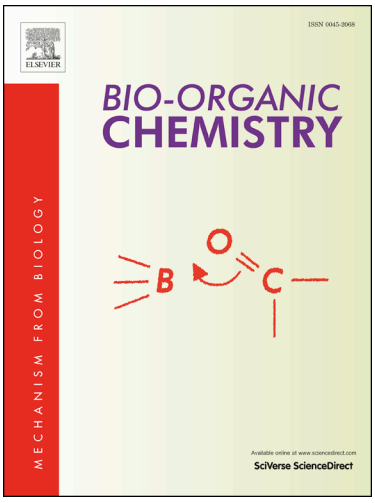

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Aminoalkoxyfluorenones and aminoalkoxybiphenyls: DNA binding modes

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A B S T R A C T

Many evidences suggest that DNA-drug interaction in the minor groove and the intercalation of drugs into DNA may play critical roles in antiviral, antimicrobial, and antitumor activities. As a continuous effort to develop novel antiviral agents, the series of planar fluorenone (**3a-7d**) were synthesized and used along with nonplanar biphenyls (**11a-d**) for the comparative analysis of their interaction with DNA. The chemical structure of new compounds was confirmed by ^{1}H NMR, ^{13}C NMR and mass spectra as well as elemental analysis. DNA affinity of **3a-7d** and **11a-d** was evaluated by ethidium bromide displacement assay. Affinity constant (lgKa) of **3a-7d** was found to be approximately two orders of magnitude higher than constants of corresponding **11a-d**. The molecular docking of aminoalkoxybiphenyls (**11a-d)** into minor grove of five different nucleotide sequences (d(CCIICICCII), d(CGCGTTAACGCG), d(CGCGATATCGCG), d(GGCCAATTGG), d(GGATATATCC)) demonstrated their binding capacity to the specific DNA site. The linear least squares fitting technique was successfully applied to derive an equation describing the relationship between lgKa and SF.

Keywords: Aminoalkoxybiphenyls, Aminoalkoxyfluorenones, DNA affinity, DNA minor grove Intercalation and molecular docking

1. Introduction

Essentially there are two chemotherapeutic approaches for treating viral diseases. The first approach is the development of selective antiviral drugs [1-3] which, however, can become redundant due to the drug resistance caused by the mutation of virus genes. Thus, establishing a new drug using this methodology is a lengthy, expensive and sometimes inefficient process. The

second one is the development of multi-target therapeutics or polypharmacological drugs that are needed to treat systematic diseases such as cancer or viral ones.

The multi-target therapy may enhance clinical efficacy, while the possibility of serious side effects is also increased. The inductors (IFNI) or non-specific resistance stimulators [4] play an important role for the clinical needs and therefore, tilorone remain a great challenge to rationally design effective multi-target therapeutics.

Two main pharmacological significant types of the drug-DNA interactions are known, it is a minor groove DNA binding and intercalation of planar polycyclic compounds between two sequential DNA base pairs [5]. Both intercalators and minor groove binders reveal antiviral activity. It was previously suggested [6, 7] that the interferon-induced inhibition of viral reproduction by planar polycyclic compounds is based on their intercalation into DNA. Thus, well known DNA intercalators acridines and their bis-analogs exhibited both antiviral [8-10] and interferon-inducing properties [11]. The same relationship between intercalation and antiviral properties revealed among fluorenone [12-15], isatin hydrazone [16], naphthylamide [17] and indoloquinoxaline [18] derivatives. We can assume that any distortion of the planarity of tilorone or tiloron-like structures should weaken or even abolish the intercalation to DNA, and consequently reduce or eliminate the interferon-inducing and antiviral activity. In order to estimate the influence of such distortion on DNA binding mode and its relationship to the antiviral activity, we have prepared nonplanar **11a-15e** structures.

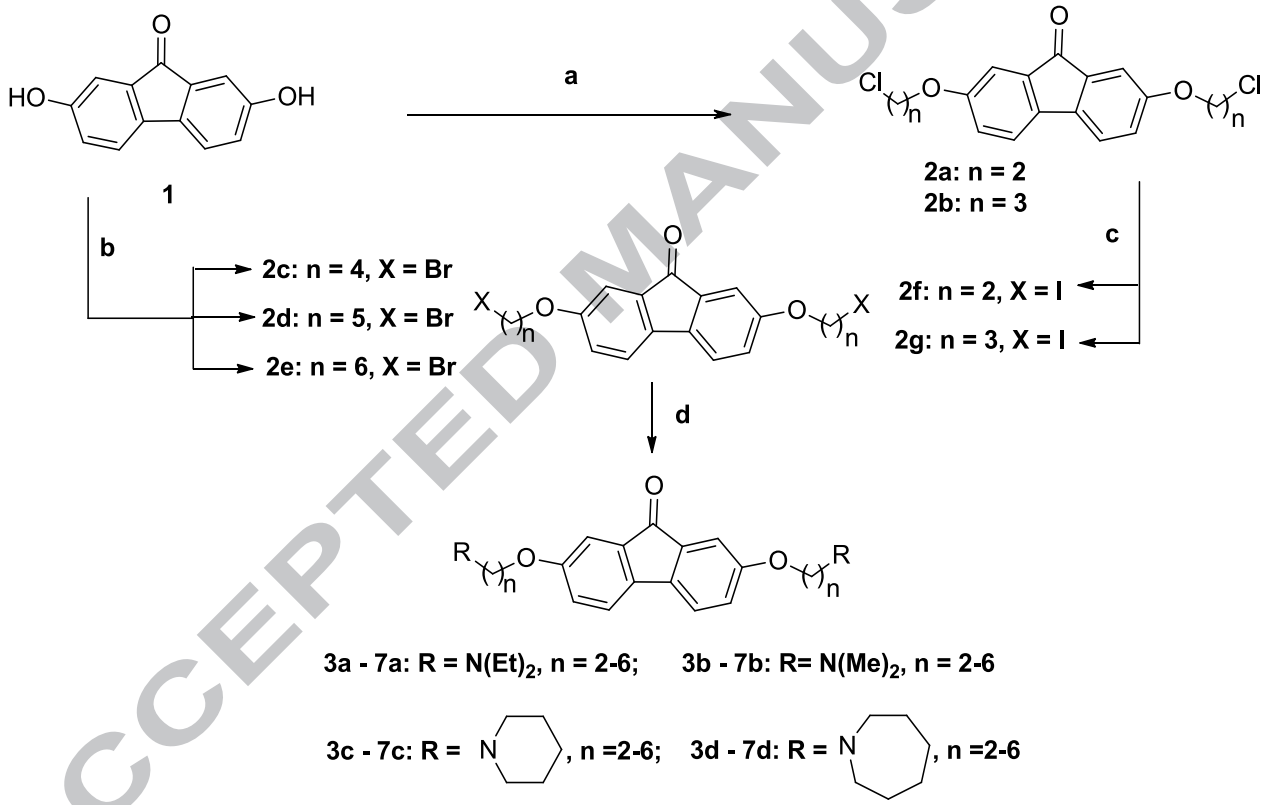
However, in contrast to our expectation, we found that these compounds have shown the antiviral activity *in vitro* on L929 *(mouse fibroblast cell line),* PST *(primary swine testicle)* , RF *(rat fibroblast cell line)* against *vesicular stomatitis virus* (VSV) and *herpes simples virus type I* (HSV) both in preventive and therapeutical modes [19, 20] and also induced interferon [21, 22].

These observations may suggest that the compounds are involved in an alternative DNA-binding mode. In this paper we specifically focus on the ability of 4,4'-dihydroxybiphenyl derivatives **11a-15e** to participate in DNA minor-groove recognition.

2. Results and Discussion

Chemistry 2.1.

The synthetic route for aminoalkoxyfluorenones **3a-7d** derivatives is outlined in Scheme 1.



Scheme 1. Reagents and conditions: (a) $(CH_2)_2Cl_2$, 20% NaOH/H_2O, TBAB, 75 C; (b) $BrCH_nBr$, K_2CO_3/DMF, 80 °C; (c) NaI, TBAI, H_2O/toluene, reflux; 10h; (d) appropriate amines/DMF, rt.

The intermediates **2a-g**, were generally obtained by O-alkylation of 2,7-dihydroxyfluoren-9-one **1** with a set of dihaloalkanes which have an straight alkyl chain from two to six methylene units. The alkylation was carried out either under phase-transfer catalysis as for **2b** or in DMF at 80 °C in the presence of anhydrous K_2CO_3 as for compounds **2b-e**. More reactive iodides **2f-g** were prepared from **2a-b** by Finkelstein type of reactions. All halide derivatives **2a-g** were then converted to the corresponding amines **3a-7d** at room temperature within 10 to 30 days.

The preparation and structural elucidation of compounds **3a, 3c, 4a-4c**, **5a** and as well as the final aminoalkoxybiphenyls **11a-15e** (Fig.1) were early reported [23, 24].

