Structural flexibility versus rigidity of the aromatic unit of DNA ligands: binding of aza- and azoniastilbene derivatives to duplex and quadruplex DNA†

H. Ihmels, M. Karbasiyoun, K. Löhl and C. Stremmel†‡

The known azastilbene (E)-1,2-di(quinolin-3-yl)ethane (2a) and the novel azoniastilbene derivatives (E)-2-(2-(naphthalen-2-yl)vinyI)quinolizinium (2b) and (E)-3,3’-(ethene-1,2-diyl)bis(1-methylquinolin-1-ium) (2c) were synthesized. Their interactions with duplex and quadruplex DNA (G4-DNA) were studied by photometric, fluorimetric, polarimetric and flow-LD analysis, and by thermal DNA denaturation studies, as well as by 1H-NMR spectroscopy. The main goal of this study was a comparison of these conformationally flexible compounds with the known G4-DNA-binding diazoniadibenzo[b,k]chrysene, that have a comparable π-system extent, but a rigid structure. We have observed that the aza- and azoniastilbene derivatives 2a–c, i.e. compounds with almost the same spatial dimensions and steric demand, bind to DNA with an affinity and selectivity that depends significantly on the number of positive charges. Whereas the charge neutral derivative 2a binds unspecifically to the DNA backbone of duplex DNA, the ionic compounds 2b and 2c are typical DNA intercalators. Notably, the bis-quinolinium derivative 2c binds to G4-DNA with moderate affinity (Kₐ = 4.8 × 10⁵ M⁻¹) and also stabilizes the G4-DNA towards thermal denaturation (ΔTₘ = 11 °C at ligand–DNA ratio = 5.0). Strikingly, the corresponding rigid counterpart, 4a,12a-diazonia-8,16-dimethyl dibenzo[b,k]chrysene, stabilizes the G4-DNA to an even greater extent under identical conditions (ΔTₘ = 27 °C). These results indicate that the increased flexibility of a G4-DNA ligand does not necessarily lead to stronger interactions with the G4-DNA as compared with rigid ligands that have essentially the same size and π system extent.

Introduction

Among the non-canonical DNA forms, the quadruplex DNA (G4-DNA), that is formed in G-rich DNA sequences upon stacking of at least two guanine quartets, is currently attracting most attention. Thus, several G-rich DNA sequences with the propensity to fold into quadruplex structures have been identified in genomic nucleic acids, for example in telomeric DNA and some promoter regions of oncogenes. Moreover, it has been shown that some biologically relevant processes are directly related to quadruplex-DNA formation, such as the suppression of gene expression, or the induction of the cellular response to DNA damage. As a result of the essential biological functionality of quadruplex DNA, the association of an exogenous ligand with G4-DNA structures may have a significant effect on the biological function of G-rich DNA sequences, either by simply blocking the binding site of enzymes, which leads to their inhibition, or by increasing the thermodynamic stability and the lifetime of the G4-DNA. In the latter case, enzyme inhibition may also occur when the enzyme requires an unwound form of the particular DNA sequence. Based on this principle, numerous G4-DNA-targeting molecules have been developed that may affect the biological activity of the DNA. Along these lines, traditional DNA intercalators, i.e. cationic, planar, polycyclic (het)arenes, figure as a promising basis for the development of G4-DNA ligands. Thus, it has been shown that such intercalators have the propensity for a terminal π-stacking at the ends of G4-DNA structures. Such as intercalation of a ligand between base pairs in duplex DNA, the terminal π-stacking is driven by dispersion interactions between the aromatic ligand and the guanine quartet and by an additional hydrophobic effect as the lipophilic ligand migrates from the aqueous solution into the hydrophobic binding site. Because of the thermodynamically

Department of Chemistry and Biology, University of Siegen, Adolf-Reichwein-Str. 2, 57068 Siegen, Germany. E-mail: ihmels@chemie.uni-siegen.de
†Electronic supplementary information (ESI) available: Absorption and emission data of 2a–c; thermal DNA denaturation analysis; NMR spectra. See DOI: 10.1039/c9ob00809h
‡Author names are in alphabetical order that does not reflect the specific contribution of each author.
due to the possible rotation about the aryl
bind to DNA. And more recently, it has been demonstrated
been shown already that pyridinium-based azoniastilbenes
π
point to check this proposal because they have a similarly
2a
have an increased a
steric demand of the binding site. Along these lines, the influ-
tation of 1,5-di(bromomethyl)naphthalene (1d) with 2-(2-
methyl-(1,3)-dioxolan-2-yl)pyridine (6) and subsequent ion
metathesis to give bis[pyridiniummethyl]naphthalene 7. The
latter was treated with polyphosphoric acid (PPA) at 150 °C
to give the cyclodehydration product 1d in 75% yield (Scheme 2).

Absorption and emission properties
The absorption and emission spectra of the aza- and azonia-
stilbene derivatives 2a-c were recorded in DMSO, MeCN, MeOH, water, and BPE buffer solution (Fig. 1, Table S1 in
ESI†). The corresponding shifts and the band structures do
not depend strongly on the solvent properties. Thus, the long-
waveband absorption maxima are lying in a rather small
range of 325–333 nm (2a), 379–396 nm (2b) and 366–369 nm
(2c), respectively. Likewise, most of the emission bands of each
compound cover the same wavelength range with small Stokes
shifts and low to moderate emission quantum yields (2a: \( \phi_l = 0.2–0.5 \); 2b: \( \lambda_l = 397–420 \) nm, \( \phi_l = 0.2–0.5 \); 2c:

\[ \lambda_l = 491–499 \) nm, \( \phi_l = 0.2 \); 2c: \]
λ_λ = 454–462 nm \( \Phi_\lambda < 0.01–0.3 \). As the only exception, the emission spectra of derivative 2a in water and MeOH deviate from the ones in the other solvents; namely the bands are significantly broader with a very pronounced red-shifted shoulder (Fig. 1A), presumably indicating aggregation.

