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Synthesis and spectroscopic properties of furyl-phenyl-acrylates and naphthofurans and their interaction with ct-*DNA*

Kristina Starčević[1], Marijana Hranjec[1], Dejana Carić[2], Grace Karminski-Zamola[1]

[1] Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Zagreb, Croatia
[2] Department of Chemistry, Faculty of Agronomy, University of Zagreb, Zagreb, Croatia

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Abstract A series of novel substituted derivatives related to furyl-phenyl-acrylates and naphthofurans, was synthesized and characterized by UV/Vis and fluorescence spectroscopy. Acyclic compounds can undergo photochemical dehydrocyclization by visible light irradiation in order to obtain their cyclic derivatives. The interactions of the prepared compounds with calf thymus *DNA* was investigated by means of electronic absorption and fluorescence spectra. It is intriguing that addition of ct-*DNA* induced a fluorescence increase of acyclic derivatives, exactly the opposite of the strong fluorescence quenching observed for cyclic derivatives **10** and **12**. Compound **11** showed decreasing fluorescence intensity for lower concentrations of ct-*DNA*, while increasing of fluorescence is observed for high excess of added ct-*DNA*.

Keywords Heterocycles; Photochemistry; UV/Vis spectroscopy; Fluorescence spectroscopy; *DNA*.

Introduction

Over the past decades, there has been great interest on the design of small molecules binding to *DNA* [1]. Generally, the interactions of small molecules with *DNA* involve three binding modes: namely intercalative binding where molecules intercalate into the base pairs of the nucleic acid, groove binding in which the molecules bound on *DNA* are located in the major or minor groove, and electrostatic binding, which takes place along the external *DNA* double helix and do not possess selectivity [2]. Understanding the modes of the binding of small molecules to *DNA* and the factors that can affect the binding is of fundamental importance in understanding drug-*DNA* interactions and in development of new efficient drugs targeted *DNA*, such as antitumor agents [3].

The constant and growing interest in the development of new efficient and general synthesis methods for the preparation of fused heterocyclic systems involving furan and thiophene subunits is justified by their well-established valuable physiological and pharmacological properties [4]; however, there is little new literature data describing the synthesis and biological activity of these systems [5, 6]. Recent technical applications of polysubstituted benzofurans and benzothiophenes, including numerous fluorescent dyes used as retrograde tracers, and Ca^{2+} and Mg^{2+} fluorescent indicator conjugates, *etc*. increase their significance. The biological activity of many arenofurane derivatives has been improved by addition of positively charged aliphatic substituents. For example, amidino-substituted heterocycles based on a furan moiety have shown a number of intriguing

Correspondence: Grace Karminski-Zamola, Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 20, HR-10000 Zagreb, Croatia. E-mail: gzamola@fkit.hr

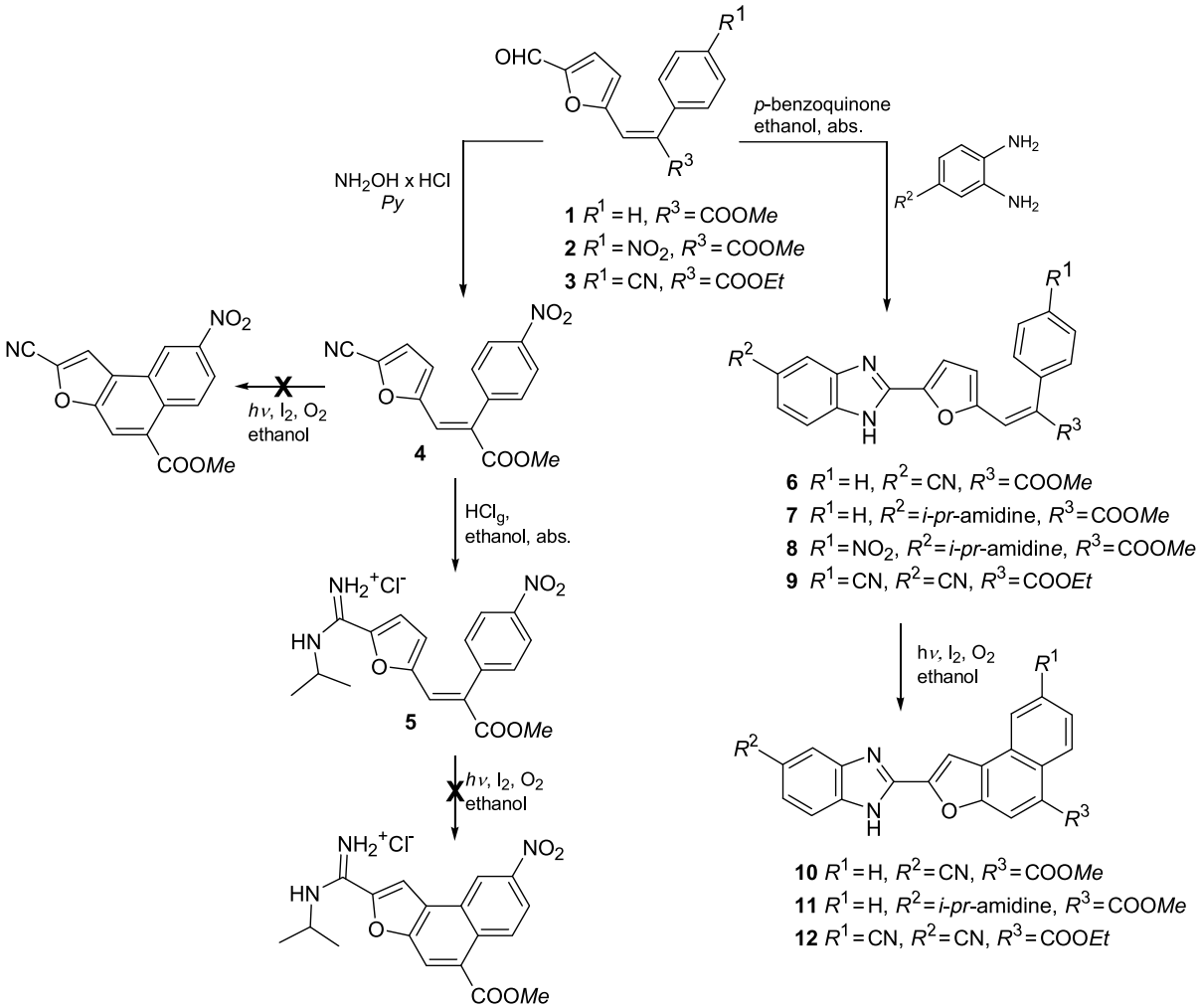
modes of biological activity, like antimicrobial [7], hallucinogenic agents [8], and some furamidines were found to be active against diverse, highly infectious parasites [9]; the best being selected for currently undergoing phase II clinical trials [10].