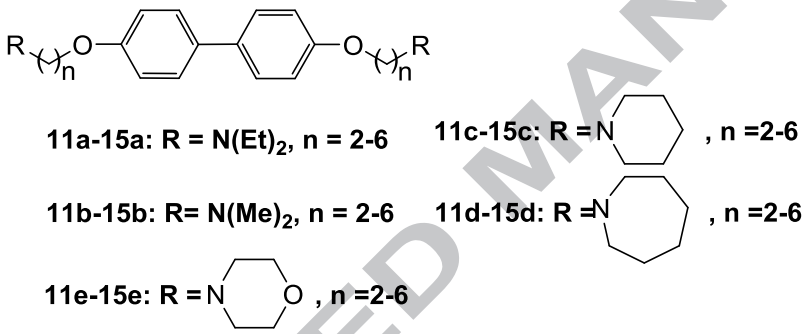
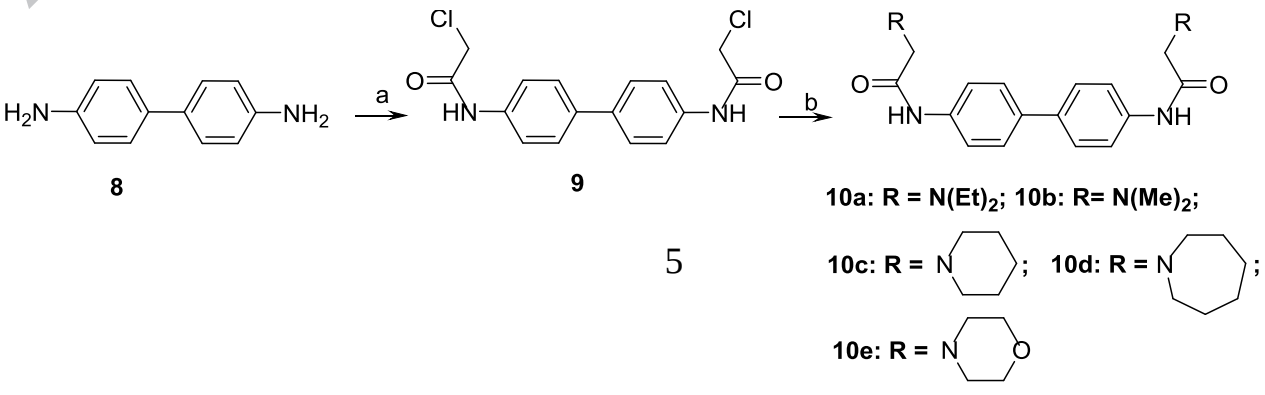


Fig. 1. Structures of aminoalkoxybiphenyls **11a-15e**

As shown in Scheme 2, benzidine derivatives **10a-e** were prepared by the acetylation of commercially available benzidine **8** with chloroacetyl chloride to afford chloroacetamide compound **9**, followed by amination with appropriate amines.



Scheme 2. Reagents and conditions: (a) $ClCH_2COCl$, toluene, reflux, 2 h; (b) appropriate amines/DMF, rt.

The chemical structures of the target compounds were confirmed by elemental analysis, mass spectrometry and NMR.

2.2.1. DNA binding studies

2.2.1.1 Analysis of DNA-ligand interaction

It is well known [25] that the compound can be considered a DNA intercalator if it meets the following criteria: (i) it can increase the specific DNA viscosity; (ii) it should show a bathochromic shift of its maximum absorption in the presentce of DNA; (iii) it is able to compete with a known intercalator for DNA binding; (iv) it is able to unwind of supercoiled plasmid.

The specific DNA viscosity measurements were carried out with compounds **11a-15e** (concentration range: 30 - 300 μmol/L) in the presence of 2.5 times excess amount of DNA under the conditions similar to those previously described [25]. None of **11a-15e** led to increase of DNA solution specific viscosity. Moreover, compound **15a** even decreases viscosity (Table 1)

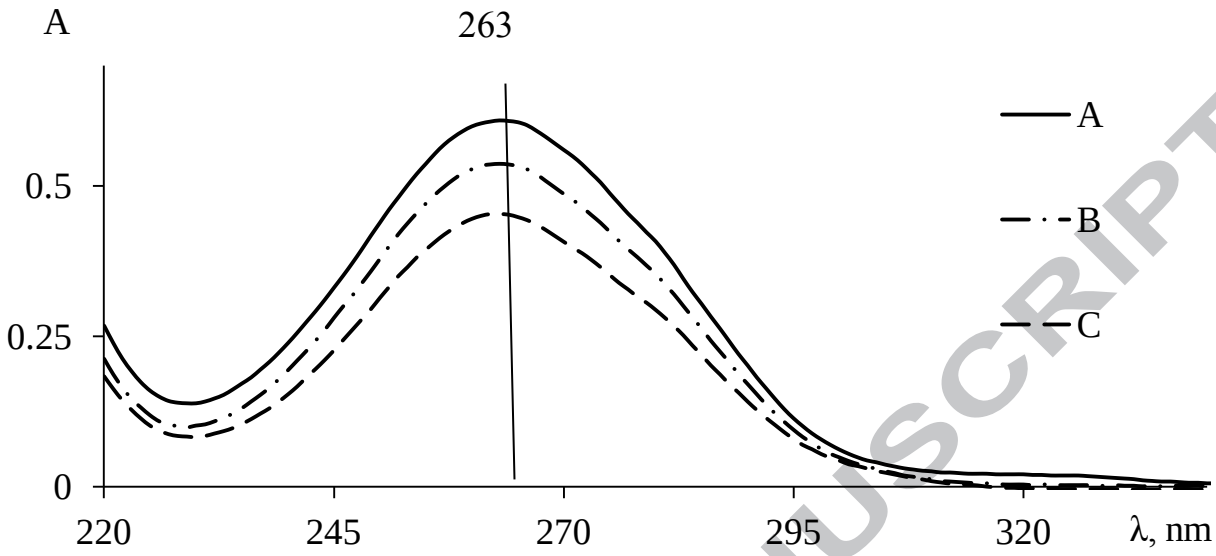
Table 1. Specific viscosity of solutions 3a-7a, 11a-15a

Compound	ŋ	σ	Compound	ŋ	σ
DNA	0.156	0.002			
3a	0.213*	0.003	**11a**	0.158**	0.003
5a	0.216*	0.003	**13a**	0.155**	0.002
7a	0.168*	0.001	**15a**	0.122*	0.002

*P < 0.05; **P > 0.05

Any bathochromic shift of maximum absorption in the electronic spectra of **11a-15d** (concentration range: 0 - 30 μmol/L) with their DNA mixtures was not observed (Fig. 2a, compound **11a**) although such shifts were found in spectra of **3a-7d** with their DNA mixtures (Fig. 2b, compound **3a**) [26].

a.



b.

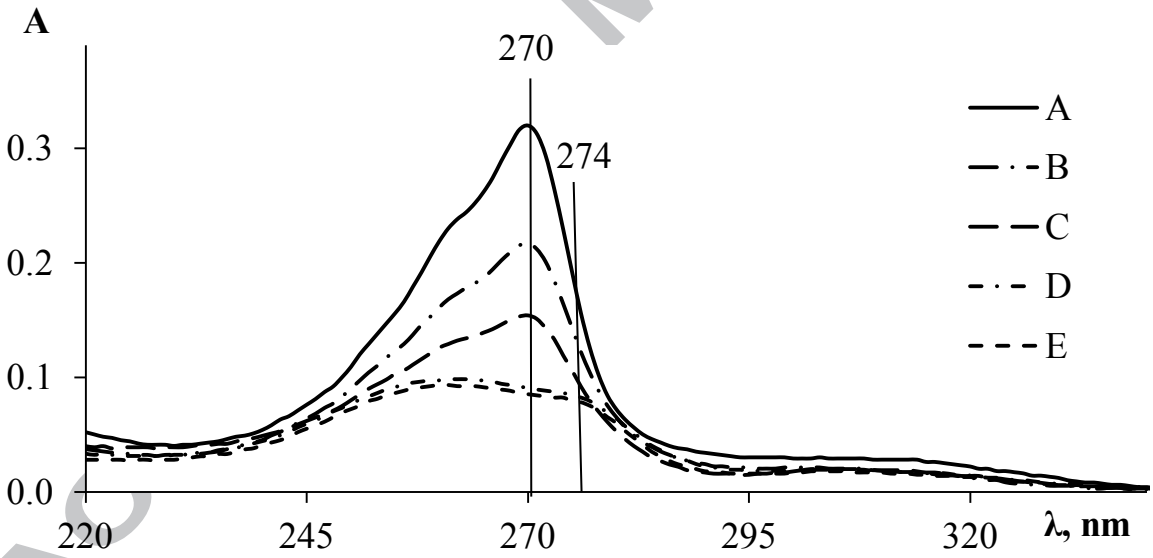


Figure 2. (a) Electron specrum in UV-range – 11a compound at C_{DNA}, μM = 0 (A); 1 (B); 10 (C); (b) – 3a compound at C_{DNA}, μM = 0 (A); 5 (B); 10 (C); 20 (D); 30 (E)

In addition, all target compounds (concentration range: 0.1-500 μmol/L) compete with ethidium bromide (EB) in binding to DNA. Based on the above experimental results, we concluded that the 4,4-aminoalkoxybiphenyl ligands **11a-15d** do not bind to DNA through the intercalation mode.

2.2.1.2 DNA affinity

The affinity of ligands for DNA (Table 2) was evaluated by the association constants (lgKa) which were calculated from their C_{50} values as early described [27, 28].

Table 2. Biphenyls and fluorenones affinity (lgK_a) to DNA.*

R	Biphenyls (11a-15d), pKa for n = 2-6					Fluorenones (3a-7d), pKa for n = 2-6				
	2	3	4	5	6	2	3	4	5	6
a	4.74	5.07	5.23	5.51	5.93	6.89	7.23	7.50	7.58	7.78
b	5.00	5.26	5.63	5.59	5.49	7.26	7.68	7.69	7.78	7.68
c	4.82	5.47	5.81	5.71	5.57	7.44	7.74	7.77	7.87	7.80
d	5.11	5.45	5.47	5.59	5.26	7.33	7.61	7.76	7.73	7.61

*$\pm\varepsilon < 0.1$

The affinity of aminoalkoxybiphenyls was dependent on the side chain length. As chain length grows, K_a increases until it reaches four to five carbon length. At this point, degrees of freedom contribution becomes dominant and K_a begins decreasing.

