two GC base pairs.**



Figures legends.

A. BENA435 at 25 μM was incubated with plasmid DNA at bp/dye ratio from 0 to 3.3 and absorption spectra were taken from 350 to 500 nm. Overlay of spectra gave a single isosbestic point at 398 nm.

B. Emission spectra of BENA435 (mixed with excess of plasmid DNA) when illuminated at different wavelengths from 300 to 450 nm. A major peak of fluorescence was observed at wavelengths 481-484 nm. At shorter excitation wavelengths (323-391), another shoulder is visible at ~ 426 nm. Numbers correspond to the excitation wavelengths.

C. Emission values of 5 μM BENA435 mixed with dsDNA or tRNA at different b(p)/dye ratios. RNA is unable to saturate BENA435 even at 256 b/dye ratio.

D. Absorption of BENA435 at 435 nm when titrated with dG/dC or dA/dT homopolymers. Lines were drawn through the points to guide the eye and do not represent a fitting to any equation.

E. Ethidium bromide (EB) or BENA435 at the same concentration were titrated with increasing amounts of ds plasmid DNA or RNA. Excitation was: for BENA435 at 435, for EB at 530 nm. Emission values were measured: for BENA435 at 484 nm, for EB at 600 nm.

F. BENA435 at 1 μM was titrated by plasmid DNA in the absence or presence of 200 mM NaCl.

G. Fluorescence of BENA435 and some of its analogues listed in the Table 1. BENA435 at 25 μM was mixed with plasmid DNA at 250 μM (bp/dye ratio 10). Only for BENA435 and compounds **8** and **12** the peak of fluorescence in complex with DNA is well separated from the peak of free dye.

H. Microscope fields showing BENA435/DNA in solution illuminated using an Alexa488/FITC filter. BENA435 at 5μM in 50 mM Na-phosphate buffer (pH 7.2) was mixed with DNA (320 μM; 64x fold molar excess of DNA over BENA435). BENA435/DNA solution contained between a glass slide and a coverslip was illuminated as described for Fig. 5B. Numbers correspond to frames. Time interval between frames is 10s.

I. Model of BENA435 intercalation between two GC base pairs. For docking of BENA435 into ds DNA we used the 3-D molecular coordinates of a DNA octamer 5'-D(GAAGCTTC)-3' crystallized with Actinomycin D (protein data bank PDB ID – 316D) (1). Prior to docking of BENA435, Actinomycin D was removed from the complex with DNA and hydrogens were added to both BENA435 and DNA. Model shows a conformation with a lowest energy (-4.47 kcal/mol). **A**. View from the major groove side. **B**. Side view (major groove on the left hand side). **C**. Stacking of BENA435 between two dG/dC pairs of bases.

References:

1. Takusagawa, F., Takusagawa, K.T., Carlson, R.G. and Weaver, R.F. (1997) Selectivity of F8-actinomycin D for RNA:DNA hybrids and its anti-leukemia activity.Bioorg Med Chem, 5, 1197-1207.