Present work is a continuation of researches aimed on the search of novel acyclic and cyclic derivatives of methyl (*E*)-3-(2-furyl)-2-phenyl-acrylate with interesting spectral-luminescent properties for biomedical application.

Results and discussion

Chemistry

All compounds **2**–**12** shown in Fig. 1 were prepared according to the Scheme 1. Compound **4** was prepared from aldehyde **2** in the reaction with hydroxylamonium hydrochloride in a yield of 90% [17]. The novel *N*-isopropylamidino-substituted acyclic compound **5** was prepared by a *Pinner* reaction [18] from cyano derivative **4** and isopropylamine in the second phase of the reaction. Acyclic cyano and amidino **6**–**9** compounds were prepared by condensation of corresponding heterocyclic aldehyde and appropriate 4-substituted-1,2-phenylendiamines in a yield of about 35–76% [16]. Compounds **7** and **11** have been prepared earlier [16] but their spectroscopic properties and interaction with *DNA* have not been examined. Attempts to isolate amidino derivatives of **6** and **9** by the *Pinner* procedure were not successful because they polymerized during the reaction and the isolation procedure owing to their instability and sensitivity to air moisture. In the reaction of photo-



Scheme 1

chemical dehydrocyclization (20–70 h) acyclic compounds **6**, **7**, and **9** were converted into cyclic products **10**, **11**, and **12**. Methyl (*E*)-3-(5-*N*-isopropylamidino-2-furyl)-2-(4-nitrophenyl)-acrylate hydrochloride (**5**) and methyl (*E*)-3-[5-(5-*N*-isopropylamidino-2-benzimidazolyl)-2-furyl]-2-(4-nitrophenyl)-acrylate hydrochloride **8** did not give desired cyclic compounds by UV irradiation under the oxidative conditions. The structures of new compounds were confirmed by elemental analysis, UV/Vis, MS, ^{1}H, and ^{13}C NMR spectra.

In our previous paper [19] we have described experiments that present the conceptual basis for development of new photoinduced anticancer therapy based on the photochemical transformation on a *DNA/RNA* inactive into an active one. Additional advantage of this concept lies in the fact the photoinduced dehydrocyclization of the 1,2-arenyl ethenes is the widely studied reaction offering thus the access to a large number of already known or new molecules with selected properties compatible with physiologically relevant conditions. In order to obtain new systems capable to undergo the photo-induced dehydrocyclization by visible light irradiation, we prepared some derivatives of methyl (*E*)-3-(2-furyl)-2-phenyl-acrylate.

*Spectroscopic characterization of **4–12** dissolved in aqueous buffer*

Since application of spectrophotometric methods in the *DNA* interaction studies was necessary, we characterized aqueous solutions of compounds by means of electronic absorption (UV/Vis) and fluorescence emission spectra. The obtained results are shown in Table 1.

Table 1 Electronic absorption and emission data of **4–12**

Compound	$\lambda_{abs.max}$/ nm	$\varepsilon \times 10^3$/ $dm^3\,mol^{-1}\,cm^{-1}$	λ_{em}/ nm	λ_{exc}/ nm	ϕ
4	309	27.2	–	–	–
5	324	24.6	–	–	–
6	366	25.4	427	365	–
7	365	33.6	425	363	–
8	366	33.9	432	367	–
9	370	26.9	424	370	–
10	353	6.1	416	354	0.043
11	360	42.2	419	359	0.452
	260	28.0			
12	365	4.0	429	363	0.064

The UV/Vis spectra of all studied compounds did not show any *pH* dependent changes in the physiologically relevant range ($pH = 3.5$–8.0). Therefore, we performed our studies at $pH = 7.0$. Absorbancies of aqueous solutions of compounds are proportional to their concentrations up to 3.69×10^{-5} mol dm^{-3} (**4**), 4.07×10^{-5} mol dm^{-3} (**5**), 4.3×10^{-5} mol dm^{-3} (**6**), 1.27×10^{-5} mol dm^{-3} (**7**), 3.7×10^{-5} mol dm^{-3} (**8**), 3.3×10^{-5} mol dm^{-3} (**9**), 2.72×10^{-5} mol dm^{-3} (**10**), 5.42×10^{-5} mol dm^{-3} (**11**), and 3.1×10^{-5} mol dm^{-3} (**12**) indicating that there is no significant intermolecular stacking which should give rise to hypochromicity effects.

All compounds showed one absorption band in the region of 309–370 nm except **11** which showed two absorption bands at 360 and 260 nm. Conversion of cyano-substituted **4** into the amidino-substituted **5** resulted in bathochromic shifts ($\Delta\lambda = 15$ nm) and decreased molar extinction coefficient values. Cyano- and amidino-substituted benzimidazole nuclei attached directly on C-5 position of methyl (*E*)-3-(2-furyl)-2-(4-nitro)-phenyl-acrylate (**6** and **7**) induced a bathochromic shift ($\Delta\lambda = 42$ nm) and increased the ε value of a compound **7**. Incorporation of a nitro substituent on the phenyl ring in **8** did not have any influence on the shift of absorption maxima or molar extinction coefficient value. Dicyano substituted **9** showed a bathochromic shift ($\Delta\lambda = 4$ nm) and decrease of the molar extinction coefficient. Photochemical cyclization of **6**, **7**, and **9** into **10**, **11**, and **12** resulted in hypsochromic shifts of the absorption maxima.

For compounds **6–12** fluorescence was excited on the wavelength of absorption maxima. All studied compounds exhibit characteristic fluorescence emission with maxima at about 416–432 nm and showed one emission band. Cyclic compounds **10** and **11** showed a hypsochromic shift of the emission maxima, while **12** showed a bathochromic shift. Their excitation spectra, except **4** and **5**, being in good agreement with the absorption spectra. Fluorescence intensities were found to be linearly concentration dependent up to 5×10^{-6} mol dm^{-3}.