2.3.Structure-DNA affinity relationships

Simple orthogonal three factor dispersion analysis determined that all three independent

structural factors (Factor A is the structure of terminal amino group, Factor B is polymethylene fragment length and Factor C is the nature of polycyclic system) had a remarkable impact on affinity. As we can see from Table 3, polycyclic fragment has the biggest impact, and the contribution of polymethylene fragment length was approximately 30 times less than that of Factor A. The structure impact of terminal amino group equals 0.64% only.

Table 3. Dispersion analysis (α = 0.05) of the contribution of structure factors to DNA affinity

Variation source	SS	DF	MS	F	P-values	F-test	Contrib., %
Factor A	0.330	3	0.110	4.344	0.011	2.9113	0.64
Factor B	1.956	4	0.489	19.295	4.66×10^{-8}	2.6787	3.80
Factor C	48.444	1	48.444	1911.383	2.01×10^{-29}	4.1596	94.04
Residual	0.786	31	0.025				1.53
Total	51.516	39					100.00

SS is the sum of squares due to the source; **DF** is degrees of freedom in the source; **MS** is the mean sum of squares due to the source; **F** is F-statistic.

2.4 Molecular docking

There are three DNA sites where interactions with ligand can occur: (i) major grove (ii) minor grove (iii) between stacked nucleic bases (DNA intercalation). It is unlikely for aminoalkoxybiphenyls to bind to the major grove, since major grove ligands are proteins or bulky molecules (e.g., Ditercalinium) of size that greatly exceeds the ones of aminoalkoxybiphenyls [29, 30]. Theoretical basis for DNA intercalation is the presence of planar polycyclic fragment of appropriate size (at least tricyclic one for a potent intercalation, in most

cases) in the ligand [31]. Aminoalkoxybiphenyls do not possess this feature, making DNA intercalation improbable despite aminoalkoxybiphenyls similarity to the known intercalators, such as actinomycins [32, 33]. Thus, we chose minor grove as the site for molecular docking.

Non-intercalative DNA binding ligands, which may show various degrees of sequence specificity for binding to the minor groove of DNA, usually represent another class of important therapeutic drugs with broad-spectrum antiviral, antibacterial, and antitumor activity. To investigate the possible involvement of the aminoalkoxybiphenyls **11a-15d** in binding to the minor groove of DNA, we analyzed the ability of these agents to recognize specific nucleotide sequence of DNA using a molecular modeling approach.

Docking of **11a-15d** into oligonucleotide [34] showed that they can potentially bind to the DNA minor groove. According to Gibbs–Helmholtz law, δG depends on $lnKa$. Scoring function (SF) reflects energy gain in the formation of complex ligand-receptor. In case of aminoalkoxybiphenyls binding to minor groove d(CCIICICCII)2 sequence (Fig.3), the correlation between SF and $lgKa$ was defined by Spearman ρ rank correlation coefficient.

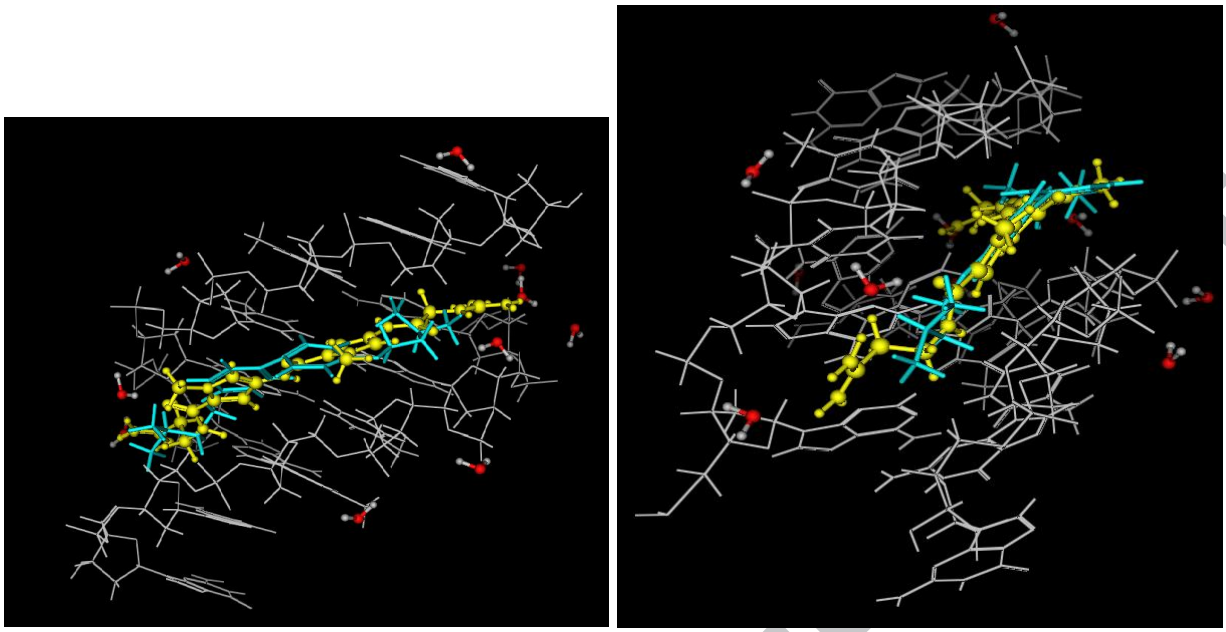


Figure 3. Binding of the aminoalkoxybiphenyls in the DNA minor groove d(CCIICICCII)$_2$ sequence (in blue – native ligand netropsin; in yellow – aminoalkoxybiphenyl, in gray – polynucleotide; on the left and on the right are two different projections of the same molecule)

SF and $lgKa$ (Table 5) distribution normality was assessed by Q-Q plot and Shapiro-Wilk test. Since most points are located near the trend line in Q-Q plot and Shapiro-Wilk W had p-value above significance level (α=0.05), we assumed that both $lgKa$ and SF data have distribution similar to normal one (Fig.4).

Table 5. $lgKa$ and SF values for biphenyls (sequence d(CCIICICCII)$_2$)

Compounds ID	$lgKa$	SF	Compounds ID	$lgKa$	SF
11a	4.74	-11.72	**13d**	5.47	-14.00
11b	4.95	-12.5	**13e**	5.12	-14.80
11c	4.82	-13.26	**14a**	5.51	-15.66
11d	5.11	-11.91	**14b**	5.59	-14.41
11e	4.94	-12.34	**14c**	5.71	-13.66
12a	5.07	-12.76	**14d**	5.59	-15.32
12b	4.85	-13.33	**14e**	5.05	-13.85
12c	5.47	-14.57	**15a**	5.31	-13.97
12d	5.45	-13.42	**15b**	5.93	-15.37
12e	5.03	-13.63	**15c**	5.57	-14.89
13a	5.23	-14.09	**15d**	5.26	-13.47
13b	5.01	-13.11	**15e**	5.05	-13.48
13c	5.81	-13.65			