Spectrometric titrations with ct DNA

The interactions of the stilbene derivatives 2a–c with double-stranded calf thymus (ct) DNA were monitored by photometric and fluorimetric titrations (Fig. 2). The absorption of derivative 2a decreased during titration with no significant shift of the absorption maximum at 329 nm (Fig. 2A1). In the case of ligands 2b and 2c, the absorption maxima at 382 nm (2b) and 366 nm (2c) also decreased on addition of up to 2.8 (2b) and 6.1 (2c) molar equivalents of ct DNA, respectively, without shift of the maxima (Fig. 2A2 and A3). On further addition of DNA the absorption increased with a bathochromic shift of approx. 7 nm in each case. It should be noted that samples of 2b were occasionally contaminated with traces of the photodimer whose formation is indicated by a weak absorption at 333 nm.

In general, the characteristic emission of the stilbene derivatives 2a–c was quenched on addition of ct DNA, which was accompanied in some cases by slight shifts of the emission maxima (Fig. 2B1–B3). In the case of ligand 2b, a very small fluorescence light-up effect was observed in the first titration steps (molar equivalent <1) before the quenching occurs. The data from the photometric or fluorimetric titrations were plotted as binding isotherms and analyzed based on the theoretical binding model (cf. ESI†). The resulting binding constants are \( 6.2 \times 10^4 \text{ M}^{-1} \) (2a), \( 5.1 \times 10^4 \text{ M}^{-1} \) (2b), and \( 2.0 \times 10^5 \text{ M}^{-1} \) (2c).

Polarimetric titrations with ct DNA

To gain further insight into the DNA-binding modes the interactions between the ligands 2a–c with ct DNA were examined by circular dichroism (CD) and flow linear dichroism (LD) spectroscopy (Fig. 3). Upon addition of DNA the compound 2a developed a significant positive induced CD (ICD) band at 344 nm and negative ICD bands at 417 nm and 307 nm, but the latter was only observed at ligand–DNA-ratio (LDR) = 0.5 and 1.0. At the same time, the characteristic positive CD band of the DNA at 277 nm fluctuated slightly with increasing LDR values. In addition, flow-LD measurements revealed a developing positive signal in the absorption range of the ligand 2a.
Fluorescence-monitored quadruplex-DNA melting

The interactions of the ligands 1d and 2a–c with quadruplex DNA were investigated with representative quadruplex-forming oligonucleotide sequences. Firstly, the established FRET melting assay\(^7\) was employed to assess the stabilization of the quadruplex structure upon association with the ligands. With this method, the propensity of a ligand to stabilize quadruplex-DNA towards unfolding was determined by monitoring the temperature-dependent emission of the dye-labeled quadruplex-forming oligonucleotide F21T, fluo-d(G\(_3\)T\(_3\))\(_{4}\)G\(_3\)-tamra (fluor: fluorescein; TAMRA: tetramethylrhodamine), because only in the quadruplex form a Förster resonance energy transfer (FRET) between the two dyes is possible. The quadruplex-forming oligonucleotide was chosen for first principal studies, as it is among the most commonly used substrates for this well-established assay.\(^17\) The FRET melting experiments revealed that the ligand 2a does not stabilize the G4-DNA F21T, even at high LDR = 5.0 (Table 1), while the ligands 1d, 2b and 2c stabilize the G4-DNA F21T towards unfolding, as clearly indicated by the increase of the melting temperature, \(\Delta T_m\), of 27 °C (1d), 3 °C (2b) and 11 °C (2c) at LDR = 5.0 (Table 1). To assess whether this stabilization of G4-DNA is a general feature of the ligands 2b and 2c the same experiments were also performed with the representative quadruplex-forming oligonucleotides FmycT [fluor-d(TGAG\(_3\)TG\(_3\))\(_7\)-tamra], FkrasT [fluor-d(A\(_2\)G\(_2\)G\(_2\)TG\(_2\)A\(_2\)GAG\(_2\)A)-tamra] and Fa2T [fluor-d(ACAC\(_2\)G\(_2\)TG\(_2\))\(_2\)-tamra] (Table 1), all of which have been used already with this assay.\(^7\)\(^18\) In all cases, the quadruplex structure is also stabilized to significantly more extent by ligand 2c as compared with 2b. Nevertheless, it was also observed that the degree of G4-DNA stabilization upon binding of ligand 2c is not the same for the different oligonucleotides. Hence, the extent of stabilization decreases in the order F21T (\(\Delta T_m = 11.0^\circ C\)), FkrasT (\(\Delta T_m = 9.9^\circ C\)), FmycT (\(\Delta T_m = 6.0^\circ C\)) and Fa2T (\(\Delta T_m = 3.1^\circ C\); all values at LDR = 5). To further assess the selectivity of the quadruplex stabilization by 2c, the melting experiments were also performed in the presence of excess of the duplex-DNA forming oligonucleotide ds26.\(^17\) Under these conditions, the ligand 2c shows a thermal stabilization of the F21T by 7.5 °C at LDR = 5.0 (Table 1).

Table 1 Shift of melting temperature, \(\Delta T_m\), of the G4-DNA F21T, FmycT, FkrasT and Fa2T in the presence of the ligands 1d and 2a–c

| LDR | \(\Delta T_m^{{\circ}C}\) |
|-----|----------------|
|     | 1d F21T | 2a  | 2b   | 2c      | 2b Fa2T | 2c  | 2b Fa2T | 2c  | 2b Fa2T | 2c  |
| 1.3 | +9.3    | −0.3 | +0.7 | +4.3 (2.9) | −0.2 | <0.1 | 0.5  | 2.4  | 1.3  | 4.6 |
| 2.5 | +21.6   | +0.2 | +1.6 | +7.6 (5.0) | 0.1  | −0.2 | −0.3 | 2.9  | 1.3  | 7.2 |
| 5.0 | +27.1   | −0.4 | +3.3 | +10.6 (7.5)| 0.3  | 3.1  | 0.3  | 6.0  | 2.2  | 9.9 |

\(c_{\text{DNA}} = 0.2 \mu M\) in Na-cacodylate buffer (pH = 7.2); estimated error: ±0.5 °C. Determined fluorimetrically based on the temperature-dependent change of FRET in F21T, FmycT, FkrasT and Fa2T.\(^\text{8}\) With 1% v/v DMSO. \(\Delta T_m\) values in parentheses determined in the presence of ds26 d(G\(_3\)T\(_3\)G\(_3\)A\(_2\)G\(_2\)A\(_2\))\(_{4}\)G\(_3\)-tamra (c = 3.0 μM).
Spectrometric titrations with quadruplex-DNA