Under the same conditions cyclic compounds **10**, **11**, and **12** exhibit stronger emission (ϕ (**10**) = 0.043, ϕ (**11**) = 0.452, ϕ (**12**) = 0.064) than their acyclic analogues **6**, **7**, and **9** (ϕ (**6**), ϕ (**7**), and ϕ (**9**) <0.001).

*Interactions of compounds **4–12** with double-stranded ct-DNA*

Interaction of all compounds with ct-*DNA* was studied by UV/Vis and fluorescence spectroscopic titrations. Addition of different concentrations of ct-DNA to **4** (1.71×10^{-5} mol dm^{-3}), **5** (1.89×10^{-5} mol dm^{-3}), **6** (1.71×10^{-5} mol dm^{-3}), **7** (2.04×10^{-5} mol dm^{-3}), **8** (1.47×10^{-5} mol dm^{-3}), **9** (1.71×10^{-5} mol dm^{-3}),

Table 2 Electronic absorption data changes of compounds **6–11** upon the addition of ct-*DNA* at different ratio *r*((compound)/(polynucleotide)) at $pH = 7.0$ (Buffer Na-cacodylate, $I = 0.05$ mol dm^{-3})

	r[a]	λ_{abs}/nm	$\Delta\lambda_{abs}$/nm	H[b]/%	$\Delta\lambda$/nm		r[a]	λ_{abs}/nm	$\Delta\lambda_{abs}$/nm	H[b]/%	$\Delta\lambda$/nm
6	0	0.457	–	–	–	**7**	0	0.690	–	–	–
	1	0.354	0.103	22.5	–		1	0.638	0.052	7.5	2
	0.25	0.279	0.178	39.0	–		0.25	0.636	0.054	8.5	3
8	0	0.477	–	–	–	**9**	0	0.482	–	–	–
	1	0.431	0.046	9.6	–		1	0.409	0.073	15.1	–
	0.25	0.411	0.066	13.8	10		0.25	0.396	0.086	17.8	–
10	0	0.298	–	–	–	**11**	0	0.532	–	–	–
	1	0.185	0.113	37.9	–		1	0.259	0.273	51.3	–
	0.25	0.180	0.118	39.6	2		0.25	0.309	0.223	42.0	8

[a] r = (compound)/(polynucleotide)

[b] Hypochromic effect, H = (Abs(compound) − Abs(complex))/Abs(compound) × 100

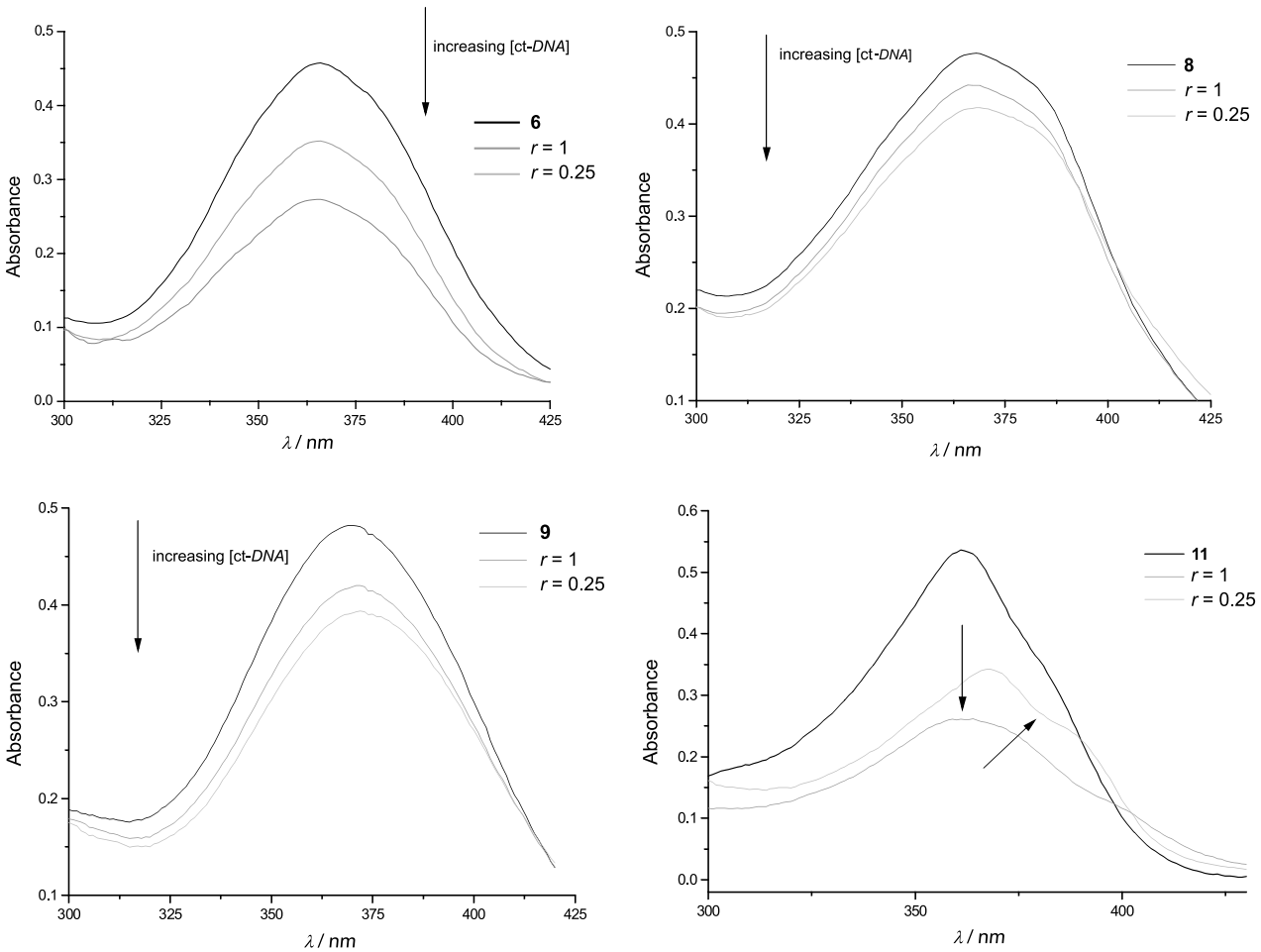


Fig. 1 Absorbance titrations of ct-*DNA* into samples of compounds **6**, **8**, **9**, and **11**. Mixing rations, compound/polynucleotide, from bottom to top are 0.25 and 1. Spectrum of free compounds **6**, **8**, **9**, and **11** in buffered aqueus solutions are indicated