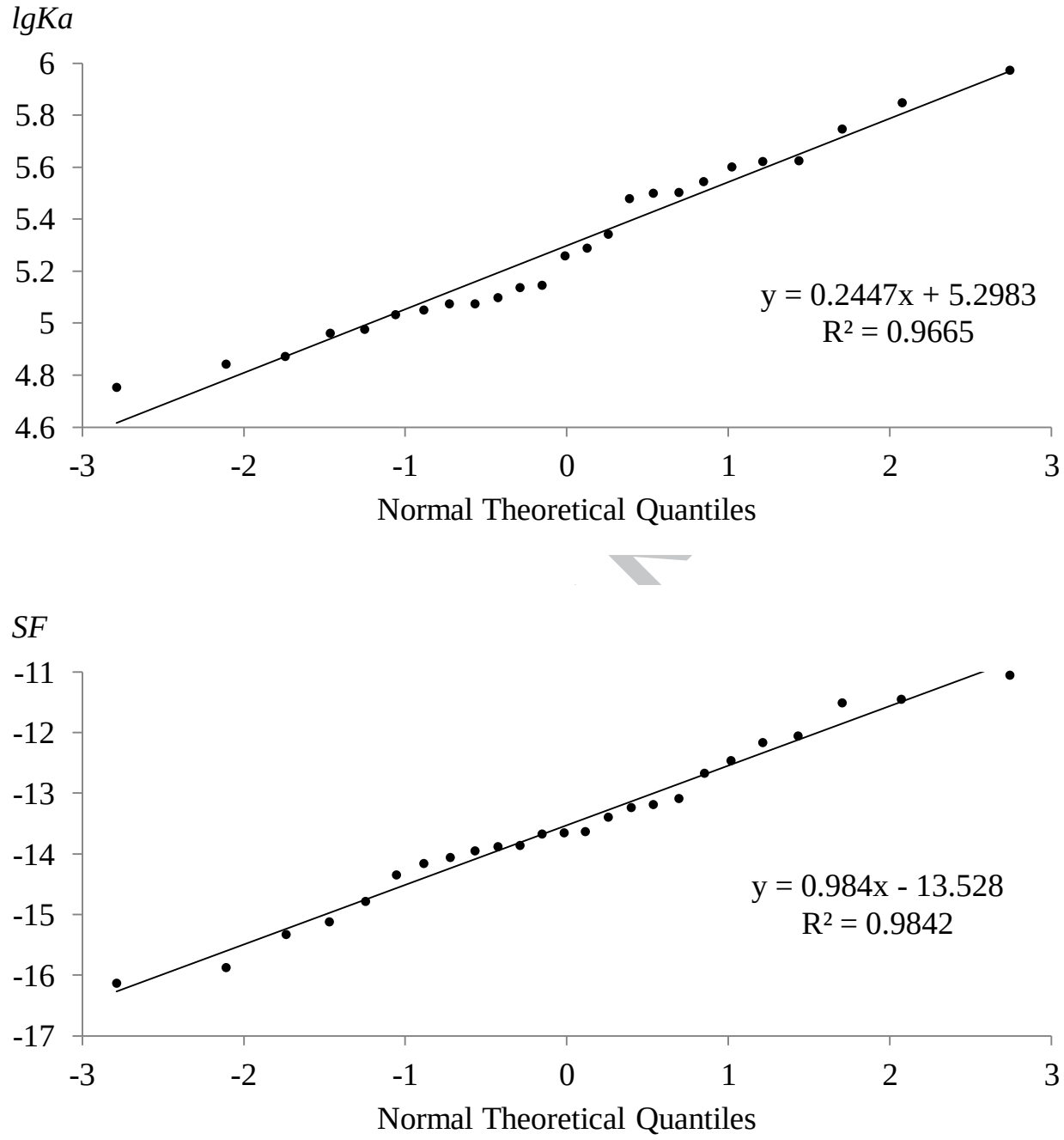


Figure 4. Quantile-quantile plots of $lgKa$ (top plot) or SF (bottom plot) data distribution vs normal distribution (solid line)

Therefore, usage of parametric statistics is justified in this case. Linear regression equation was calculated using least squares method (eq.1).

$$lgKa = -0.21639\ (\pm 0.04821)SF + 2.29530(\pm 0.66351) \quad (1)$$

Measurement of linear regression goodness-of-fit was crucial, as well as checking, if there are any outlier prediction values for this regression (Table 6).

Table 6. Statistic assessment of linear regression goodness-of-fit (sequence d(CCIICICCII)$_2$)

MAE	0.18
MAPE	3.43%
RMSE	0.24
R^2	0.467
outliers	0%

It is worth noticing that SF data has low scattering with $max\ (lgKa) = 5.93$, $min\ (lgKa) = 4.74, mean\ (lgKa) = 5.26\ and\ St.dev.(lgKa) = 0.33$. Overall statistic parameters show acceptable level of regression fit (Fig. 5). Outliers were defined using the Grubbs test for outliers [35], i. e. compound is an outlier if difference between observed and predicted values exceeds triple RMSE. Total number of outlier is given as percent of all data points.

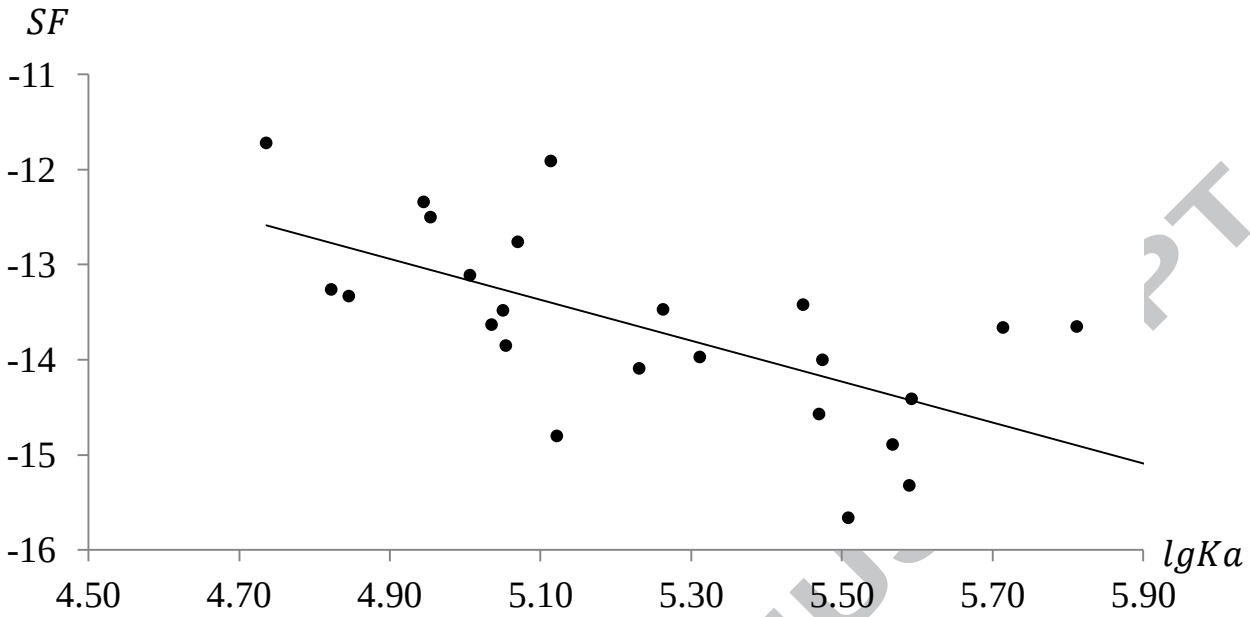


Figure 5. ???????? vs ???? plot. Solid line is a trend line which corresponds to regression equation (sequence d(CCIICICCII)$_2$)

This regression equation was used for prediction of $lgKa$ of benzidine derivatives (**10a-10e**). **10a-10e** were synthesized, tested for DNA affinity and docked into the same oligonucleotide as previously described compounds (Table 7), exhibiting the same minor groove binding mode. Comparison of $lgKa$ experimental value and $lgKa$ calculated using regression equation showed that calculated value is close to modelled one with MAE = 0.15 and RMSE = 0.11.

Table 7. $lgKa$ and SF values for benzidines (sequence d(CCIICICCII)$_2$)

Compounds ID	$lgKa$	$lgKa$ (theoretical)	SF
10a	4.74 ± 0.02	4.83 ± 1.40	-11.72
10b	4.95 ± 0.09	5.00 ± 1.15	-12.5
10c	4.82 ± 0.02	5.16 ± 1.12	-13.26
10d	5.11 ± 0.06	4.87 ± 1.36	-11.91
10e	4.94 ± 0.02	4.96 ± 1.23	-12.34

Such good prediction encourages us to presume that applicability domain of above-mentioned equation can comprise compounds of classes other than biphenyl as long as they tend to have similar binding mode.

Aminoalkoxybiphenyls **11a-15e** were also docked in another oligonucleotide (Table 8) [36], where N,N'-bis[3-(4,5-dihidro-1H-imidazol-2-yl)phenyl]biphenyl-4,4'-dicarboxamide was bound to d(CGCGAATTCGCG)$_2$ sequence (Fig.6).

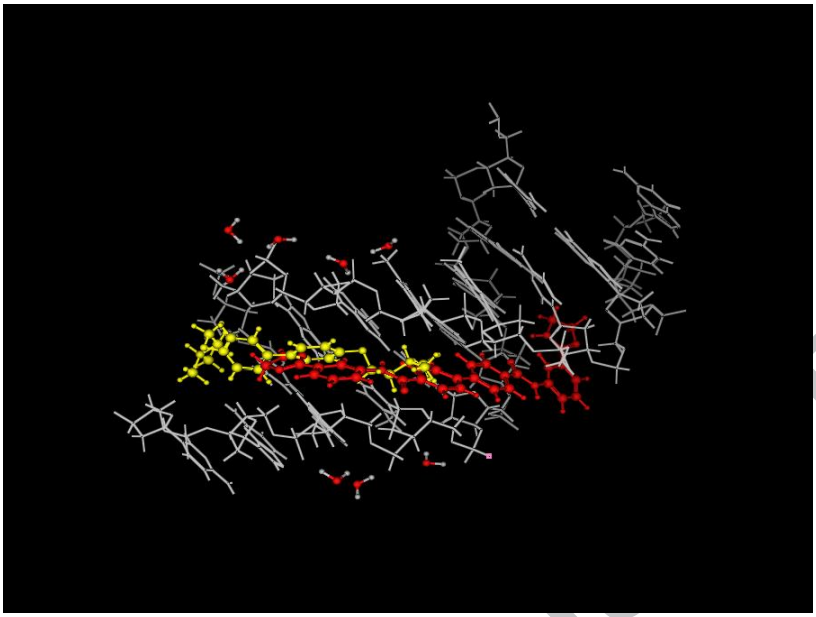


Figure 6. Binding of the aminoalkoxybiphenyls in the DNA minor groove sequence d(CGCGAATTCGCG)$_2$ (in red – native ligand netropsin; in yellow – aminoalkoxybiphenyl, in gray – polynucleotide)

Table 8. $lgKa$ and minimum SF values for biphenyls (sequence d(CGCGAATTCGCG)$_2$)

Compounds ID	SF	Compounds ID	SF	Compounds ID	SF
11a	-11.77	**12e**	-11.54	**14c**	-12.09
11b	-12.34	**13a**	-11.63	**14d**	-12.60
11c	-12.30	**13b**	-12.57	**14e**	-14.46
11d	-9.73	**13c**	-13.65	**15a**	-13.50
11e	-14.10	**13d**	-12.93	**15b**	-12.85
12a	-12.07	**13e**	-12.57	**15c**	-12.60
12b	-12.55	**14a**	-14.15	**15d**	-12.23
12c	-13.00	**14b**	-13.19	**15e**	-14.31
12d	-12.20				

In this case correlation between SF and lgK_a is absent (R^2 = 0.014). The correlation between SF and lgK_a in case of d(CCIICICCII)$_2$ sequence and formation of complexes aminoalkoxybiphenyls with DNA d(CGCGAATTCGCG)$_2$ sequence in the rich GC DNA regions in favor of selective binding aminoalkoxybiphenyls with GC DNA sequences.

