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Development of novel cell-permeable DNA sensitive dyes using combinatorial synthesis and cell-based screening†

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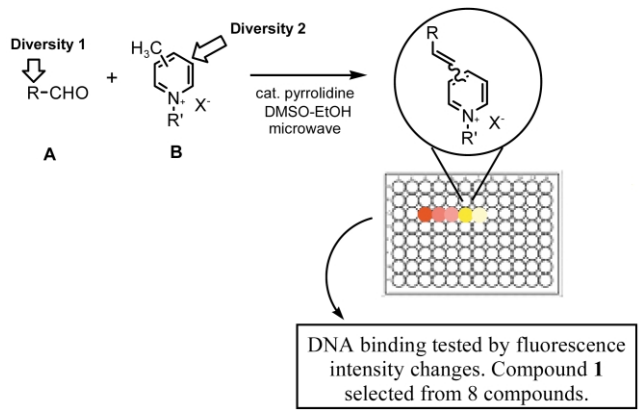
A novel cell-permeable DNA fluorescence sensor was developed based on combinatorially-created styryl dyes and cell-based localization screening.

DNA-sensitive fluorescent probes have been widely used for cell imaging[1] and DNA sequencing on gels.[2] As most of the commonly used dyes, such as ethidium bromide[3] and Sytox Green,[4] are not cell permeable, these cell imaging processes require damage to the membrane or separation of the DNA from the cell in order to stain the nucleic acids. Only a couple of cell permeable dyes, such as Hoechst 33258[5] and DAPI,[6] are available, and they permeate the membrane and localize in the nuclei of living cells.[7] The development of novel DNA sensitive dyes with higher selectivity and sensitivity has been of great interest.

In our previous research, we developed a unique combinatorial approach to the synthesis of styryl dyes (574 compounds) and their subcellular localization including nuclear, mitochondrial, cytosolic, vesicular, granular and reticular structures.[8] The nuclear staining of the dyes may have two different mechanisms: (1) binding to DNA or other nucleic targets with high affinity, and/or (2) increasing their fluorescence intensity upon binding to DNA. We envisioned that the latter case would provide novel DNA sensors. Herein, we present the discovery of novel DNA sensitive styryl dyes by extended combinatorial synthesis, cell-based screening and the fluorescence property measurement upon binding to DNA.

An extended styryl dye library, composed of 855 compounds, was synthesized (for details see ESI†) and screened for the subcellular localization in live UACC-62 human melanoma cells on glass bottom 96-well plates by the previously reported procedure (Scheme 1).[8,9] Only 8 out of 855 compounds showed strong nuclear localization (Fig. 1). The compounds were resynthesized on large scale for further study.

The synthesis of compounds **B** was achieved by refluxing with the pyridine derivatives and iodomethane for 2 h. Compound **B** crystallized out in ethyl acetate. The condensation with aldehydes (**A**) and compound **B** was performed by refluxing with piperidine for 2 h in EtOH. After cooling to room temperature, the crystallized compounds were filtered and washed with ethyl acetate. With these purified compounds, we tested the fluorescence intensity change upon addition of DNA. Out of 8 nuclear localizing compounds, only compound **1** showed a strong fluorescence increase.



Scheme 1 General procedure of the synthesis and screening of the styryl dyes in 96-well format.

Compound **1** is an orange solid that exhibits an excitation wavelength of λ = 413 nm and an emission wavelength of λ = 583 nm (Table 1). A linear fluorescence response was observed in the 0.05–100 μM range (in PBS: phosphate-buffered saline) without self-quenching or shifts in emission or excitation wavelengths. With a series of concentrations of dsDNA (double stranded DNA) added to compound **1**, a linear increase in the fluorescence intensities was observed (Fig. 2). At the highest

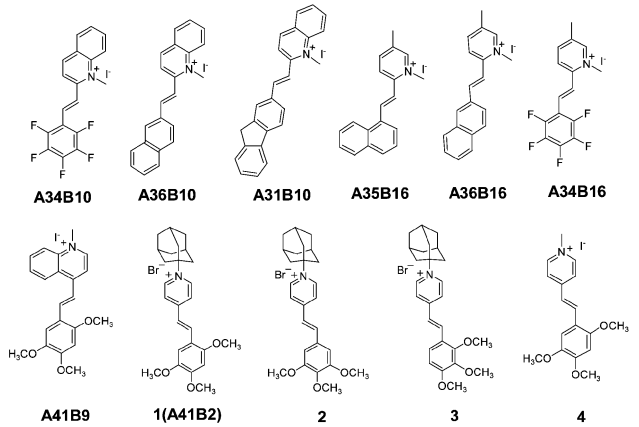


Fig. 1 Eight nuclear localizing compounds and three derivatives of **1**.