10 (4.98×10^{-5} mol dm^{-3}), and **11** (1.26×10^{-5} mol dm^{-3}) resulted in significant hypochromic effects of some compounds (39% for **6**, 8.5% for **7**, 14% for **8**, 18% for **9**, 40% for **10**, and 42% for **11**) which pointed to their strong interaction with the *DNA*. Compounds **7**, **8**, **10**, and **11** showed also bathochromic shifts of absorption maxima ($\Delta\lambda_{max} = 2$–10 nm) in the range of $\lambda > 300$ nm at which ct-*DNA* does not absorb light, according to Table 2. Addition of ct-*DNA* to buffered solution of **12** (5.88×10^{-5} mol dm^{-3}) at a ratio ((**10**)/(polynucleotide)) > 0.1 induced precipitation.

Addition of ct-DNA into buffered aqueous solutions of cyano-substituted methyl-*E*-3-(2-furyl)-2-(4-nitrophenyl)acrylate **4** and its amidino-substituted derivative **5** did not yield any measurable changes in the UV/Vis spectra. As it is shown in Fig. 1, compounds **6**, **8**, and **9** ($\sim 2 \times 10^{-5}$ mol dm^{-3}) showed decreasing of absorption maxima upon the addition of different concentration ($\sim 8.5 \times 10^{-6}$–1×10^{-4} mol dm^{-3}) of ct-*DNA* solution, while **7** and **11** showed increasing of absorption maxima and bathochromic shifts at large excess of ct-*DNA*.

Since aqueous solutions of cyclic compounds **10** and **12** exhibit strong fluorescence, it was possible to perform fluorimetric titrations at 60 times lower concentration for **12** and 100 times lower concentration for **10** than used in the UV/Vis experiments. Acyclic compounds **6**, **7**, and **8** and cyclic **11** exhibit lower fluorescence so we performed fluorimetric titrations at 10 times lower concentration than used in the UV/Vis experiments. Increasing the amount of ct-*DNA*, F/F_0 of **6**, **7** and **8** reached *ca*. 2.29, 3.91, and 9.72 at large excess of ct-*DNA*. F/F_0 of cyclic compounds **10**, **11**, and **12** reached *ca*. 0.2, 0.7, and 0.16, according to Table 3.

As shown in Fig. 2, compounds **6**, **7**, and **8** ($\sim 1.4 \times 10^{-6}$ mol dm^{-3}) showed an increasing of fluorescence intensity upon the addition of different concentrations ($\sim 1.4 \times 10^{-6}$–1.4×10^{-4} mol dm^{-3}) of ct-*DNA* solutions, while **10** and **12** at the same conditions showed decreasing of fluorescence intensity. Compound **11** showed a decrease of the fluorescence intensity for $r > 0.25$, while further addition of ct-*DNA* ($r = 0.25$) yielded an increase of fluorescence intensity. Increasing of fluorescence intensity of **11** at $r < 0.25$ resulted in appearance of two maxima. One of maxima appeared at lower wavelength (406 nm) and the other one at higher wavelength (426 nm) compared to emission maxima of **11**.

Conclusion

The main aim of the present study was to prepare small series of substituted methyl (*E*)-3-(2-furyl)-2-phenylacrylates which can undergo photochemical dehydrocyclization by visible light irradiation in order to obtain its cyclic derivatives. Some changes in

Table 3 Fluorescence intensity changes of compounds **6**–**8** and **10**–**12** upon the addition of ct-*DNA* at different ratio r((compound)/(polynucleotide)) at $pH = 7.0$ (buffer Na-cacodylate, $I = 0.05$ mol dm^{-3})

	r[a]	Int/a.u.	ΔInt/a.u.	F/F_0[b]		r[a]	Int/a.u.	ΔInt/a.u.	F/F_0[b]
6	0	–	–	–	**7**	0	181.3	–	–
	1	116.7	7.5	0.06		1	218.8	37.5	0.21
	0.25	124.2	44.2	0.38		0.25	405.1	223.8	1.23
	0.05	267.7	151.0	1.30		0.05	891.0	709.7	3.91
	0.01	383.9	267.2	2.29		0.01	–	–	–
8	0	61.9	–	–	**10**	0	498.8	–	–
	1	68.8	6.9	0.11		1	517.0	18.2	0.04
	0.25	105.4	43.5	0.70		0.25	474.3	24.5	0.05
	0.05	235.5	174.6	2.80		0.05	448.3	50.5	0.10
	0.01	663.7	601.7	9.72		0.01	398.0	108.0	0.20
11	0	149.8	–	–	**12**	0	638.9	–	–
	1	52.3	97.5	0.65		1	738.9	100.0	0.16
	0.25	44.0	105.8	0.71		0.25	693.1	54.2	0.08
	0.05	68.6	81.2	0.54		0.05	657.9	19.0	0.03
	0.01	145.3	4.5	0.03		0.01	587.0	51.9	0.08

[a] r = (compound)/(polynucleotide); [b] F = fluorescence of compound-*DNA* complex, F_0 = fluorescence of compound without ct-*DNA* addition