3. Conclusion

The series of planar fluorenones **(3a-7d)** and nonplanar biphenyls **(11a-d)** were prepared for the comparative analysis of interaction with DNA. Initial evaluation of the affinity for these two series of compounds to DNA revealed the deference between the classic intercalation of

fluorenones and non-intercalating mechanism of action for biphenyls. Molecular docking studies allowed us to suggest that aminoalkoxybiphenyls can potentially bind to a minor grove of DNA, particularly to d(CCIICICCII)$_2$ sequence. The negative correlation between lgKa and docking SF was defined and a regression equation, which was capable of lgKa prediction for the structures similar to biphenyls was derived. Despite the fact that the two series of compounds demonstrated substantially different in affinity and binding mode to DNA, their pharmacological properties were similar. Further studies on these DNA minor groove binders and their analogues, along with extended structural investigation are required to better understand regulatory factors guiding the ability to develop antiviral activity.

4 Experimental

4.1. Chemistry

All reagents and solvents were purchased from commercial sources and were used as received, unless noted otherwise. Melting points were determined with a Kofler bench melting point apparatus, and are uncorrected. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on Bruker Avance 500 MHz spectrometer with TMS as an internal standard. Mass-spectra (MS) were obtained with VG 70 70 EQ spectrometer (ionization was carried out by Ar atomic beam with 10 kV energy; compounds were dissolved in 3-nitrobenzyl alcohol. All reactions and the purity of the synthesized compounds were controlled by TLC on Merck Aluminum Foil Silica gel 60 F_{254}. UV-Vis spectroscopy was performed on a Shimadzu UV-2401PC spectrophotometer. Microanalyses were performed for the indicated elements, and the results are within 0.4% of calculated values. All buffers, intermediates and working solutions were prepared using chemically pure grade reagents and distilled water.

4.1.1. 2,7-Bis(2-chloroethoxy)fluoren-9-one (**2a**)

A mixture of 2,7-dihydroxyfluorenone (**1**) (23.34 g, 0.11 mol), 1,2-dichloroethane (250 mL), 20% NaOH aqueous solution (50 mL) and tetrabutylammonium chloride (7.50 g, 0.027 mol) were vigorously stirred at 75 °C for one day. After the reaction mixture was cooled, organic layer was separated and washed with 5% NaOH (3 × 20 mL) and H_2O (3 × 20 mL), dried ($CaCl_2$) and filtered. The filtrate was evaporated under reduced pressure to afford the pure **2b** as a red solid after recrystallization from *i*-PrOH (32.27 g, yield: 87%); mp 145 – 146 °C (Lit. mp 142 – 144 °C, [23]).

4.1.2. 2,7-Bis(2-chloropropoxy)fluoren-9-one (**2b**).

A mixture of 2,7-dihydroxyfluorenone (**1**) (23.34 g, 0.11 mol), 1,3-bromochloropropane (157.44 g, 1.0 mol) and anhydrous K_2CO_3 (64 g, 0.46 mol) in DMF (150 mL) was stirred at 80°C for one day. Upon completion of reaction, the mixture was diluted with H_2O (600 mL). The precipitate was filtered and recrystallized from EtOH to give a solid (33.33 g, yield: 83%); mp: 124 – 125 °C.

4.1.3. 2,7-Bis(4-bromobutoxy)fluoren-9-one (**2c**).

A mixture of 2,7-dihydroxyfluorenone (**1**) (23.34 g, 0.11 mol), 1,4-dibromobutane (216 g, 1.00 mol) and anhydrous K_2CO_3 (64 g, 0.46 mol) in DMF (150 mL) was stirred at 80º C for one day. Upon completion of reaction, the mixture was diluted with H_2O (600 mL). The precipitate was filtered and recrystallized from EtOH to give a solid; (39.25 g, yield: 74%); mp 109 – 110° C.

Intermediates **2d – 2e** were papered in a similar manner to **2b – 2c**

4.1.4. 2,7-Bis(4-bromopentoxy)fluoren-9-one (**2d)**

Yield: 78%; mp 96–97 °C.

4.1.5. 2,7-Bis(4-bromohexoxy)fluoren-9-one (**2e**)

Yield: 71%; mp 83–84 °C.

4.1.6. 2,7-Bis(2-iodoethoxy)fluoren-9-one (2**f**).

A solution of NaI (24.00 g, 0.16 mol) and tetrabutylammonium iodide (29.55 g, 0.08 mol) in H_2O (70 mL) was added to a solution of **2b** (26.98 g 0.08 mol) in toluene (200 mL). The mixture was refluxed with vigorous stirring for 10 h and then cooled to the room temperature. The organic layer was separated, washed with 5% $Na_2S_2O_3$, H_2O, dried (Na_2SO_4), and filtered. The filtrate was evaporated under reduced pressure to provide a solid material; (39.95 g, Yield: 96 %); mp 154 – 155 °C.

Compound **2g** was papered in a similar manner to **2f.**

4.1.7. 2,7-Bis(3-iodopropoxy)fluoren-9-one (**2g**)

Yeild: 95%; mp 120 – 121 °C.

4.1.8. 2,7-Bis[2-(diethylamino)ethoxy]fluoren-9-one Dihydrochloride (**3a**)

Diethylamine (0.73 g, 0.01 mol) was added to a solution of **2f** (1.10 g 0.002 mol) in DMF (5 mL). The reaction mixture was kept at room temperature for 5 days, diluted with H_2O (200 mL), and then acidified with HCl to achieve a pH of 2-3. The acidic solution was washed with $CHCl_3$ (3 × 50 mL), and then basified with aqueous NaOH until pH was 12 – 13. The product was

extracted with $CHCl_3$ (3 × 50 mL). The combined $CHCl_3$ layers were washed with H_2O until pH of aqueous layer was about 7, dried (Na_2SO_4) and evaporated under reduced pressure. The resultant residue was dissolved in anhydrous dioxane (20 mL) and then saturated anhydrous solution of HCl in dioxane was added to the resulting solution while stirring. The solvent was evaporated under reduced pressure to give a crude gummy residue that was suspended in acetone. A stirred suspension was refluxed for 15 min, followed by filtration to give a solid material. The procedure was repeated until a pure reddish solid product **(3a)** was afforded; (0.80 g, Yield: 83%); mp 236 – 237 °C (Lit. mp 235 – 236 °C [23]; FAB-MS m/z: M^+ = 411.

Compounds **3b – 7d** were papered in a similar manner to **3a.**

4.1.9. 2,7-Bis[2-(dimethylamino)ethoxy]fluoren-9-one Dihydrochloride (**3b**)

Yield: 78%; mp 272 – 273 °C; FAB-MS m/z: M+ = 355; Lit. mp 278 – 280 °C [23].

4.1.10. 2,7-Bis(2-piperidin-1ylethoxy)fluoren-9-one Dihydrochloride (**3c**)

Yield: 87%; mp 304 – 305 °C; FAB-MS m/z: M+ = 435; Lit. mp 304 – 306 °C [23].

4.1.11. 2,7-Bis(2-azepan-1-ylethoxy)fluoren-9-one Dihydrochloride (**3d**)

Yield: 85%; mp 291 – 292 °C; ^{1}H NMR ($CDCl_3$) δ: 1.6 (m, 8 H, $2CH_2$+$2CH_2$), 1.6 (m, 8 H, $2CH_2$+$2CH_2$), 2.7 (t, 8 H, $2CH_2$+$2CH_2$), 2.9 (t, *J*=5.8 Hz, 4 H, $2CH_2$), 4.0 (t, *J*=5.8 Hz, 4 H, $2CH_2$), 6.9 (dd, *J*=7.8, 2.3 Hz, 2 H, 2CH), 7.1 (d, *J*=2.0 Hz, 2 H, 2CH), 7.2 (d, *J*=7.8 Hz, 2 H, 2CH); ^{13}C NMR ($CDCl_3$) δ: 27.0, 28.0, 55.8, 56.3, 67.1, 110.4, 120.4, 120.9, 135.9, 137.5, 159.3, 193.7; FAB-MS m/z: M+ = 463; Anal. calc. for $C_{29}H_{38}N_2O_3$·2HCl: C 65.04, H 5.23, N 7.53; found C 64.70, H 5.57, N 7.87.

4.1.12. 2,7-Bis[3-(diethylamino)propoxy]fluoren-9-one Dihydrochloride (**4a**)

Yield: 90%; mp 252–253 °C; FAB-MS m/z: M+ = 439; Lit. mp 256–257 °C [23].

4.1.13. 2,7-Bis[3-(dimethylamino)propoxy]fluoren-9-one Dihydrochloride (**4b**).

Yield: 80%; mp 280 – 281 °C; FAB-MS m/z: M+ = 383; Lit. mp 282 – 283 °C [23].

4.1.13. 2,*7-Bis(3-piperidin-1-ylpropoxy)fluoren-9-one Dihydrochloride* (**4c**)

Yield: 90%; mp 278 – 279 °C; FAB-MS m/z: M+ = 463; Lit. mp 279 – 280 °C [23].