The interactions of the ligands 1d, 2b and 2c with the quadruplex-forming oligonucleotides 22AG d(G1TTA)G3 and a2 d(ACAG4TGTG4)2 were investigated by photometric and fluorimetric titrations (Fig. 4 and 5). Upon the addition of 22AG or a2, the absorption maxima of 2b (381 nm) and 2c (366 nm) decreased with the development of red-shifted absorption maxima at 401 nm (22AG) and 406 nm (a2) for 2b (Fig. 4A1 and B1), and 379 nm (22AG) and 382 nm (a2) for 2c (Fig. 4A2 and B2). In both titrations of the ligand 2c an isosbestic point was observed.

At the same time, the emission of derivatives 1d, 2b and 2c is significantly quenched upon binding to 22AG and a2 (Fig. 5). The analysis of the resulting binding isotherms from the fluorimetric titrations of the ligands 1d, 2b and 2c revealed binding constants of $1.5 \times 10^6$ M$^{-1}$ (1d), $2.1 \times 10^5$ M$^{-1}$ (2b) and $4.8 \times 10^5$ M$^{-1}$ (2c) with 22AG and $5.8 \times 10^5$ M$^{-1}$ (2b) and $4.0 \times 10^5$ M$^{-1}$ (2c) with a2.

1H-NMR spectroscopic studies

Titrations of the diazoniastilbene 2c to the quadruplex-forming oligonucleotide Tel6 d(T$_2$AG$_3$) was monitored by 1H-NMR spectroscopy (Fig. 6). This hexanucleotide forms an equilibrium between monomeric and a terminally π-stacked dimeric G4-DNA [d(T$_2$AG$_3$)], in aqueous solution$^{16,19}$ Although Tel6 is only a very simplified quadruplex model as it does not contain the loop structures, such as e.g. 22AG, this oligonucleotide or variations thereof are often employed for the NMR spectroscopic detection of terminally stacking G4-DNA ligands$^{20}$ because the interpretation of the NMR data is straightforward. It should be noted that due to limited solubility at the employed concentration range only up to 1.5 molar equivalents of the ligand 2c could be added. The titration of ligand 2c to Tel6 led to significant shifts of the 1H NMR signals of the G4-DNA Tel6 (Fig. 6). Thus, NMR signals of the guanine imino protons of the dimeric quadruplex broadened and shifted to high-field by 0.10–0.12 ppm on addition of up to 0.4 molar equivalents of 2c and eventually disappeared during the course of titration. At the same time, the imino protons of the monomeric quadruplex were shifted by 0.27–0.32 ppm to higher field. Except for the proton signals of A3H2 and T1H6, most of the bands of the aromatic protons in the range of 6–8 ppm remained relatively sharp up to 0.8 molar equivalents of 2c (cf. ESI, Fig. S2†). In addition, the
1H-NMR signals of ligand 2c also developed and shifted during the titration. As a general trend, the signals of the protons H2, H4, H6, and H7 were shifted to lower field by up to 0.4 ppm upon association of the ligand with the DNA, whereas the proton H8 is slightly shifted to higher field and H5 does not experience a significant shift (cf. ESI, Fig. S2†).

**Discussion**

The results from the spectrometric DNA titrations with ct DNA clearly indicate the binding interactions between duplex DNA and the azo- and azonastilbene derivatives 2a-c; however, with significantly different affinity and binding mode. Thus, the association of the charge neutral derivative 2a with ct DNA is rather weak, as shown by the lack of a shift of absorption band during the photometric DNA titration and by the inefficient fluorescence quenching by DNA. Moreover, the positive LD signal of the DNA-bound ligand indicates groove binding. As the development and structure of the ICD bands does not reveal a clear trend and depends strongly on the ligand the hardly soluble compound along the DNA backbone, as shown by the plain decrease of absorption band with no significant shift. Therefore the determined binding constant is rather an aggregation constant of the ligand. This undirectional binding mode of this ligand to DNA is explained by the lack of a positive charge, which is usually required for high affinity DNA ligands. In contrast, the ligands 2b and 2c exhibit the characteristic spectroscopic features of DNA intercalators, specifically polycyclic azoniahetarenes,10c,21 upon complex formation22 namely a hypochromic effect and red shift of the absorption band, fluorescence quenching, the development of a weak positive or negative ICD band, typical binding constants (2b: 5.1 × 10^4 M^-1, 2c: 2.0 × 10^5 M^-1) and – mostly indicative of the binding mode – a negative LD band in the ligand absorption range. It is tempting to conclude that the ligands 2b and 2c have different alignments in the intercalation site as the ICD signals develop with different phase. However, the sign of the ICD signals depends on the relative orientation of the transition dipole moments of the ligand and the DNA base pairs. Considering dipole moments of the ligands that are aligned along the long molecular axis in 2b and along the short molecular axis in 2c, as deduced from the substitution pattern, both ligands intercalate into DNA with the long molecule axis perpendicular to the long axis of the binding site.