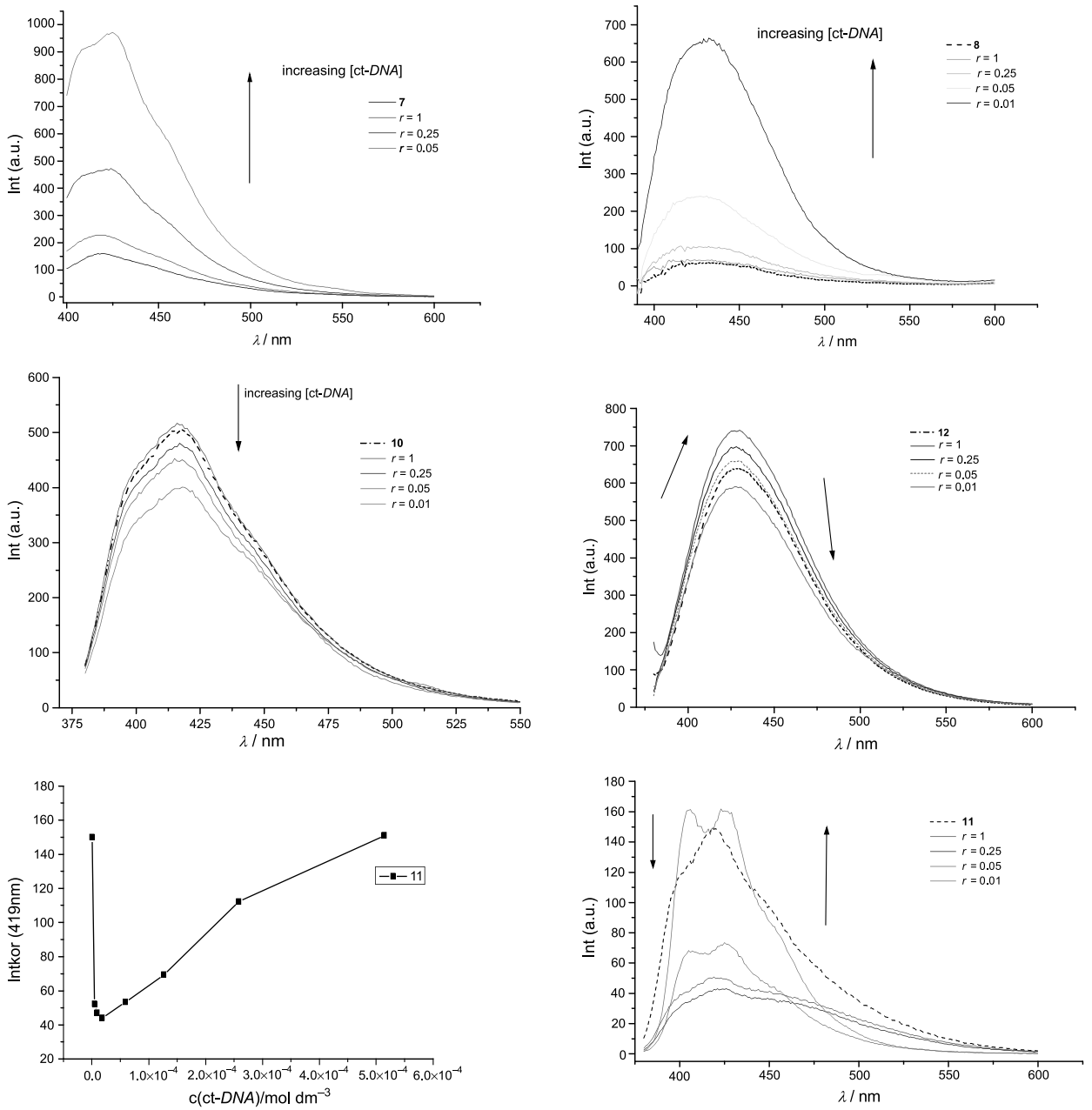


Fig. 2 Fluorescence titrations of ct-*DNA* into samples of compounds **7**, **8**, **10**, **11**, and **12**. Mixing rations, compound/polynucleotide, are 0.01, 0.05, 0.25, and 1. Spectrum of free compounds in buffered aqueus solutions is indicated

structure had an impact on reaction of photochemical dehydrocyclization. Compounds **4**, **5**, and **8** were not capable of undergoing the aformentioned photochemical dehydrocyclization, while acyclic compounds **6**, **7**, and **9** were efficiently converted into the corresponding naphthofurans **10**, **11**, and **12**. All prepared compounds had interesting spectroscopic properties and showed one absorption and emission band (**11** showed two absorption bands). Cyano- and amidino substituents induced bathochromic shift and mostly increasing of molar extinction coefficient. We wanted to shift absorption maxima to longer wavelengths, possibly in visible region, but introducing of a nitro group did not yield any important shift of absorption maxima. Compounds **6**–**12** showed moderate changes of their spectral-fluorescent properties in the presence of ct-*DNA*, except **4** and **5** which did not yield any measurable changes. In the presence of different ratios of ct-*DNA*, acyclic compounds **6**, **7**, and **8** increased fluorescence inten-

sity, while its cyclic derivatives **10** and **12** at the same conditions showed exactly opposite quenching of fluorescence intensity. The obtained results revealed the possibility of two different types of interactions with *DNA*, probably minor groove binding and intercalation. Compounds **9** and **11** showed both changes of fluorescence intensity, fluorescence quenching at lower concentration of added ct-*DNA*, and increase of fluorescence intensity at high excess of ct-*DNA*. Since results obtained from spectroscopic titrations of prepared compounds with ct-*DNA* are interesting and might revealed the possibility for biomedical application, detailed binding studies using some other spectroscopic methods will be further investigated.

Experimental

Chemistry

Melting points were obtained on an Original Kofler Mikroheitztisch apparatus (Reichert, Wien). The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini 300 or a Bruker Avance DPX 300 at 300 and 75 MHz. All NMR spectra were measured in *DMSO*-d_6 solutions using *TMS* as an internal standard. Elemental analysis for carbon, hydrogen and nitrogen were performed on a Perkin-Elmer 2400 elemental analyzer. Where analyses are indicated only as symbols of elements, analytical results obtained are within 0.4% of the theoretical value. All compounds were routinely checked by TLC with Merck silica gel 60F-254 glass plates and HPLC-MS.

*Preparation of compounds **2** and **4–12***

Methyl (E)-3-(5-formyl-2-furyl)-2-(4-nitro)-phenyl-acrylate (**2**, $C_{15}H_{11}NO_6$)