Table 1 Spectrophotometric properties of the styryl dyes

Dye	λ_{max}/ nm	λ_{em}^{free}/ nm	λ_{em}^{DNA}/ nm	ϕ_F^{free}	ϕ_F^{DNA}	$\phi_F^{DNA}/\phi_F^{free}$
1	413	583	566	0.00024	0.0032	13.3
2	366	553	520	0.0051	0.022	4.3
3	370	491	502	0.0024	0.0037	1.5

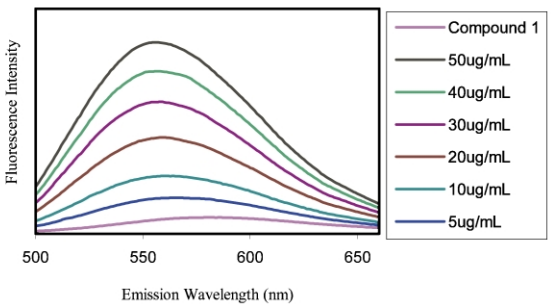


Fig. 2 Fluorometric titration of compound **1** with dsDNA in a buffer solution (λ_{ex} = 394 nm, compound **1** (5 μM)).

† Electronic supplementary information (ESI) available: experimental section. See http://www.rsc.org/suppdata/cc/b3/b303960a/

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concentration of DNA tested (50 μg mL^{-1}), the fluorescence emission reached up to 13.3 times higher than that of the free compound (Fig. 3). A blue shift of 17 nm in the emission wavelength upon DNA addition was observed, without a significant excitation wavelength shift. The structure of compound **1** includes a 2,4,5-trimethoxy group from the benzaldehyde moiety and a unique adamantyl pyridinium functionality.

Different trimethoxy isomers, **2** (3,4,5-trimethoxy) and **3** (2,3,4-trimethoxy), were synthesized to compare the positional effects of the methoxy groups in compound **1** (Fig. 1). While the responses of compounds **2** and **3** to DNA treatment were similar to that of compound **1**, the fluorescence emission increase was much smaller in **2** (4.3 fold) and **3** (1.5 fold). It is noteworthy that the intrinsic fluorescence intensities of compounds **2** and **3** are higher than that of compound **1**, but DNA treated samples showed comparable quantum yields (Table 1). Compound **4** was also resynthesized and tested to study the structural importance of the adamantyl group in compound **1**.

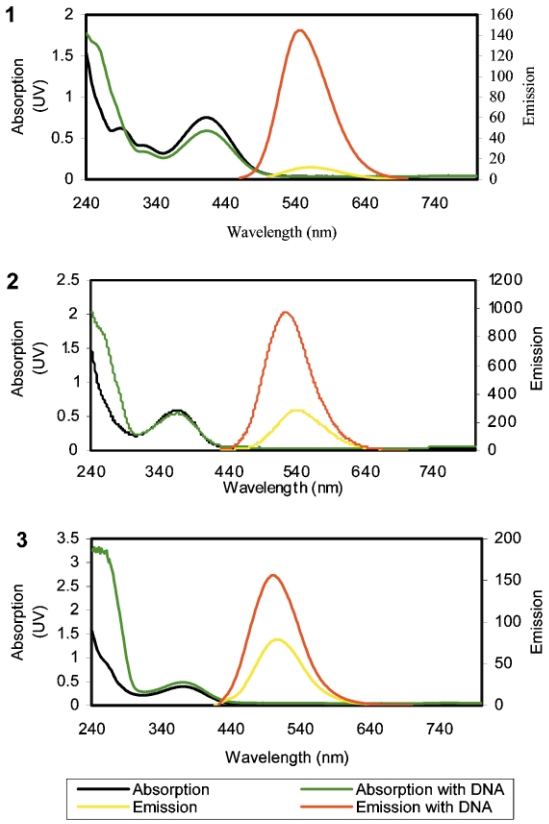

Fig. 3 Absorption and fluorescence spectra of compounds **1–3**. Dye **1, 2, 3** (50 μM), dsDNA (50 μg mL^{-1}).

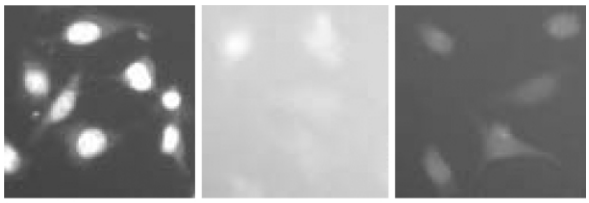

Fig. 4 Nuclear staining of compounds **1**, **2**, and **3** (500 μM).

Interestingly, the simple exchange of the adamantyl with a methyl group significantly reduced the DNA response in compound **4**. Therefore, both 2,4,5-trimethoxy groups and the adamantyl group are important in the specific interaction of compound **1** and DNA.

The three related compounds **1**, **2**, and **3** were incubated in live UACC-62 human melanoma cells to compare their nuclear localization properties (Fig. 4). In comparison to compound **1** in the same concentration, compounds **2** and **3** showed stronger fluorescence backgrounds and spread throughout the cytoplasm. However, compound **1** clearly stains the nucleus of live cells more selectively.

In summary, a combined combinatorial synthesis of styryl dyes and cell-based screening provided a novel structural motif for a DNA sensor, compound **1**. Compound **1** is one of the rare cell permeable nuclear staining dyes and would be useful for live cell imaging purposes. The general strategy developed in this study will be applied to novel organelle specific sensor development.

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