4.1.14. 2,*7-Bis(3-azepan-1-ylpropoxy)fluoren-9-one Dihydrochloride* (**4d**)

Yield: 76%; mp 266 – 267 °C; FAB-MS m/z: M^+ = 491; ^{1}H NMR ($CDCl_3$) δ: 1.6 (m, 8 H, $2CH_2$+$2CH_2$), 1.7 (m, 8 H, $2CH_2$+$2CH_2$), 2.0 (t, 4 H, $2CH_2$), 2.7 (t, *J*=4.9 Hz, 8H, $2CH_2$+$2CH_2$), 2.8 (t, *J*=4.9 Hz, 4H, $2CH_2$), 4.0 (t, *J*=5.9 Hz, 4 H, $2CH_2$), 6.9 (d, *J*=8.0 Hz, 2 H, 2CH), 7.1 (s, 2 H, 2CH), 7.2 (d, *J*=8.0 Hz, 2 H, 2CH); ^{13}C NMR ($CDCl_3$) δ: 26.6, 27.3, 46.4, 54.2, 55.1, 66.4, 110.0, 120.1, 120.3, 135.5, 137.0, 159.0, 193.4; Anal. calc. for $C_{31}H_{42}N_2O_3$·2HCl: C 66.06, H 4.97, N 7.87; found C 66.38, H 4.79, N 7.91.

4.1.15. 2,7-Bis[4-(diethylamino)butoxy]fluoren-9-one Dihydrochloride (**5a**)

Yield: 77%; mp 230 – 231 °C; FAB-MS m/z: M+ = 467; Lit. mp 233–234 °C; [23]

4.1.16. 2,7-Bis[4-(dimethylamino)butoxy]fluoren-9-one Dihydrochloride (**5b**)

Yeild: 75%; mp 299–300 °C; FAB-MS m/z: M^+ = 411; ^{1}H NMR ($CDCl_3$) δ: 1.6 (m, *J*=6.0 Hz, 4 H, $2CH_2$), 1.8 (m, *J*=6.0 Hz, 4 H, $2CH_2$), 2.2 (s, 12 H, $4CH_3$), 2.3 (t, 4 H, $2CH_2$), 4.0 (t, 4 H, $2CH_2$), 6.9 (d, *J*=8.0 Hz, 2 H, 2CH), 7.1 (s, 2 H, 2CH), 7.2 (d, *J*=8.0 Hz, 2 H, 2CH); ^{13}C NMR ($CDCl_3$) δ: 23.8, 26.6, 45.1, 58.9, 67.8, 109.8, 120.0, 120.4, 135.5, 137.0, 159.0, 193.4; Anal. calc. for $C_{25}H_{34}N_2O_3 \cdot 2HCl$: C 62.11, H 5.79, N 7.51; found C 62.08, H 5.59, N 7.28.

4.1.17. 2,7-Bis(4-piperidin-1-ylbutoxy)fluoren-9-one Dihydrochloride (**5c**)

Yeild: 80%; mp 253 – 254 °C; FAB-MS m/z: M^+ = 491; ^{1}H NMR ($CDCl_3$) δ: 1.4 (m, 4 H, $2CH_2$), 1.6 (m, *J*=4.9 Hz, 9 H, $2CH_2$+$2CH_2$), 1.6 (m, 4 H, $2CH_2$), 1.8 (m, *J*=6.6 Hz, 4 H, $2CH_2$), 2.3 (t, 4 H, $2CH_2$), 2.3 (t, *J*=7.7 Hz, 8 H, $2CH_2$+$2CH_2$), 4.0 (t, *J*=6.0 Hz, 4 H, $2CH_2$), 6.9 (d, *J*=7.8 Hz, 2 H, 2CH), 7.1 (s, 2 H, 2CH), 7.2 (d, *J*=8.0 Hz, 2 H, 2CH); ^{13}C NMR ($CDCl_3$) δ: 23.0, 24.1, 25.6, 26.9, 54.2, 58.6, 67.9, 109.8, 120.0, 120.4, 135.5, 137.0, 159.0, 193.4; Anal. calc. for $C_{31}H_{42}N_2O_3 \cdot 2HCl$: C 66.06, H 4.97, N 7.87; found C 66.14, H 4.80, N 7.59.

4.1.18. 2,7-Bis(4-azepan-1-ylbutoxy)fluoren-9-one Dihydrochloride (**5d**)

Yield: 74%; mp 248 – 249 °C; FAB-MS m/z: M^+ = 519; ^{1}H NMR ($CDCl_3$) δ: 1.6 (m, 12 H, $2CH_2$+$2CH_2$, $2CH_2$), 1.6 (m, 8 H, $2CH_2$+$2CH_2$), 1.8 (m, 4 H, $2CH_2$), 2.5 (t, *J*=7.4 Hz, 4 H, $2CH_2$), 2.6 (t *J*=5.8 Hz, 8 H, $2CH_2$+$2CH_2$), 4.0 (t, *J*=6.4 Hz, 4 H, $2CH_2$), 6.9 (dd, *J*=8.2, 2.5 Hz, 2 H, 2CH), 7.1 7.1 (d, *J*=2.5 Hz, 2 H, 2CH), 7.2 (d, *J*=8.0 Hz, 2 H, 2CH); ^{13}C NMR ($CDCl_3$) δ: 23.6, 26.6, 26.8, 27.6, 55.1, 57.4, 68.0, 109.8, 120.0, 120.4, 135.5, 137.0, 159.0, 193.4; Anal. calc. for $C_{33}H_{46}N_2O_3 \cdot 2HCl$: C 66.99, H 4.73, N 8.18; found C 66.74, H 4.93, N 8.26.

4.1.19. 2,7-Bis[5-(diethylamino)pentoxy]fluoren-9-one Dihydrochloride (**6a**)

Yield: 72%; mp 212 – 213 °C; FAB-MS m/z: M^+ = 495; ^{1}H NMR ($CDCl_3$) δ: 1.0 (t, *J*=7.1 Hz, 12 H, $4CH_3$), 1.4 (m, *J*=13.7, 7.1, 6.9 Hz, 4 H, $2CH_2$), 1.5 (m, 4 H, $2CH_2$), 1.8 (m, *J*=14.0, 6.9, 6.6 Hz, 4 H, $2CH_2$), 2.4 (m, 4 H, $2CH_2$), 2.5 (q, *J*=7.1 Hz, 8 H, $4CH_2$), 4.0 (t, *J*=6.4 Hz, 4 H, $2CH_2$), 6.9 (dd, *J*=8.1, 2.1 Hz, 2 H, 2CH), 7.1 (d, *J*=2.2 Hz, 2 H, 2CH), 7.2 (d, *J*=8.0 Hz, 2 H, 2CH); ^{13}C NMR ($CDCl_3$) δ: 11.2, 23.7, 26.4, 28.7, 46.5, 52.4, 68.0, 109.8, 120.0, 120.4, 135.5, 137.0, 159.0, 193.4; Anal. calc. for $C_{31}H_{46}N_2O_3$·2HCl: C 65.59, H 4.94, N 8.52; found C 65.90, H 5.25, N 8.52.

4.1.20 2,7-Bis[5-(dimethylamino)pentoxy]fluoren-9-one Dihydrochloride (**6b**)

Yield: 68 %; mp 214 – 215 °C; FAB-MS m/z: M^+ = 439; ^{1}H NMR ($CDCl_3$) δ: 1.4 (m, *J*=7.4, 7.1, 6.9, 6.9 Hz, 4 H, $2CH_2$), 1.5 (m, 4 H, $2CH_2$), 1.8 (m, 4 H, $2CH_2$), 2.3 (s, 12 H, $4CH_2$), 2.3 (m, 4 H, $2CH_2$), 3.9 (t, *J*=6.4 Hz, 4 H, $2CH_2$), 6.9 (dd, *J*=8.2, 2.4 Hz, 2 H, 2CH), 7.1 (d, *J*=2.3 Hz, 2 H, 2CH), 7.2 (d, *J*=8.3 Hz, 2 H, 2CH); ^{13}C NMR ($CDCl_3$) δ: 23.8, 27.0, 29.0, 45.1, 59.4, 68.3, 110.2, 120.5, 120.7, 135.9, 137.4, 159.4, 193.8; Anal. calc. for $C_{27}H_{38}N_2O_3$·2HCl: C 63.40, H 5.48, N 7.88; found C 63.28, H 5.29, N 7.91.

4.1.21. 2,7-Bis(5-piperidin-1ylpentoxy)fluoren-9-one Dihydrochloride (**6c**)

Yield: 73%; mp 231 – 232 °C; FAB-MS m/z: M^+ = 519; ^{1}H NMR ($CDCl_3$) δ:1.4 (m, 8 H, $2CH_2$; $2CH_2$), 1.6 (m, 12 H, $2CH_2$+$2CH_2$; $2CH_2$), 1.8 (m, 4 H, $2CH_2$), 2.3 (t, 4 H, $2CH_2$), 2.4 (t, 8 H, $2CH_2$+$2CH_2$), 4.0 (t, *J*=6.4 Hz, 4 H, $2CH_2$), 6.9 (dd, *J*=8.2, 2.5 Hz, 2 H, 2CH), 7.1 (d, *J*=2.2 Hz, 2 H, 2CH), 7.2 (d, *J*=8.0 Hz, 2 H, 2CH); ^{13}C NMR ($CDCl_3$) δ: 23.8, 24.1, 25.6, 26.3, 28.7, 54.3, 59.0, 68.0, 109.8, 120.0, 120.4, 135.5, 137.0, 159.0, 193.4; Anal. calc. for $C_{33}H_{46}N_2O_3$·2HCl: C 66.99, H 4.73, N 8.18; found C 67.04, H 4.60, N 8.49.