The corresponding methyl (*E*)-3-(2-furyl)-2-(4-nitro)-phenyl-acrylate was formylated by *Vilsmeier* formylation. Phosphorus oxychloride (1.58 cm^3, 17.3 mmol) was added drop-wise with cooling to 1.3 cm^3 *DMF* (16.3 mmol). The reaction mixture was stirred for 0.5 h by cooling on ice, the apparatus being protected with a $CaCl_2$ tube. A solution of methyl (*E*)-3-(2-furyl)-2-phenylacrylate (2 g, 7.33 mmol) in 5 cm^3 *DMF* was added drop-wise to the mixture. After the addition was completed, the mixture was stirred for 1 h at room temperature, then heated at 90°C for 3 h, cooled and poured onto crushed ice and made weakly alkaline with sodium carbonate solution, and left over-night on ice. The solid was filtered off, washed with water and recrystallized from methanol. Yield 0.602 g (27.1%) yellow-orange crystals; mp 166–167°C; ^{1}H NMR (300 MHz, *DMSO*): $\delta = 9.37$ (s, 1H), 8.22 (d, $J = 8.5$ Hz, 2H), 7.69 (s, 1H), 7.53 (d, $J = 8.5$ Hz, 2H), 7.35 (d, $J = 3.7$ Hz, 1H), 6.49 (d, $J = 3.7$ Hz, 1H), 3.38 (s, CH_3) ppm; ^{13}C NMR (75 MHz, *DMSO*): $\delta = 175.31$ (d), 154.40 (s), 149.42 (s), 141.53 (s), 132.11 (s), 131.36 (d, 2C), 126.57 (s), 125.21 (d), 124.40 (d, 2C), 118.42 (d), 116.73 (d), 111.28 (s), 59.79 (q) ppm; MS: m/z (%) = 301.2 (M +, 100).

Methyl (E)-3-(5-cyano-2-furyl)-2-(4-nitro)-phenyl-acrylate (**4**, $C_{15}H_{10}N_2O_5$)

Compound **2** (0.6 g, 2.0 mmol) and hydroxyl ammonium hydrochloride (0.24 g, 14.3 mmol) were heated in 1.5 cm^3 pyridine at 60°C for 30 min, then 1 cm^3 acetic anhydride was successively added at a temperature not exceeding 95°C, which was being kept for additional 2 h. The mixture was cooled to 20°C, poured into 37.5 cm^3 water and the separated nitrile was stirred for 1 h, filtered, washed with water, dried, and recrystallized from petrolether. Yield 0.541 g (90.2%) yellow powder; mp 113–114°C; ^{1}H NMR (300 MHz, *DMSO*): $\delta = 8.30$ (d, $J = 8.7$ Hz, 2H), 7.70 (s, 1H), 7.60 (d, $J = 8.7$ Hz, 2H), 7.55 (d, $J = 3.8$ Hz, 1H), 6.62 (d, $J = 3.8$ Hz, 1H), 3.73 (s, CH_3) ppm; ^{13}C NMR (75 MHz, *DMSO*): δ=165.58 (s), 153.02 (s), 148.50 (s), 141.85 (s), 131.40 (s), 130.91 (d, 2C), 126.11 (d), 125.92 (s), 125.84 (d), 123.41 (d, 2C), 117.32 (d), 111.75 (s), 52.81 (q) ppm; MS: m/z (%) = 298.2 (M +, 100).

Methyl (E)-3-(5-N-isopropylamidino-2-furyl)-2-(4-nitro)-phenyl-acrylate hydrochloride (**5**, $C_{18}H_{20}ClN_3O_5$)

A stirred suspension of 0.324 g **4** (1.1 mmol) in 5 cm^3 anhydrous *Et*OH was cooled in an ice-salt bath and was saturated with HCl gas. The flask was then tightly stoppered and the mixture was maintained at room temperature for 2 days, until nitrile band disappeared (monitored by IR analysis at 2200 cm^{-1}). The reaction mixture was purged with N_2 gas and diluted with ether (30 cm^3). The crude imidate was filtered off and was immediately suspended in 5 cm^3 anhydrous ethanol. The 0.52 cm^3 isopropylamine (6.1 mmol) was added and the mixture was stirred for one day at room temperature. The reaction mixture was diluted with 50 cm^3 diethylether to give a precipitate. The precipitate was collected and recrystallized from ethanol/ether. Yield 0.120 g (27.8%) yellow powder; mp 225–226°C; ^{1}H NMR (300 MHz, *DMSO*): $\delta = 9.51$ (bs, 3H), 8.31 (d, $J = 8.8$ Hz, 2H), 7.76 (s, 1H), 7.64 (d, $J = 3.6$ Hz, 1H), 7.61 (s, 1H), 6.20 (d, $J = 3.6$ Hz, 1H), 4.05–3.95 (m, CH), 3.75 (s, CH_3), 1.20 (d, $J = 6.2$ Hz, 6H, $(CH_3)_2$) ppm; MS: m/z (%) = 358.2 (M + 1, 100).

Methyl (E)-3-[5-(5-cyano-2-benzimidazolyl)-2-furyl]-2-phenyl-acrylate (**6**, $C_{22}H_{15}N_3O_3$)

A mixture of 0.5 g **1** (1.95 mmol), 0.256 g 4-cyano-1,2-phenylenediamine (1.95 mmol), and 0.211 g *p*-benzoquinone (1.95 mmol) in 10 cm^3 absolute *Et*OH was stirred under nitrogen at reflux for 6 h. The reaction mixture was evaporated in vacuum and the oily residue was dissolved in diethylether. Petrolether was added and the precipitated product was filtered off. Yield 0.550 g (76%) yellow powder; mp 169–170°C; ^{1}H NMR (300 MHz, *DMSO*): $\delta = 13.56$ (s, NH, 1H), 8.11 (s, 1H), 7.71 (s, 1H), 7.69 (d, $J = 8.6$ Hz, 1H), 7.60 (d, $J = 8.6$ Hz, 1H), 7.55–7.48 (m, 3H), 7.29 (d, $J = 7.6$ Hz, 2H), 7.21 (d, $J = 3.8$ Hz, 1H), 5.66 (d, $J = 3.8$ Hz, 1H), 3.80 (s, CH_3) ppm; ^{13}C NMR (75 MHz, *DMSO*): $\delta = 168.22$ (s), 166.36 (s), 151.75 (s), 145.32 (s), 144.71 (s), 135.11 (s), 134.77 (s), 131.72 (s), 128.99 (d, 2C), 128.76 (d, 3C), 128.44 (d), 126.65 (d), 125.67 (d), 119.84 (s), 119.77 (s), 115.61 (d), 115.15 (d), 114.75 (s), 52.4 (q) ppm; MS: m/z (%) = 370.2 (M + 1, 100).

