INTERAKCIJE NOVIH AMIDINO-SUPSTITUIRANIH ARIL-BIS(BENZAZOLA) S DNK I RNK

INTERACTIONS OF NOVEL AMIDINO-SUBSTITUTED ARYL-BIS(BENZAZOLES) WITH DNA AND RNA

L. Ptiček[1], L. Racané[1], I. Zonjić[2], L.-M. Tumir[2], M. Radić Stojković[2]

[1]Sveučilište u Zagrebu Tekstilno-tehnološki fakultet, Prilaz baruna Filipovića 28a, Zagreb

[2]Institut Ruđer Bošković, Bijenička cesta 54, Zagreb

INTRODUCTION

In the field of drug discovery and development, biologically important heterocyclic molecules known as "privileged structures" play an important role as promising future drug candidates because of their versatile binding properties for different biotargets. In continuation of our previous research in the synthesis and evaluation of antiproliferative activities of amidino-substituted 2-aryl(heteroaryl)bisbenzothiazole derivatives [1,2], we present here study of binding abilities for DNA and RNA of a series of eleven amidino-substituted bis(benzazoles), differing in heteroaromatic scaffolds as well as in type and position of amidine moiety. We studied the impact of benzazole derivatives on interactions with polynucleotides by a set of spectrophotometric methods - competition dialysis, fluorimetry, circular dichroism, and thermal melting experiments.