In addition, it was demonstrated that the ligands 2b and 2c bind to quadruplex DNA also, as shown exemplarily with the two representative DNA forms 22AG and a2 as well as with the dye labeled quadruplex-forming oligonucleotides F21T, FmycT, FkrasT and Fa2T. With regard to the association with quadruplex DNA, the azo- and azonastilbene derivatives 2a-c show a similar trend of binding constants as with duplex DNA. It should be noted that the binding constants of the ligands with ct DNA and quadruplex DNA appear to be the same (e.g. for 2c: \( K_B^{DNA} = 2.0 \times 10^5 \text{ M}^{-1} \), \( K_B^{22AG} = 4.8 \times 10^5 \text{ M}^{-1} \)), however; they were determined in solutions with different ionic strength (10 mM BPE buffer versus 95 mM K-phosphate buffer). Therefore, they cannot be directly compared because it is known that the binding constants, especially the ones of cationic ligands, decrease with increasing ionic strength. In fact, a control experiment showed that 2c has a significantly smaller binding constant with ct DNA of \( K_B = 6.7 \times 10^4 \text{ M}^{-1} \) at higher ionic strength (cf. ESI†). In general, with the increasing number of positive charges in the molecule the binding interaction with the DNA is getting stronger; however, this effect is more pronounced in the case of quadruplex DNA 22AG. Thus, the uncharged derivative 2a does not have a stabilizing effect on quadruplex DNA which indicates a very weak binding interaction. In contrast, the positively charged stilbene derivatives 2b and 2c induce a significant stabilization of the quadruplex DNA F21T, FmycT, FkrasT and Fa2T toward unfolding, and this effect is much stronger in the case of the dicationic derivative 2c than with the monocationic one. This difference may be explained by the effect of the positive charge, namely attractive Coulomb interactions with the phosphate backbone as well as thermodynamically favorable release of counter ions from the grooves and their subsequent solvation in the aqueous medium.9 The ligands 2b and 2c also bind to the ILPR DNA a2 as indicated by the spectrometric titrations and binding constants that are in the same range as the ones observed with 22AG. However, the stabilization of the ILPR quadruplex toward unfolding is not very pronounced according to the relatively low shifts of melting temperature of Fa2T upon ligand binding. This difference between binding constants and \( \Delta T_m \) values may be explained by the different buffer solutions that are used in each experiment.18 Moreover, even the rather moderate increase of the DNA melting temperature of Fa2T is indicative of ligand binding, because similar results were obtained with the well-established quadruplex ligands thiazole orange (\( \Delta T_m = 3.1 \text{ °C} \)), porphyrine TMPyP4 (\( \Delta T_m = 6.3 \text{ °C} \)) and coralyne (\( \Delta T_m = 1.8 \text{ °C} \)) under identical conditions.18

In the case of ligand 2c, the association with the quadruplex-forming oligonucleotide Tel6 as simple model was further confirmed by NMR-spectroscopic analysis. The broadening and significant upfield-shift of the imino protons of the guanine residues of the quadruplex usually indicate a terminal π-stacking of the ligand. Although it may be tempting to assume intercalation, this mode of binding is usually not favorable in quadruplex structures.23 Moreover, the significant shifts of the ligand protons during complex formation may be explained by the π-stacking to a terminal quartet and the positioning of the ligand protons in the anisotropic cones of the guanine quartet.
The initial goal of this study was the comparison of the DNA-binding properties of the stilbene derivatives 2a-c with the ones of the structurally resembling, quadruplex-binding dibenzochrysenes 1a-c. Specifically, it should be tested whether the increased structural flexibility of the stilbenes – while maintaining a similar longitudinal extension of the π-system – increases the affinity of the former ligands to DNA. Indeed, it was observed that the structurally flexible diazonialkylbenzene 2c has a stabilizing effect on quadruplex DNA F21T (ΔTm = 11 °C; LDR = 5.0); but the diazonialkylbenzene derivatives 1a-c have been shown to induce an even larger increase of the melting temperature of quadruplex DNA F21T under almost identical conditions (ΔTm = 6–19 °C, LDR = 5.0). In the case of 1a and 2c (Table 1), the thermal stabilization of the quadruplex is only marginally influenced by the presence of duplex DNA ds26, indicating a high selectivity of these ligands for quadruplex DNA relative to duplex. Nevertheless, this selectivity appears to be slightly more pronounced for the rigid diazonialkylbenzene 1a because the decrease of the melting temperature is smaller (ΔΔTm = −2.5 °C, LDR = 5.0) than the observed with 2c (ΔΔTm = −3.1 °C). Moreover, the binding constant of 2c with quadruplex DNA 22AG is about half as large as the ones of the ligands 1a-c (2c: 4.8 × 10^5 M^-1 versus 1a-c: 2.5–3 × 10^6 M^-1). These observations imply that the gain in flexibility in ligand 2c as compared to the structurally rigid dibenzochrysenes 1a-c does not compensate the loss of overall π-surface. Thus, these results show in a direct comparison of two ligands with resembling extent and shape of the π system that at least under equilibrium conditions the π-stacking or dispersion interactions of a ligand contribute more to the affinity of a ligand to terminal binding sites of the quadruplex structure than its flexibility, although the latter would allow the ligand to adjust its conformation within the binding site in an induced-fit process. Although it may be obvious from literature data^1,7 that both rigid and flexible ligands can bind to quadruplex DNA with reasonable affinity and selectivity, to the best of our knowledge there is only one direct and explicit comparison reported between two types of ligands with closely resembling ligand structures that mainly differ in terms of rigidity of the π system.12 However, it should be emphasized that the latter study is not directly comparable with the results presented herein, because even the “rigid” ligands contain flexible substituents that may influence the overall binding affinity to G4-DNA. Complementary to those findings, our results could be helpful for ligand design, because we present the counterintuitive observation that the affinity and selectivity of a given flexible ligand may be even increased by the introduction of more rigidity. This small but significant effect may be explained by thermodynamic factors, because the rigid ligand does not lose conformational freedom, thus causing no additional entropic penalty, upon transfer from the solution to the constrained binding site.

Notably, the differences of the ligand-induced shifts of quadruplex melting temperatures on addition of 2b and 2c (ΔΔTm = 7 °C (F21T) and ΔΔTm = 3 °C (Fa2T) at LDR = 5) appear to be too large to be solely caused by the different charges and may need further attention. Considering the known, but still rather unexplored “methyl effect” of DNA ligands, the increased affinity of 2c may also be caused by its methyl substituents, i.e. supported by additional dispersion interactions between the methyl groups and the hydrophobic binding site. To assess whether this methyl effect also affects the quadruplex-stabilizing properties of the diazonialkylbenzo[b,k]chrysene scaffold and to have a better comparison with 2c, the dimethyl-substituted derivative 1d was also investigated in this study as it resembles the methyl-substitution pattern of 2c. Moreover, it was assumed that due to its close structural resemblance with the parent compounds 1a-c the derivative 1d also binds to quadruplex DNA through terminal π stacking. In fact, the methyl-substituted derivative 1d also induces a much better stabilization of the quadruplex DNA F21T toward thermal unfolding as the parent compounds 1a-c (ΔTm = 27 °C; LDR = 5.0), although with slightly smaller binding constant. Unfortunately, the bad solubility of some 1d-DNA complexes hampered its complete investigation. But at least the obtained results are in agreement with the ones with ligand 2c, which indicates that a methyl effect may operate in quadruplex ligands, presumably based on attractive dispersion interactions of the methyl substituents with the hydrophobic binding site. Nevertheless, this assumption has to be verified in a systematic study with a larger series of methyl-substituted ligand derivatives.