Methyl (E)-3-[5-(5-N-isopropylamidino-2-benzimidazolyl)-2-furyl]-2-phenyl-acrylate hydrochloride (**7**, $C_{25}H_{27}N_4O_3Cl_3 \times 3H_2O$) [16]
A mixture of 1.01 g **1** (4.0 mmol), 0.90 g 4-*N*-isopropylamidino-1,2-phenylenediamine (4.0 mmol), and 0.43 g *p*-benzoquinone (4.0 mmol) in 25 cm^3 absolute *Et*OH was stirred under nitrogen at reflux for 4 h. The reaction mixture was cooled to room temperature, diethylether was added and the resulting brown solid was filtered off. The crude product was suspended in absolute ethanol and cooled to 0–5°C. Into the suspension was introduced HCl gas until the suspension was saturated and the content was stirred over night on the room temperature. Dry diethylether was added, the precipitated yellow-green powder was filtered off and washed with dry diethyl-ether. Yield 0.95 g (41%) slightly green powder; mp 236–238°C; ^{1}H NMR (300 MHz, *DMSO*): $\delta = 13.54$ (s, NH, 1H), 9.60 (s, NH, 1H), 9.57 (s, NH, 1H), 9.49 (bs, NH, 1H), 8.01 (s, 1H), 7.77 (d, $J = 8.7$ Hz, 1H), 7.73 (s, 1H), 7.61 (d, $J = 8.8$ Hz, 1H), 7.60 (s, 1H), 7.54–7.46 (m, 3H), 7.38 (d, $J = 3.8$ Hz, 1H), 7.31 (d, $J = 7.8$ Hz, 2H), 5.71 (d, $J = 3.8$ Hz, 1H), 4.07 (m, CH), 3.90 (s, CH_3), 1.34 (d, $J = 6.2$ Hz, 6H, $(CH_3)_2$) ppm; ^{13}C NMR (75 MHz, *DMSO*): $\delta = 166.9$ (s), 162.5 (s), 152.9 (s), 145.6 (s), 144.4 (s), 135.5 (s), 134.45 (s), 132.9 (s), 129.6 (d, 2C), 129.3 (d), 129.1 (d, 2C), 127.1 (d), 124.7 (d), 124.2(s), 119.87 (s), 116.8 (d), 116.6 (d), 116.2 (d), 115.3 (d), 53.1 (q), 45.7 (d), 21.9 (q, 2C) ppm; MS: m/z (%) = 429.5 (M + 1, 100).

Methyl (E)-3-[5-(5-N-isopropylamidino-2-benzimidazolyl)-2-furyl]-2-(4-nitrophenyl)-acrylate hydrochloride (**8**, $C_{25}H_{24}ClN_5O_5$)
A mixture of 0.2 g **2** (0.66 mmol), 0.150 g 4-*N*-isopropylamidino-1,2-phenylenediamine (0.66 mmol), and 0.072 g *p*-benzoquinone (0.66 mmol) in 10 cm^3 absolute was stirred under nitrogen at reflux for 3 h. The reaction mixture was cooled to the room temperature, diethyl-ether was added and the resulting product was filtered off and washed with diethylether and recrystallized from ethanol/acetone. Yield 0.220 g (65%) green powder; mp 221–222°C; ^{1}H NMR (300 MHz, *DMSO*): $\delta = 13.65$ (bs, NH, 1H), 9.54 (bs, NH, 1H), 9.40 (bs, NH, 1H), 9.02 (bs, NH, 1H), 8.34 (d, $J = 8.6$ Hz, 2H), 7.96 (s, 1H), 7.79 (s, 1H), 7.70 (d, $J = 8.6$ Hz, 2H), 7.63 (d, $J = 8.6$ Hz, 2H), 7.35 (d, $J = 3.2$ Hz, 1H), 6.26 (d, $J = 3.2$ Hz, 1H), 4.10–4.06 (m, CH), 3.75 (s, CH_3), 1.29 (d, $J = 6.3$ Hz, 6H, $(CH_3)_2$) ppm; ^{13}C NMR (75 MHz, *DMSO*): $\delta = 168.76$ (s), 166.28 (s), 162.77 (s), 151.39 (s), 147.89 (s), 147.32 (s), 146.73 (s), 146.490 (s), 142.92 (s), 141.66 (s), 131.56 (d, 2C), 129.32 (s), 127.91 (d), 127.17 (d), 124.74 (d), 124.48 (d, 2C), 118.38 (d), 116.09 (d), 114.95 (d), 53.86 (q), 45.48 (d), 21.70 (q, 2C) ppm; MS: m/z (%) = 474.3 (M + 1, 100).

Ethyl (E)-3-[5-(5-cyano-2-benzimidazolyl)-2-furyl]-2-(4-cyanophenyl)-acrylate (**9**, $C_{24}H_{16}N_4O_3$)
A mixture of 0.2 g **3** (0.68 mmol), 0.091 g 4-cyano-1,2-phenylenediamine (0.68 mmol), and *p*-benzoquinone (0.073 g, 0.68 mmol) 10 cm^3 in absolute *Et*OH was stirred under nitrogen at reflux for 12 h. The reaction mixture was evaporated in vacuum and the crude product was recrystallizated from petrolether. Yield 0.096 g (35%); yellow crystals; mp 142–144°C; ^{1}H NMR (300 MHz, *DMSO*): $\delta = 13.54$ (s, NH, 1H), 8.15 (bs, 1H), 7.96 (d, $J = 8.2$ Hz, 2H), 7.75 (s, 1H), 7.71–7.65 (m, 2H), 7.54 (d, $J = 8.2$ Hz, 2H), 7.26 (d, $J = 3.4$ Hz, 1H), 6.06 (d, $J = 3.4$ Hz, 1H), 4.15 (q, $J = 7.0$ Hz, 2H, CH_2CH_3), 1.27 (t, $J = 7.1$ Hz, 3H, CH_2CH_3) ppm; MS: m/z (%) = 409.2 (M + 1, 100).

Methyl (E)-2-[(5-cyano)benzimidazolyl]naphtho[2,1-b]furan-5-carboxylate (**10**, $C_{22}H_{13}N_3O_3$)
An ethanolic solution (35 cm^3) of 0.10 g **6** (0.27 mmol), and iodine (0.005 g) was irradiated at room temperature with 400-W high-pressure mercury lamp using a Pyrex filter for 50 h. The air was bubbled through the solution. The solution was concentrated and resulting product was filtered off. Yield 0.050 g (50%) light yellow powder; mp 189–190°C; ^{1}H NMR (300 MHz, *DMSO*): $\delta = 13.81$ (bs, NH, 1H), 8.79 (d, $J = 8.6$ Hz, 1H), 8.45 (d, $J = 8.3$ Hz, 1H), 8.43 (s, 1H), 8.37 (s, 1H), 7.72 (t, $J = 8.7$ Hz, 2H), 7.63–7.61 (m, 3H), 3.74 (s, CH_3) ppm; MS: m/z (%) = 368.3 (M + 1, 100).