1. BINDING OF STUDIED COMPOUNDS

COMPETITION DIALYSIS

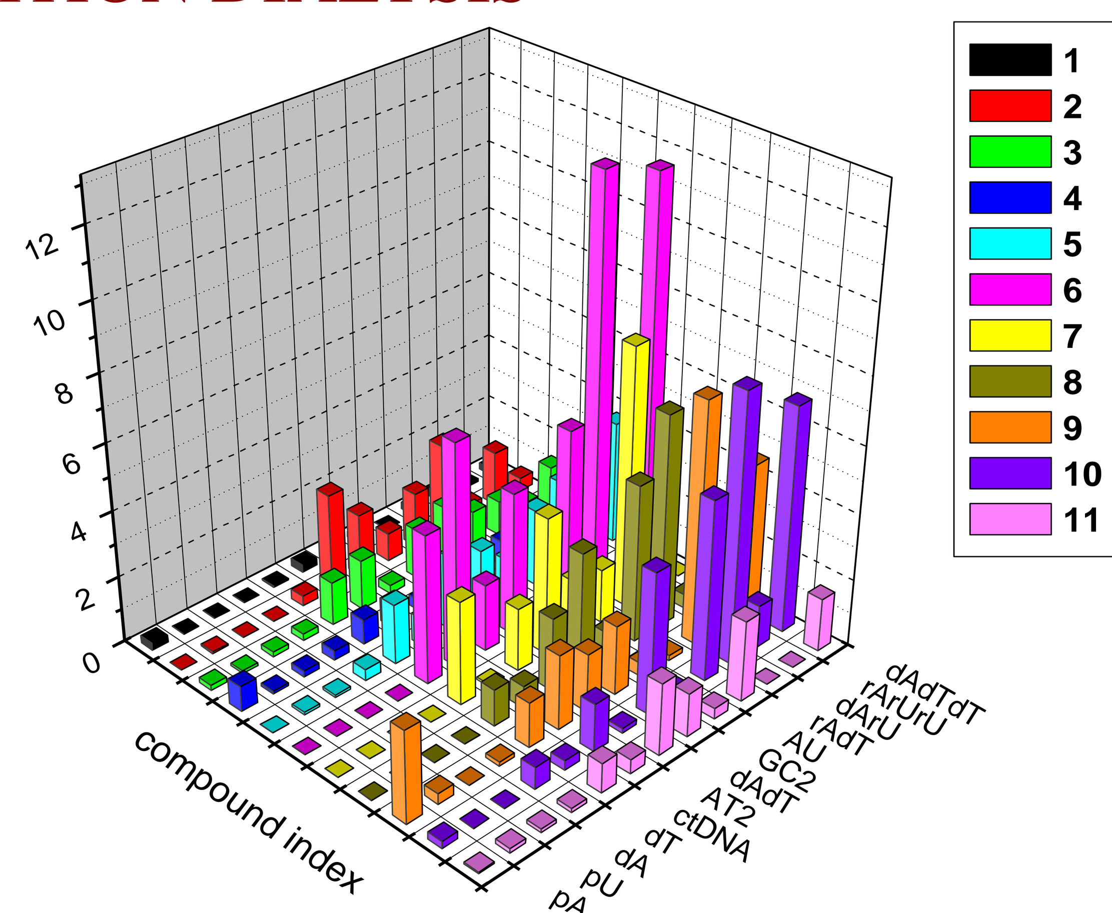

Figure 1. Competitive dialysis results for binding of eleven compounds with 13 different polynucleotides; Na-cacodylate buffer, I=0.05 mol dm^{-3} + 1 mM EDTA, pH=7

FLUORIMETRY

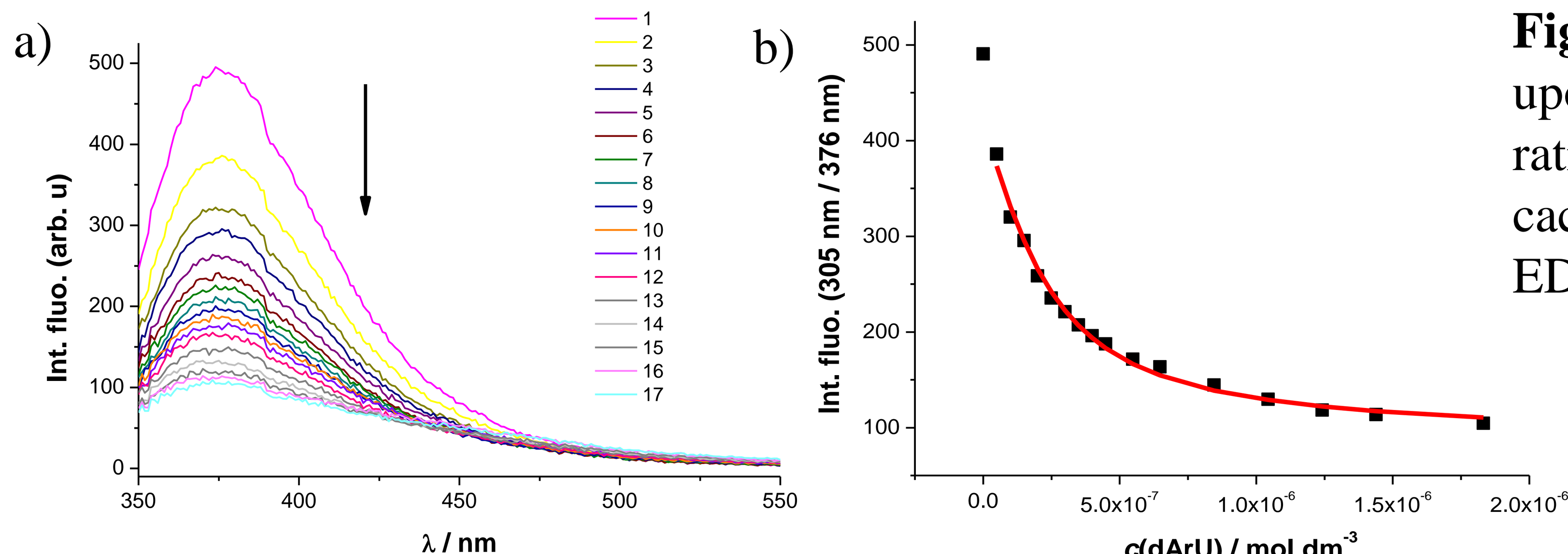

Figure 2. a) Changes in fluorescence spectrum of compound **9** (c=1×10^{-7} mol dm^{-3}, λ_{exc}=305 nm) upon titration with dArU hybrid (c=0.5×10^{-7} – 1.8×10^{-6} mol dm^{-3}); b) Dependence of compound **9** absorbance at λ=376 nm on c(dArU), at pH=7, Na-cacodylate buffer, I=0.1 mol dm^{-3}

Table 1. Stability constants (log K_s)^{a,b} calculated from fluorimetric titrations for compounds **2**, **6**, **7**, **8**, **9**, and **10** with polynucleotides at 25 °C and pH 7 (Na-cacodylate buffer, I=0.05 mol dm^{-3})