4.1.22. 2,7-Bis(5-azepan-1-ylpentoxy)fluoren-9-one Dihydrochloride (**6d**)

Yield: 69%; mp 223 – 224 °C; FAB-MS m/z: M^+ = 547; ^{1}H NMR ($CDCl_3$) δ: 1.4 (m, 4 H, $2CH_2$), 1.5 (m, 12 H, $2CH_2$+$2CH_2$, $2CH_2$), 1.6 (m, *J*=4.7 Hz, 4 H, $2CH_2$), 1.8 (m, 8 H, $2CH_2$+$2CH_2$), 2.5 (t, *J*=7.7 Hz, 4 H, $2CH_2$), 2.6 (t, *J*=5.8 Hz, 8 H, $2CH_2$+$2CH_2$), 4.0 (t, *J*=6.4 Hz, 4 H, $2CH_2$), 6.9 (dd, *J*=8.1, 2.3 Hz, 2 H, 2CH), 7.1 (d, *J*=2.5 Hz, 2 H, 2CH), 7.2 (d, *J*=8.2 Hz, 2 H, 2CH); ^{13}C NMR ($CDCl_3$) δ: 23.6, 26.6, 26.9, 27.5, 28.7, 55.2, 57.7, 68.0, 109.8, 120.0, 120.4, 135.5, 137.0, 159.0, 193.4; Anal. calc. for $C_{35}H_{50}N_2O_3$·2HCl: C 67.83, H 4.52, N 8.46; found C 68.03, H 4.43, N 8.15.

4.1.23. 2,7-Bis[6-(diethylamino)hexoxy]fluoren-9-one Dihydrochloride (**7a**)

Yield: 72%; mp 177 – 178 °C; FAB-MS m/z: M^+ = 523; ^{1}H NMR ($CDCl_3$) δ: 1.1 (t, *J*=7.0 Hz, 12 H, $4CH_3$), 1.3 (m, 4 H, $2CH_2$), 1.5 (m, 4 H, $2CH_2$), 1.5 (m, 4 H, $2CH_2$), 1.7 (m, *J*=14.1, 6.8 Hz, 4 H, $2CH_2$), 2.5 (t, 4 H, $2CH_2$), 2.6 (q, *J*=6.9 Hz, 8 H, $4CH_2$), 3.9 (t, *J*=6.1 Hz, 4 H, $2CH_2$), 6.9 (d, *J*=7.8 Hz, 2 H, 2CH), 7.1 (s, 2 H, 2CH), 7.2 (d, *J*=8.0 Hz, 2 H, 2CH); ^{13}C NMR ($CDCl_3$) δ: 11.0, 25.9, 26.1, 27.2, 29.1, 46.8, 52.5, 68.4, 110.2, 120.5, 120.8, 135.9, 137.4, 159.4, 193.8; Anal. calc. for $C_{33}H_{50}N_2O_3$·2HCl: C 66.54, H 4.70, N 8.80; found C 66.61, H 4.98, N 9.05.

4.1.24. 2,7-Bis[6-(dimethylamino)hexoxy]fluoren-9-one Dihydrochloride (**7b**)

Yield: 65 %; mp 197 – 198 °C; FAB-MS m/z: M^+ = 467; ^{1}H NMR ($CDCl_3$) δ: 1.3 (m, *J*=6.8, 6.8, 6.8, 6.8 Hz, 4 H, $2CH_2$), 1.4 (m, 4 H, $2CH_2$), 1.5 (dt, *J*=14.9, 7.5 Hz, 4 H, $2CH_2$), 1.7 (m, 4 H, $2CH_2$), 2.3 (s, 12 H, $4CH_2$), 2.4 (m, 4 H, $2CH_2$), 3.9 (t, *J*=6.4 Hz, 4 H, $2CH_2$), 6.9 (dd, *J*=8.2, 2.4 Hz, 2 H, 2CH), 7.1 (d, *J*=2.0 Hz, 2 H, 2CH), 7.2 (d, *J*=8.3 Hz, 2 H, 2CH); ^{13}C NMR ($CDCl_3$) δ:

25.9, 27.0, 27.0, 29.1, 45.0, 59.4, 68.4, 110.2, 120.5, 120.8, 135.9, 137.4, 159.4, 193.8; Anal. calc. $C_{29}H_{42}N_2O_3 \cdot 2HCl$: for C 64.55, H 5.19, N 8.22; found C 64.21, H 5.20, N 8.12.

4.1.25. 2,7-Bis(6-piperidin-1ylhexoxy)fluoren-9-one Dihydrochloride (**7c**)

Yield: 79%; mp 224 – 225 °C; FAB-MS m/z: M^+ = 547. ^{1}H NMR ($CDCl_3$) δ: 1.3 (m, 4 H, $2CH_2$), 1.4 (m, *J*=4.5 Hz, 8 H, $2CH_2$+$2CH_2$), 1.5 (m, *J*=7.3, 3.0 Hz, 4 H, $2CH_2$), 1.6 (d, *J*=5.0 Hz, 8 H, $2CH_2$; $2CH_2$), 1.8 (m, 4 H, $2CH_2$), 2.3 (t, 4 H, $2CH_2$), 2.4 (s, 8 H, $2CH_2$+$2CH_2$), 3.9 (t, *J*=6.1 Hz, 4 H, $2CH_2$), 6.9 (dd, *J*=7.8, 2.3 Hz, 2 H, 2CH), 7.1 (d, *J*=2.0 Hz, 2 H, 2CH), 7.2 (d, *J*=7.8 Hz, 2 H, 2CH); ^{13}C NMR ($CDCl_3$) δ: 24.3, 25.7, 25.9, 26.6, 27.4, 29.1, 54.5, 59.3, 68.4, 110.2, 120.4, 120.8, 136.0, 137.4, 159.5, 193.9; Anal. calc. for $C_{35}H_{50}N_2O_3 \cdot 2HCl$: C 67.83, H 4.52, N 8.46; found C 67.95, H 4.19, N 8.57.

4.1.26. 2,7-Bis(6-azepan-1-ylhexoxy)fluoren-9-one Dihydrochloride (**7d**)

Yield: 71%; mp 212–214 °C; FAB-MS m/z: M^+ = 575; ^{1}H NMR ($CDCl_3$) δ: 1.4 (m, 4 H, $2CH_2$), 1.5 (m, 4 H, $2CH_2$), 1.6 (m, 12 H, $2CH_2$+$2CH_2$, $2CH_2$), 1.8 (m, 12 H, $2CH_2$+$2CH_2$, $2CH_2$), 2.7 (m, 4 H, $2CH_2$), 2.9 (m, 8 H, $2CH_2$+$2CH_2$), 3.9 (t, *J*=6.1 Hz, 4 H, $2CH_2$), 6.9 (dd, *J*=8.0, 2.3 Hz, 2 H, 2CH), 7.1 (d, *J*=2.0 Hz, 2 H, 2CH), 7.2 (d, *J*=8.0 Hz, 2 H, 2CH); ^{13}C NMR ($CDCl_3$) δ: 25.5, 25.8, 25.9, 25.9, 27.0, 29.0, 55.0, 57.8, 68.3, 110.3, 120.5, 120.8, 135.9, 137.4, 159.4, 193.8; Anal. calc. for $C_{37}H_{54}N_2O_3Cl_2$ 2HCl: C 68.61, H 4.32, N 8.71; found C 68.46, H 4.31, N 8.85.

4.1.27. N,N'-Biphenyl-4,4'-diylbis(2-chloroacetamide) (**9**)

Chloroacetyl chloride (35.24 g, 0.312 mol) was added dropwise to a stirred solution of benzidine (**8**) (22.11 g, 0.12 mol) in toluene (500 mL) at room temperature. The resulting mixture was

refluxed for 2 h, cooled to room temperature, and concentrated under reduced pressure. The resultant residue was suspended in $CHCl_3$ (500 mL) and refluxed for an additional 0.5 h. The suspension was cooled to room temperature, and then the precipitate was filtered off and washed with $CHCl_3$ (3 × 50 mL) yielded 32.38 g, 80%; mp. 275–276 °C. The product **9** was used in the next step without further purification.

4.1.28. N,*N'-Biphenyl-4,4'-diylbis[2-(diethylamino)acetamide] Dihydrochloride* (**10a**)

Diethylamine (1.10 g, 0.015 mol) was added to a solution of **9** (1.01 g 0.003 mol) in DMF (5 mL). After the reaction was completed, the reaction mixture was diluted with H_2O (10 mL) and product was extracted with $CHCl_3$ (3 × 20 mL). The combined $CHCl_3$ layers were washed with H_2O until pH of aqueous layer was 7, dried (Na_2SO_4) and evaporated under reduced pressure to dryness. The residue was dissolved in benzene (20 mL) and added to a stirred saturated anhydrous solution of HCl in dioxane. The resultant precipitate was filtered off and suspended in acetone. A stirred suspension was refluxed for 15 min, followed by filtration to give a solid material. The procedure was repeated until a pure solid product **(10a)** was afforded; (0.99 g, Yield: 68 %); mp 274 – 275 °C; FAB-MS m/z: MH^+ = 411; ^{1}H NMR ($CDCl_3$) δ: 1.1 (t, *J*=7.3 Hz, 12 H, $4CH_3$), 2.6 (q, 8 H, $2CH_2$+$2CH_2$), 3.2 (s, 4 H, $2CH_2$), 7.5 (d, *J*=8.2 Hz, 4 H, 4CH), 7.6 (d, *J*=8.2 Hz, 4 H, 4CH), 9.4 (s, 2 H, NH). Anal. calc. for $C_{24}H_{34}N_4O_2$·2HCl: C 59.62, H 11.59, N 7.51; found C 59.46, H 11.76, N 7.21.