Conclusions

We have shown that aza- and azoniastilbene derivatives 2a-c, i.e. compounds with almost the same spatial dimensions and steric demand, bind to DNA with an affinity and selectivity that depends significantly on the number of positive charges. Whereas the charge neutral derivative 2a only binds nonspecifically to the DNA backbone of duplex DNA, the ionic compounds 2b and 2c are typical DNA intercalators. Most notably, the bis-quinoxalinium derivative 2c binds to quadruplex DNA with moderate affinity and also induces a pronounced stabilization of the quadruplex DNA towards thermal denaturation, presumably caused by an additional methyl effect. In contrast to the proposed properties of the stilbene derivatives 2b and 2c, these ligands have a significantly weaker stabilizing effect on quadruplex DNA than the dibenzochrysenes derivatives 1a-d. From this observation we carefully conclude that the increased flexibility of a quadruplex-DNA ligand does not lead to stronger interactions with the quadruplex DNA as compared with rigid ligands that have essentially the same size and extent of π-system. Certainly, this finding has to be further substantiated with a larger series of ligands and quadruplex forms; however, the present study already reveals that structural rigidity and lack of conformational freedom, as in the diazonialkylbenzene series, is not a disadvantage with respect to quadruplex-DNA binding.
Experimental

Equipment

Melting points were determined with a BÜCHI 545 (Büchi, Flawil, CH) and are uncorrected. NMR spectra were recorded on a Bruker AV 400 (1H: 400 MHz and 13C: 100 MHz) or on a Varian VNMRS 600 (1H: 600 MHz, 13C: 150 MHz) at 20 °C; chemical shifts are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard (δ = 0.0). Combustion analyses were carried out by Mr. Rochus Breuer (Organic Chemistry I, University of Siegen). Mass spectra were recorded on a Finnigan LCQ deca (driving voltage: 6 kV, impingement gas: argon, capillary temperature: 200 °C, auxiliary gas: nitrogen). Photometric titrations were recorded with a Varian Cary Eclipse spectrometer for the spectrophotometric analyses. The CD and flow-LD measurements were performed on a Chirascan spectrometer (Applied Photophysics).

Materials

Commercially purchased reagents were used without further purification. Chemicals were obtained from Alfa Aesar GmbH & Co. KG, Karlsruhe, Germany (N-bromosuccinimide, 3-bromoquinoline, ethylenetriylsilane), Acros Organics [n-butyl-lithium solution (2.5 M in hexane), 2-butenoic acid, 4-pyridylcarbonitrile]. 1,5-di(bromomethyl)naphthalene (5)11 2-(2-methyl-[1,3]-dioxolan-2-yl)pyridine (6)13 2-methylquinolinizinium bromide (3)15 and (E)-1,2-di(quinolin-3-yl)ethane (2a)15 were prepared according to literature protocols. The bispyridinium bis(tetrafluoroborate) (1d).

Synthesis of 1,5-bis[[1-(2-methyl-1,3-dioxolan-2-yl)pyridinyl]methyl]naphthalene (7). A solution of 1,5-di(bromomethyl)naphthalene (3)11 (0.75 g, 2.39 mmol) and 2-(2-methyl-[1,3]-dioxolan-2-yl)pyridine (6)13 (1.09 g, 6.60 mmol) in DMSO (15 mL) were stirred at r.t. for 10 d. The yellow brown solution was added into EtoAc (100 mL). The precipitated crude solid was filtered and washed with EtoAc and Et2O. The solid was recrystallized from MeOH (0.5 mL, with added HBF4) to obtain product 7 (600 mg, 0.92 mmol, 36%); mp = 252–254 °C (dec.).1 -1H NMR (400 MHz, DMSO-d6): δ = 1.84 (6 H, s, CH3), 3.73–3.76 (4 H, m, CH2), 4.02–4.08 (4 H, m, CH2), 6.25 (4 H, s, CH2N), 7.43 (1 H, d, J = 7 Hz, CH), 7.63 (2 H, dd, J = 8 Hz, CH), 7.79 (2 H, d, J = 9 Hz, CH), 8.20 (2 H, ddd, J = 8 Hz, J = 6 Hz, J = 1 Hz, CH), 8.39 (2 H, d, J = 8 Hz, CH), 8.74 (2 H, dd, J = 9 Hz, J = 8 Hz, CH), 9.05 (2 H, d, J = 6 Hz, CH). - 13C-NMR (DMSO-d6, 100 MHz): 26.0, 61.1, 65.3, 106.1, 109.1, 125.5, 125.7, 126.9, 128.3, 132.2, 133.6, 147.6, 148.8, 156.3. - El. anal. for C26H20N2B2F8·2 H2O (563.33) calcd (%): C 52.93; H 4.13; N 4.21.