Methyl (E)-2-[(5-N-isopropylamidino)benzimidazolyl]naphtho[2,1-b]furan-5-carboxylate hydrochloride
(**11**, $C_{25}H_{24}N_4O_3Cl_2 \times 2H_2O$) [16]
An ethanolic solution 140 cm^3 of 0.90 g **7** (2.0 mmol) was irradiated at room temperature with 400-W high-pressure mercury lamp using a Pyrex filter for 20 h. The air was bubbled through the solution. The solution was concentrated, diethyl-ether was added to the solution and the precipitated powder was filtered off and recrystallized from ethanol. Yield 0.37 g (35%); yellow powder; mp 255–257°C; ^{1}H NMR (300 MHz, *DMSO*): $\delta = 13.65$ (s, NH, 1H), 9.62 (bs, NH, 2H), 9.52 (s, NH, 1H), 9.07 (s, NH, 1H), 8.88 (d, $J = 8.7$ Hz, 1H), 8.63 (s, 1H), 8.50 (d, $J = 8.4$ Hz, 1H), 8.47 (d, $J = 7.6$ Hz, 1H), 8.11 (s, 1H), 7.87–7.70 (m, 3H), 7.63 (bs, 1H), 4.46 (m, CH), 4.07 (s, CH_3), 1.24 (d, 6H, $J = 6.2$ Hz, $(CH_3)_2$) ppm; ^{13}C NMR (75 MHz, *DMSO*): $\delta = 167.5$ (s), 162.7 (s), 158.9 (s), 151.2 (s), 148.7 (s), 145.6 (s), 143.8 (s), 141.7 (s), 128.3 (d), 128.1 (s), 127.5 (d), 127.0 (d), 126.3 (d), 124.9 (s), 124.3 (d), 123.7 (d), 117.3 (s), 116.3 (d), 115.7 (d), 114.4 (d), 53.1 (q), 45.6 (d), 21.9 (q, 2C) ppm; MS: m/z (%) = 427.4 (M + 1, 100).

Ethyl (E)-2-[(5-cyano)benzimidazolyl-]-4-cyanonaphtho[2,1-b]furan-5-carboxylate (**12**, $C_{24}H_{14}N_4O_3$)
An ethanolic solution (20 cm^3) of 0.05 g **9** (0.13 mmol), and iodine (0.002 g) was irradiated at room temperature with 400-W high-pressure mercury lamp using a Pyrex filter for 70 h. The air was bubbled through the solution. The solution was concentrated and dark residue was treated with ethanol. The resulting product was filtered off. Yield 0.020 g (40%) light brown powder; mp 167–168°C; ^{1}H NMR (300 MHz, *DMSO*): $\delta = 13.60$ (s, NH, 1H), 8.63 (s, 1H), 8.50–8.47 (m, 2H), 8.16 (s, 2H), 7.98 (d, $J = 8.5$ Hz, 1H), 7.75 (d, $J = 8.3$ Hz, 1H), 7.60 (d, $J = 8.5$ Hz, 1H), 4.20 (q, $J = 7.1$ Hz, 2H, CH_2CH_3), 1.20 (t, $J = 7.1$ Hz, 3H, CH_2CH_3) ppm; MS: m/z (%) = 407.2 (M + 1, 100).

Spectroscopic measurements

UV absorption spectra were recorded against the solvent at 25 ± 0.1°C, using a Varian Cary 50 spectrophotometer operated in double-beam mode. The wavelength range covered was 200–450 nm. Quartz cells of 1-cm path length were used throughout and absorbancies were sampled at 0.1 nm intervals. Samples were prepared by diluting the respective stock solutions (in *DMSO*) with the appropriate aqueous buffer.

Fluorescence measurements were carried out on a Varian Cary Eclipse fluorescence spectrophotometer at 25°C using 1-cm path quartz cells. Excitation maxima were determined from excitation spectra covering the range of 200–400 nm. Emission spectra were recorded from 375 to 600 nm and corrected for the effects of time- and wavelength-dependent light-source fluctuations using a standard of rhodamine 101, a diffuser provided with the fluorimeter and the software supplied with the instrument. The measurements were performed in aqueous buffer solution ($pH = 7.0$, sodium cacodylate buffer, $I = 0.05\,\mathrm{mol\,dm^{-3}}$). Relative fluorescence quantum yields were determined according to *Miller* [13] using Eq. (1):

$$\phi_x = \phi_s \times A_s D_x n_x^2 / A_x D_s n_s^2 \qquad (1)$$

wherein ϕ is the emission quantum yield, A is the absorbance at the excitation wavelength, D is the area under the corrected emission curve and n is the refractive index of the solvents used. The subscripts s and x refer to the standard and to the unknown. The standard we used was *N*-acetyl-l-tryptophanamide with a published fluorescence quantum yield of 0.14 [14, 15]. All samples were purged with argon to displace oxygen. The reproducibility (difference between the largest and the smallest value in a series of three independent measurements, divided by their arithmetic mean) of quantum yield measurements was better than 10%.

Calf thymus (ct-*DNA*) was purchased from Fluka, dissolved in the sodium cacodylate buffer, $I = 0.05\,\mathrm{mol\,dm^{-3}}$, $pH = 7.0$ and additionally sonicated and filtered through the 0.45 μm filter. The final concentration of the calf thymus (ct-*DNA*) solution expressed as the concentration of phosphates was determined spectroscopically [11, 12] by measuring the absorbance of buffered solution at $\lambda = 260$ nm for at least five independent additions of the *DNA* stock solution aliquots and dividing the averaged absorbance value by molar extinction coefficient $\varepsilon_{(\text{ct-}DNA)} = 6600\,\mathrm{mmol^{-1}\,cm^{-2}}$. All reagents are of analytical reagent grade and the doubly distilled water is used all along.

Stock solutions were prepared by dissolving of compounds **4**–**12** in *DMSO*. Before experiment respective aliquots were added to the aqueous buffer, partial volume of *DMSO* in water not exceeding 0.1%. Stock solutions in *DMSO* of all studied compounds were shown to be stable over longer periods.

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