Compound	ATT		dArU		rAdT	
	logK_s	I/I_0^{c}	logK_s	I/I_0^{c}	logK_s	I/I_0^{c}
2	8.1	0.15	7.4	0.10	8.1	0.06
6	7.5	0.1	8.1	0.33	8.4	0.19
7	7.8	0.18	7.4	0.3	7.3	0.27
8	7.2	0.24	7.03	0.34	7.2	0.52
9	7.2	0.15	7.2	0.21	8.2	0.15
10	8.1^d	-^{d}	-^{e}	-^{e}	-^{e}	-^{e}

^{a}log K_s according to Scatchard's equation for fixed value of $n_{[compound]/[polynucleotide]}$

^{b}for easier comparison, all logKs values, calculated by Scatchard's equation, were recalculated for a given ratio of n = 0.5

^{c}I_0 denotes the initial fluorescence intensity of compounds; I denotes the fluorescence intensity of the compound-polynucleotide complex calculated using Scatchard's equation

^{d}due to the initial fluorescence intensity close to zero, it was not possible to calculate I/I_0 for compound **10**

^{e}due to the formation of several types of complexes during binding to the polynucleotide, it was not possible to calculate the stability constant

2. DETERMINATION OF BINDING MODES

MELTING TEMPERATURE EXPERIMENTS

Table 2. Melting temperatures of three-stranded polynucleotide (ATT^a) and of a mixture of four polynucleotides (rAdT, rArU, dArU, dAdT^b) due to addition of compounds to polynucleotide solution at compound/polynucleotide concentration ratio r=0.1 and r=0.025 at pH=7 (Na-cacodylate buffer, I=0.05 mol dm^{-3} + 1mM EDTA)

Polynucleotide	$^c\Delta T_m$/ °C					
	2	6	7	8	9	10
ATT^d	19.4/22.7	10.3/>24.8	44.9/18.5	5.3/7.3	14.8/21.7	0/>29.5
rAdT^e	2.9	1.2	2.3	0	0.7	0
rArU^e	2.8	0	1.8	0	1.0	0
dArU^e	6.3	2.6	9.3	0	2.5	0
dAdT^e	> 29.4	22.7	16.5	1.6	18.1	> 29.4

^{a}T_m values for the ATT polynucleotide were 21.7 °C and 70.5 °C

^{b}T_m values for mixture of polynucleotides: dArU 48.2 °C; rArU 57.4 °C; rAdT 65.3 °C; dArU 70.6 °C