Synthesis of 4a,12a-diazonia-8,16-dimethylbifenzo[bf,k][1,4]chrysenebis(tetrafluoroborate) (1d). The bispyridinium 7 (295 mg, 0.45 mmol) was stirred in PPA (3.06 g) at 150 °C for 24 h. The mixture was cooled to 100 °C and saturated aqueous solution of NaBF4 (10 mL) was added. After cooling slowly to r.t. the aqueous solution was extracted with MeNO2. The organic layer was separated, dried with Na2SO4, and the solvent was evaporated to obtain the product 1d as yellow needles (179 mg, 0.34 mmol, 75%); mp > 300 °C. - 1H NMR (400 MHz, DMSO-d6): δ = 3.49 (6 H, s, CH3), 8.34 (2 H, dd, J = 7 Hz, CH), 8.49 (2 H, dd, J = 8 Hz, CH), 9.14 (2 H, d, J = 9 Hz, CH), 9.17 (2 H, d, J = 9.4 Hz, CH), 9.69 (2 H, d, J = 9 Hz, CH), 9.77 (2 H, d, J = 7 Hz, CH), 11.62 (2 H, s, CH). - 13C-NMR (DMSO-d6, 100 MHz): δ = 13.4 (CH3), 122.8 (Cq), 125.0 (CH), 125.3 (CH), 128.2 (CH), 128.8 (Cq), 132.4 (Cq), 133.9 (Cq), 135.8 (CH). - El. anal. for C26H20N2B2F8·2 H2O (563.33) calcd (%): C 54.78; H 4.24, N 4.91; found: C 55.37; H 4.01, N 5.44.

Synthesis of (E)-1,2-(napthalena-2-yl)vinyl]quinoizinimidizinium bromide (2b). A solution of 2-methylquinolinizinium bromide (3)15 (448 mg, 2.00 mmol), 2-naphthaldehyde (4) (469 mg, 3.00 mmol) and piperidine (0.20 mL) in MeOH (5 mL) was stirred under reflux for 5 h. The reaction mixture was cooled to r.t. and filtered. The filter cake was washed with cold MeOH and then with Et2O. The obtained orange-colored solid was recrystallized from MeOH to give the product 2b (450 mg, 1.24 mmol, 62%) as orange crystals (note: due to its photoreactivity this compound should be handled in the dark); mp = 272–274 °C (dec.).1 -1H NMR (600 MHz, DMSO-d6): δ = 7.55–7.60 (2 H, m, 1′-H, 2′-H), 7.70 (1 H, d, J = 16 Hz, 4′-H), 7.92–8.04 (5 H, m, 3′-H, 9′-H, 7′-H, 8′-H, 9′-H), 8.12 (1 H, d, J = 16 Hz, 5′-H), 8.19 (1 H, s, 10′-H), 8.31 (1 H, t, J = 8 Hz, 8-H), 8.43–8.50 (2 H, m, 7-H, 6′-H), 8.60 (1 H, s, 1-H), 9.26 (1 H, d, J = 8 Hz, 4-H), 9.33 (1 H, d, J = 7 Hz, 6-H). - El. NMR (150 MHz, DMSO-d6): δ = 120.3, 122.8, 123.0, 123.6, 124.3, 126.9, 126.9, 127.3, 127.7, 128.4, 128.7, 129.1, 133.0, 133.1, 133.6, 133.6, 136.9, 138.4, 142.8, 144.9. - MS (ESI): mz (rel. intensity) = 282 (100) [M+-]. - El. anal. for C12H15BrN × H2O (368.28) calcd (%): C 66.33; H 4.77, N 3.68; found: C 66.41; H 4.71; N 3.64.
Synthesis of (E)-3,3′-(ethane-1,2-diyl)bis(1-methylquinolin-1-ium) (2c). In a 200 mL sealed tube, a mixture of 2a15 (141 mg, 0.50 mmol) in MeI (5.0 mL) was stirred at 140 °C for 5 h. The reaction mixture was cooled to r.t. and Et₂O (30 mL) was added. The yellow precipitate was filtered and washed with Et₂O. The remaining solid was dissolved in a small amount of MeOH (100 mL) and the solid was passed through a bromide-saturated ion-exchange column (DOWEX® 1 × 8) three times. The solvent was evaporated and the solid was re-crystallized from MeOH/MeCN to obtain product 2c as a yellow solid (note: due to its photoreactivity this compound was handled in the dark); mp = 292–294 °C. 1H NMR (600 MHz, DMSO-d₆): δ = 4.71 (6 H, s, 2 × CH₃), 7.94 (2 H, s, 1′-H, 2′-H), 8.11 (2 H, t, J = 8 Hz, 6-H, 6′-H), 8.28–8.32 (2 H, m, 7-H, 7′-H), 8.53 (2 H, d, J = 8 Hz, 5-H, 5′-H), 8.55 (2 H, d, J = 9 Hz, 8-H, 8′-H), 9.40 (2 H, s, 4-H, 4′-H), 9.92 (2 H, s, 2-H, 2′-H). - 13C NMR (150 MHz, DMSO-d₆): δ = 45.8, 119.3, 127.8, 129.1, 129.9, 130.5, 130.6, 135.5, 137.5, 142.9, 149.2. - El. anal. for C₂₂H₁₉Br₂·0.5 H₂O (508.25) calcd (%): C 51.99, H 4.76, N 5.51, found: C 52.28; H 4.78; N 5.55.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Generous support by the Deutsche Forschungsgemeinschaft is gratefully acknowledged. We thank Ms Jennifer Hermann, Ms Sandra Uebach and Dr Stefanie Müller for technical assistance.

Notes and references

1 (a) C. K. Kwok and C. J. Merrick, Trends Biotechnol., 2017, 35, 997; (b) S. Neidle, Nat. Rev. Chem., 2017, 1, 0041; (c) S. Neidle, J. Med. Chem., 2016, 59, 5987; (d) D. Rhodes and H. J. Lipps, Nucleic Acids Res., 2015, 43, 8627; (e) S. Balasubramanian, L. H. Hurley and S. Neidle, Nat. Rev. Drug Discovery, 2011, 10, 261; (f) G. W. Collie and G. N. Parkinson, Chem. Soc. Rev., 2011, 40, 5867.

2 (a) T. Caneque, S. Müller and R. Rodriguez, Nat. Rev. Chem., 2018, 2, 202; (b) A. Laguerre, J. M. Y. Wong and D. Monchaud, Sci. Rep., 2016, 6, 32141; (c) V. S. Chambers, G. Marsico, J. M. Boutell, M. Di Antonio, G. P. Smith and S. Balasubramanian, Nat. Biotechnol., 2015, 33, 877.