Compounds **10b-e** were papered in a similar manner to **10a**

4.1.29. N,N'-Biphenyl-4,4'-diylbis[2-(dimethylamino)acetamide] Dihydro-chloride (**10b**)

Yield: 65%; mp 265 – 266 °C; FAB-MS m/z: MH^+ = 355; ^{1}H NMR ($CDCl_3$) δ: 2.4 (s, 12 H,

$4CH_3$), 3.1 (s, 4 H, $2CH_2$), 7.6 (d, J=8.1 Hz, 4 H, 4CH), 7.7 (d, J=8.1 Hz, 4 H, 4CH), 9.1 (s, 2 H, NH); Anal. calc. for $C_{20}H_{26}N_4O_2 \cdot 2HCl$: C 56.21, H 13.11, N 6.60; found C 56.29, H 13.04, N 6.80.

4.1.30. N,N'-Biphenyl-4,4'-diylbis(2-piperidin-y1lacetamide) Dihydrochloride (**10c**)

Yield: 62%; mp 280 – 281 °C; FAB-MS m/z: MH^+ = 435; ^1H NMR ($CDCl_3$) δ: 1.5 (m, 4 H, $2CH_2$), 1.6 (m, 8 H, $2CH_2$+$2CH_2$), 2.5 (t, 8 H, $2CH_2$+$2CH_2$), 3.1 (s, 4 H, $2CH_2$), 7.5 (d, *J*=8.2 Hz, 4 H, 4CH), 7.6 (d, *J*=8.7 Hz, 4 H, 4CH), 9.3 (s, 2 H, NH); Anal. calc. for $C_{26}H_{34}N_4O_2 \cdot 2HCl$: C 61.53, H 11.04, N 7.15; found C 61.41, H 11.02, N 7.44.

4.1.31. N,N'-Biphenyl-4,4'-diylbis(2-azepan-1-ylacetamide) Dihydrochloride (**10d**)

Yield: 70%; mp 263 – 264°C; FAB-MS m/z: MH^+ = 463; ^1H NMR ($CDCl_3$) δ: 1.7 (m, 16 H, $2CH_2$+$2CH_2$, $2CH_2$+$2CH_2$); 2.8 (t, *J*=5.5 Hz, 8 H, $2CH_2$+$2CH_2$); 3.3 (s, 4 H, $2CH_2$); 7.5 (d, *J*=9.1 Hz, 4 H, 4CH); 7.6 (d, *J*=8.2 Hz, 4 H, 4CH), 9.4 (s, 2 H, NH); Anal. calc. for $C_{28}H_{38}N_4O_2 \cdot 2HCl$: C 62.80, H 10.46, N 7.53; found C 63.02, H 10.33, N 7.19.

4.1.32. N,N'-Biphenyl-4,4'-diylbis(2-morpholin-1-ylacetamide) Dihydrochloride (**10e**)

Yield: 71 %; mp 285–286 °C; FAB-MS m/z: MH+ = 439; ^1H NMR ($CDCl_3$) δ: 2.6 (t, 8 H, $2CH_2$+$2CH_2$), 3.1 (s, 4 H, $2CH_2$), 3.8 (t, 8 H, $2CH_2$+$2CH_2$), 7.5 (d, *J*=8.2 Hz, 4 H, 4CH), 7.6 (d, *J*=8.2 Hz, 4 H, 4CH), 9.1 (s, 2 H, NH); Anal. calc. for $C_{24}H_{30}N_4O_4 \cdot 2HCl$: C 56.36, H 10.95, N 6.31; found C 56.46, H 10.79, N 6.19.

4.2. DNA-ligand interaction

4.2.1. Spectrophotometric study of DNA interaction

UV-spectra were recorded in quartz cells with 1 cm optical path length in the 0.02 M ionic strength buffer (0.1 M Hepes, 9.3 mM NaCl, pH 7.0). Concentrations of **3a – 7d** were 5 μM and **11a – 15d** 30 μM, respectively.

4.2.2. Viscosity study

The specific viscosity of solutions was determined by a Cannon-Manning semi-micro type 75 viscometer at 25 °C. All solutions passed through the membrane filter 0.4-μm for particulate matter removal. Experiment was carried out under 2.5-fold test substance excess to DNA, using 200 base pairs of long DNA which was prepared at pH 7.4 [25].

4.2.3. DNA affinity study

C_{50} values for the **3a-7d**, **11a-15e**, **10a-10e** were determined by the ethidium displacement method using DNA 10.6 μM in nucleotides, 12.7 μM EB in 0.02 M ionic strength buffer (4 mM AcONa, 18.66 mM NaCl, 0.25 mM EDTA, pH 5.5). $lgKa$ were calculated [28].

4.3. Molecular docking techniques

Aminoalkoxybiphenyls were docked using MOE 2011.10 software. The PDB data files on the DNA sequence from DNA complexes with netropsin were obtained from http://www.rcsb.org/pdb and used them as targets for docking. The targets were adjusted by protonation, deletion of unbound water, and oligonucleotides energy minimization. Energy

minimization was conducted by using the AMBER99 force field. In order to define if a target can be potentially docked, re-docking procedure was performed.

Table 9. Re-docking results

PDB	195D	1DNE	1Z8V	1DVL	2LWH	4U8B
SF	-18.87	-15.30	-16.19	-16.73	-16.96	-13.98
RMSE	1.65	1.99	2.08	1.78	2.13	0.92

Re-docking results showed (Table 9) that all targets can be potentially used for docking. Docking of **3a–7d** and **11a-d** was accomplished by using London dG scoring function [37] and Triangle Matcher Placement. Two approaches were used for best ligand pose definition: choosing minimal SF pose or pose when binding mode of docked ligand is mostly similar to binding mode of native ligand. Re-docking allowed to define that native ligand binds to dCA7, dIA6, dIA3 (d – deoxy, C – cytosine, I – inosine, numeral - the serial number of the base in the DNA sequence. All of this netropsin binding centers are on one DNA sequence (A). Therefore, those poses of docked ligands which bind to $d(CCIICICCII)_2$ were more favorable in terms of this approach.

Table 10. Spearman's ρ correlation coefficient values

PDB	195D	1DNE	1Z8V	1DVL	2LWH
Spearman's ρ^a	0.19	-0.05	-0.04	-0.75	-0.08
p-value	0.36	0.79	0.86	1.7×10^{-4}	0.7
Spearman's ρ^b	-0.24	-0.19	0.18	-0.82	-0.13
p-value	0.25	0.36	0.4	1.2×10^{-5}	0.52

[a] minimum SF approach, [b] similar binding approach, [*] at significance level (α) = 0.01 Spearman's ρ should be less than -0.64* for a confirmed negative correlation (Grubbs et al., 1950).

Finally, correlation between calculated SF and $lgKa$ was defined by applying the minimum energy pose which was chosen because almost identical Spearman's ρ for both approaches made simpler (minimum SF) method favorable (Table 10).

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Conflict of Interest

No conflict of interest.

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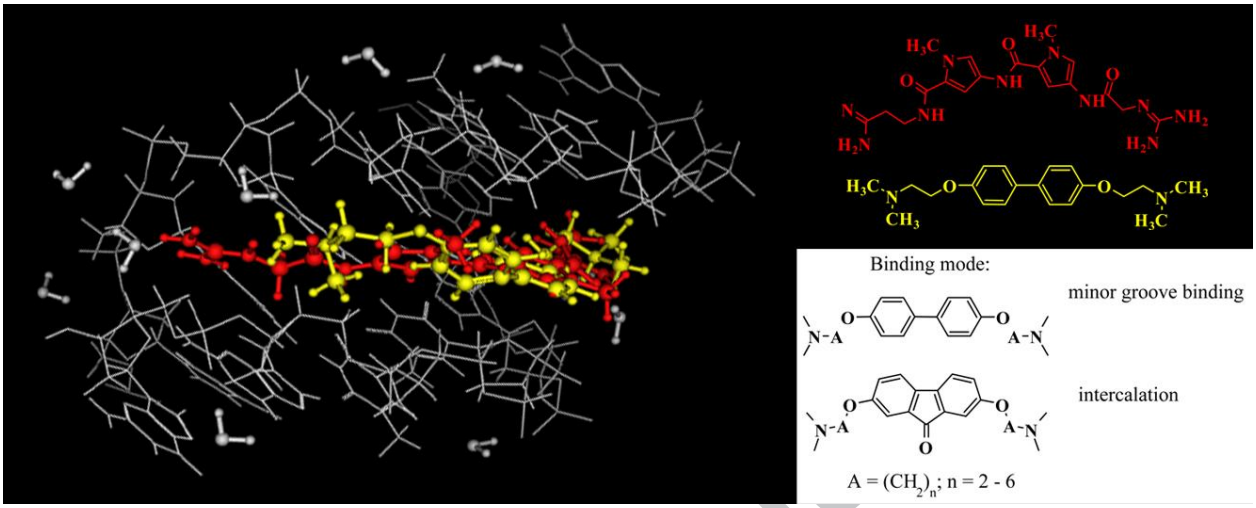
Graphical abstract



Highlights

- DNA-drug interaction in the minor groove and the intercalation of drugs into DNA may play critical roles in antiviral, antimicrobial, and antitumor activities
- The series of planar fluorenones and nonplanar biphenyls were prepared for the comparative analysis of interaction with DNA
- Despite the fact that the two series of compounds demonstrated substantially different in affinity and binding mode to DNA, their pharmacological properties were similar
- Molecular docking studies suggest that aminoalkoxybiphenyls can potentially bind to a minor grove of DNA