^{c}measurement error of T_m is ± 0.5 °C

^{d}concentration ratio of compound and polynucleotide was r = 0.1

^{e}concentration ratio of compound and polynucleotide was r = 0.025

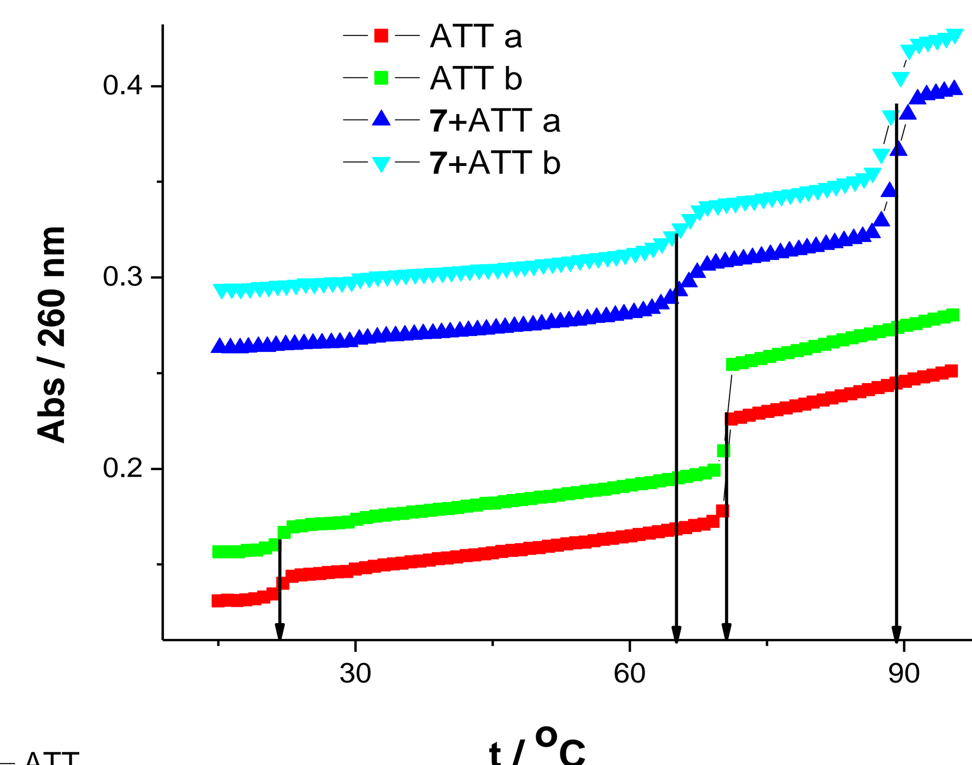

Figure 3. Melting curve of ATT triplex upon addition of compound **7** at molar ratio $r_{[compound]/[polynucleotide]}$=0.1 (pH=7, Na-cacodylate buffer, I=0.1 mol dm^{-3} + 1 mM EDTA).

CD SPECTROSCOPY

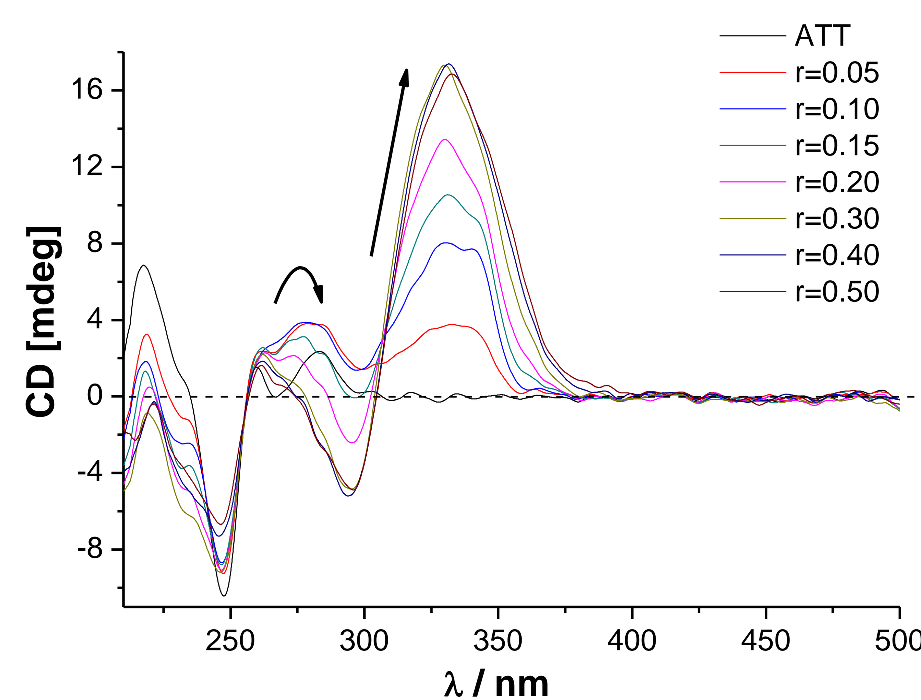

Figure 4. CD titration of ATT triplex (c=4.5×10^{-5} mol dm^{-3}) with compound **7** at molar ratios $r_{[compound]/[polynucleotide]}$=0.05 – 0.5 (pH=7, Na-cacodylate buffer, I=0.05 mol dm^{-3})

CONCLUSION

Compounds **6**, **8**, **9** and **10** showed high binding affinities for dArU, rAdT i ATT polynucleotides and compound **2** for AT$_2$ and rAdT. All obtained stability constants were of the same order of magnitude, which suggests that all tested compounds have a similar affinity for binding to polynucleotides dArU, rAdT and ATT. Compound **7** was particularly interesting since it showed a higher stabilization effect with ATT triplex than dAdT duplex. The appearance of a bisignate signal with maxima at 294 nm and 333 nm at higher ratios, (r ≥ 0.15) and a CD signal intensity equal to zero at the absorption maximum of the compound (λ=305 nm) suggests binding of **7** dimer inside the minor groove of ATT triplex.

REFERENCES **[1]** L. Racané, L. Ptiček, G. Fajdetić, et al, *Bioorg. Chem.* 95 (2020) 103537. **[2]** L. Racané, L. Ptiček, M. Sedić, et al, *Mol. Divers.* 22 (2018) 723.

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