3 (a) J. L. Huppert and S. Balasubramanian, Nucleic Acids Res., 2007, 35, 406; (b) J. L. Huppert, A. Bugaut, S. Kumari and S. Balasubramanian, Nucleic Acids Res., 2008, 36, 6260; (c) A. Verma, V. K. Yadav, R. Basundra, A. Kumar and S. Chowdhury, Nucleic Acids Res., 2009, 37, 4194.

4 (a) C. L. Grand, T. J. Powell, R. B. Nagle, D. J. Bearss, D. Tye, M. Gleason-Guzman and L. H. Hurley, Proc. Natl. Acad. Sci. U. S. A., 2004, 101, 6140; (b) A. Siddiqui-Jain, C. L. Grand, D. J. Bearss and L. H. Hurley, Proc. Natl. Acad. Sci. U. S. A., 2002, 99, 11593; (c) S. Balasubramanian, L. H. Hurley and S. Neidle, Nat. Rev. Drug Discovery, 2011, 10, 261; (d) Y. Li, J. Syed, Y. Suzuki, S. Asamitsu, N. Shioda, T. Wada and H. Sugiyama, ChemBioChem, 2016, 17, 928; (e) G. W. Collie and G. N. Parkinson, Chem. Soc. Rev., 2011, 40, 5867; (f) S. J. Adam, C. L. Grand, D. J. Bearss and L. H. Hurley, Proc. Natl. Acad. Sci. U. S. A., 2002, 99, 11593; (g) S. Kumari, A. Bugaut, J. L. Huppert and S. Balasubramanian, Nat. Chem. Biol., 2007, 3, 218.

5 (a) S. Balasubramanian and S. Neidle, Curr. Opin. Chem. Biol., 2009, 13, 345; (b) R. Hänsel-Hertrs, M. Di Antonio and S. Balasubramanian, Nat. Rev. Mol. Cell Biol., 2017, 18, 279.

6 (a) R. C. Monsen and J. O. Trent, Biochimie, 2018, 152, 134; (b) H.-S. Lee, M. Carmen, M. Liskowky, E. Peat, J.-H. Kim, M. Oshima, H. Masumoto, M.-P. Teulade-Fichou, Y. Pommier, W. C. Earnshaw, V. Larionov and N. Kouprina, Cancer Res., 2018, 78, 6282.

7 (a) S. Asamitsu, T. Bando and H. Sugiyama, Chem. – Eur. J., 2019, 25, 417; (b) E. Ruggiero and S. N. Richter, Nucleic Acids Res., 2018, 46, 3270; (c) T. Che, Y.-Q. Wang, Z.-L. Huang, J.-H. Tan, Z.-S. Huang and S.-B. Chen, Molecules, 2018, 23, 493; (d) Y. Xu, Chem. Soc. Rev., 2011, 40, 2719; (e) A. Ali and S. Bhattacharya, Bioorg. Med. Chem. Lett., 2014, 22, 4506; (f) P. Murat, Y. Singh and E. Defrancq, Chem. Soc. Rev., 2011, 40, 5293.

8 (a) S. Bhaduri, N. Ranjan and D. P. Arya, Beilstein J. Org. Chem., 2018, 14, 1051; (b) A. Recsifina, C. Zagni, M. G. Varrica, V. Pistarà and A. Corsaro, Eur. J. Med. Chem., 2014, 74, 95.

9 H. Ihmels and L. Thomas, in Materials Science of DNA Chemistry, ed. J.-I. Jin, CRC Press, Boca Raton, 2011, p. 49.

10 (a) A. Granzhan, H. Ihmels and K. Jäger, Chem. Commun., 2009, 1249; (b) K. Jäger, J. W. Bats, H. Ihmels, A. Granzhan, S. Uebach and B. O. Patrick, Chem. – Eur. J., 2012, 18, 10903; (c) A. Granzhan and H. Ihmels, Synlett, 2016, 1775; (d) H. Ihmels, K. Löhl, T. Paululat and S. Uebach, New J. Chem., 2018, 42, 13813.

11 A. de Cian, E. DeLemos, J.-L. Mergny, M.-P. Teulade-Fichou and D. Monchaud, J. Am. Chem. Soc., 2007, 129, 1856.

12 B. Prasad, J. Jamroskovic, S. Bhowmik, R. Kumar, T. Romell, N. Sabouri and E. Chorell, Chem. – Eur. J., 2018, 24, 7926.

13 W. Sbliwa, G. Matusiak and B. Bachowska, Croat. Chem. Acta, 2006, 79, 513.

14 (a) M.-Q. Wang, Y. Wu, Z.-Y. Yang, Q.-Y. Chen, Y.-C. Jiang and A. Sang, Dyes Pigm., 2017, 145, 1; (b) M.-Q. Wang, J. Xu, L. Zhang, Y. Liao, H. Wei, Y.-Y. Yin, Q. Liu, M.-Q. Wang, W.-X. Zhu, Z.-Z. Song, S. Li and Y.-Z. Zhang, Bioorg. Med. Chem. Lett., 2015, 25, 5672; (c) R. Chennoufi, H. Bougherara, N. Gagey-Eilstein, B. Dumat, E. Henry, F. Subra, S. Bury-Moné, F. Mahuteau-Betzner, P. Tauc, M.-P. Teulade-Fichou and E. Deprez, Sci. Rep., 2016, 6, 21458; (d) M.-Q. Wang, J. Xu, L. Zhang,
Y. Liao, H. Wei, Y.-Y. Yin, Q. Liu and Y. Zhang, *Bioorg. Med. Chem.*, 2019, 27, 552.

15 X. Zhang, E. L. Clennan, N. Arulswamy, R. Weber and J. Weber, *J. Org. Chem.*, 2016, 81, 5474.

16 Y. Wang and D. J. Patel, *Biochemistry*, 1992, 31, 8112.

17 (a) D. Renčík, J. Zhou, L. Beaupaire, A. Guédin, A. Bourdoncle and J.-L. Mergny, *Methods*, 2012, 57, 122; (b) A. de Cian, L. Guittat, M. Kaiser, B. Saccà, S. Amrane, A. Bourdoncle, P. Alberti, M.-P. Teulade-Fichou, L. Lacroix and J.-L. Mergny, *Methods*, 2007, 42, 183.

18 (a) D. Dzubieli, H. Ihmels, M. M. A. Mahmoud and L. Thomas, *Beilstein J. Org. Chem.*, 2014, 10, 2963; (b) J. W. Yan, S.-B. Chen, H.-Y. Liu, W.-J. Ye, T.-M. Ou, J.-H. Tan, D. Li, L.-Q. Gu and Z.-S. Huang, *Chem. Commun.*, 2014, 50, 6927; (c) S. Paramasivan and P. H. Bolton, *Nucleic Acids Res.*, 2008, 36, e106; (d) J. Carvalho, T. Quintela, N. M. Gueddouda, A. Bourdoncle, J. Mergny, G. F. Salgado, J. A. Queiroz and C. Cruz, *Org. Biomol. Chem.*, 2018, 16, 2776; (e) A. Chauhan, S. Paladhi, M. Debnath and J. Dash, *Org. Biomol. Chem.*, 2016, 14, 5761; (f) J. Lavrado, P. M. Borralho, S. A. Ohnmacht, R. E. Castro, C. M. P. Rodrigues, R. Moreira, D. J. V. A. dos Santos, S. Neidle and A. Paolo, *ChemMedChem*, 2013, 8, 1648.

19 G. A. Michelotti, E. F. Michelotti, A. Pullner, R. C. Duncan, D. Eick and D. Levens, *Mol. Cell. Biol.*, 1996, 16, 2656.

20 See e.g. (a) P. Kumar and R. Barthwal, *Biochimie*, 2018, 147, 153; (b) K. Padmapiya and R. Barthwal, *Biochim. Biophys. Acta*, 2017, 1861, 37; (c) L. Scaglioni, R. Mondelli, R. Artali, F. R. Sirtori and S. Mazzini, *Biochim. Biophys. Acta, Gen. Subj.*, 2016, 1860, 1129; (d) K. Padmapiya and R. Barthwal, *Bioorg. Med. Chem. Lett.*, 2016, 26, 4915; (e) S. Tripathi, T. P. Pradeep and R. Barthwal, *ChemBioChem*, 2016, 17, 554; (f) T. P. Pradeep and R. Barthwal, *Biochimie*, 2016, 128, 59; (g) W. Gai, Q. Yang, J. Xiang, W. Jiang, Q. Li, H. Sun, A. Guan, Q. Shang, H. Zhang and Y. Tang, *Nucleic Acids Res.*, 2013, 41, 2709; (h) R. Ferreira, R. Artali, J. Farrera-Sinfreu, F. Albericio, M. Rojo, R. Eritja and S. Mazzini, *Biochim. Biophys. Acta, Gen. Subj.*, 2011, 1810, 769; (i) E. Gavathiotis, R. A. Heald, M. F. G. Stevens and M. S. Searle, *Angew. Chem., Int. Ed.*, 2001, 40, 4749.

21 (a) S. K. Manna, A. Mandal, S. K. Mondal, A. K. Adak, A. Jana, S. Das, S. Chattopadhyay, S. Roy, S. K. Ghorai, S. Samanta, M. Hossain and M. Baidya, *Org. Biomol. Chem.*, 2015, 13, 8037; (b) B. Abarca, R. Custodio, A. M. Cuadro, D. Sucunza, A. Domingo, F. Mendiciti, J. Alvarez-Builla and J. J. Vaquero, *Org. Lett.*, 2014, 16, 3464; (c) R. M. Suárez, P. Bosch, D. Sucunza, A. M. Cuadro, A. Domingo, F. Mendiciti and J. J. Vaquero, *Org. Biomol. Chem.*, 2015, 13, 527; (d) P. Bosch, D. Sucunza, F. Mendiciti, A. Domingo and J. J. Vaquero, *Org. Chem. Front.*, 2018, 5, 1916.

22 M. M. Aleksic and V. Kapetanovic, *Acta Chim. Slov.*, 2014, 61, 555.

23 (a) E. Gavathiotis, R. A. Heald, M. F. G. Stevens and M. S. Searle, *Angew. Chem., Int. Ed.*, 2001, 40, 4749; (b) H. Mita, T. Ohyama, Y. Tanaka and Y. Yamamoto, *Biochemistry*, 2006, 45, 6765; (c) d. S. M. Webba, *Methods*, 2007, 43, 264.

24 (a) A. K. F. Martensson and P. Lincoln, *Phys. Chem. Chem. Phys.*, 2018, 20, 11336; (b) E. J. Barreiro, A. E. Kümmere and C. A. M. Fraga, *Chem. Rev.*, 2011, 111, 5215; (c) C. S. Leung, S. S. F. Leung, J. Tirado-Rives and W. L. Jorgensen, *J. Med. Chem.*, 2012, 55, 4489; (d) B. Rajendran, Y. Sato, S. Nishizawa and N. Teramai, *Bioorg. Med. Chem. Lett.*, 2007, 17, 3682; (e) B. Rajendran, A. Rajendran, Y. Sato, S. Nishizawa and N. Teramai, *Org. Biomol. Chem.*, 2009, 17, 351; (f) B. Rajendran, A. Rajendran, Z. Ye, E. Kanai, Y. Sato, S. Nishizawa, M. Sikorski and N. Teramai, *Org. Biomol. Chem.*, 2010, 8, 4949; (g) K. Benner, H. Ihmels, S. Kölsch and P. M. Pithan, *Org. Biomol. Chem.*, 2014, 12, 1725.

25 D. V. Berrndikova, O. A. Fedorova, E. V. Tulyakova, H. Li, S. Kölsch and H. Ihmels, *Photochem. Photobiol.*, 2014, 91, 723.

26 (a) M. M. Paquette, R. A. Kopelman, E. Beiter and N. L. Frank, *Chem. Commun.*, 2009, 5424; (b) W. Paw and R. Eisenberg, *Inorg. Chem.*, 1997, 36, 2287; (c) J.-L. Pozzo, A. Samat, R. Guglielmetti and D. D. Keukeleire, *J. Chem. Soc., Perkin Trans. 2*, 1992, 1327.

27 A. Granzhan, H. Ihmels and G. Viola, *J. Am. Chem. Soc.*, 2007, 129, 1254.