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Improving the affinity of naphthalene diimide ligand to telomeric DNA by incorporating Zn^{2+} ions into its dipicolylamine groups

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ABSTRACT

N,*N*′-bis[3-[3-(2,2′-dipicolyl)methylaminopropyl]-methylaminopropyl]naphthalene-1,4,5,8-tetracarboxylic acid diimide, **1**, and its complex with zinc ions, **2**, were investigated against telomeric sequences, [TAGGG(TTAGGG)$_3$] and [AGGG(TTAGGG)$_3$], which reveal different G-quadruplex structures depending on the conditions. Spectrophotometric, SPR, and CD techniques revealed that both ligands showed large binding constants to hybrid-type G-quadruplexes formed in the presence of K^+ ions. Moreover, **2** revealed higher affinity to investigated oligonucleotides suggesting that complex of naphthalene diimide derivative with Zn^{2+}, comparing to **1**, provided additional electrostatic or coordination interactions between positively charged zinc ions and condensed negative charged phosphate anions from G4 DNA.

1. Introduction

The structure that attracted a particular research interest in the past decade was the four-stranded DNA (alternatively termed G-quadruplex, tetraplex or G4 DNA structure). A number of studies have been reported in an attempt to characterize G-quadruplex structures in vitro. Studies revealed that G4 structure can be formed by telomeric DNA, which occurred at the end of human chromosomes. This telomeric DNA exhibits structural polymorphism, which depends on strand orientation and arrangement of loops. Moreover, the G-quadruplex structure may be stabilized by monovalent cations. The ionic radius of stabilizing cations greatly influences G-quadruplex topology. The most studied structure is formed by the sequence AGGG(TTAGGG)$_3$, adopting different topology depending on the experimental conditions. Under physiologically relevant conditions this oligonucleotide forms mixed hybrid and chair-type G-quadruplexes,[1] while in the presence of Na^+ ions its structure is antiparallel 'basket'.[2] Structural features of telomeric G-quadruplexes are important due to exploration of these structures for drug discovery since stabilization of G-quadruplex by small molecule may inhibit telomerase, a reverse transcriptase that is active in most cancer cells. For this purpose naphthalene diimide derivatives have been investigated extensively.[3–6] Neidle's group reported that binding affinity of naphthalene-based ligands depends on structural constituents of ligand as well as the arrangement of the phosphate and other polar groups in DNA structure since four groups from tetra-substituted naphthalene diimide contact with each G-quadruplex groove interacting through hydrogen bonds, water bridges and electrostatic contact. They also suggested that increasing the size of terminal groups is a good strategy for improving the affinity of investigated ligands.[7] Taking into account these highlights we propose naphthalene diimide derivative carrying dipicolylamine moieties (**1**) as a G-quadruplex stabilizer (Fig. 1). In order to enhance the affinity of ligand to telomeric DNA by increasing potential binding sites, we also incorporated Zn^{2+} ions into dipicolylamine moieties, **2** (Fig. 1) because of its high affinity to phosphate anion.[8] This assumption also stems from our previous results, which revealed that ligand **2** showed the improved binding affinity to calf thymus DNA having larger amount of phosphate anions.[9] It is also known that phosphate ions are condensed in G-quadruplex structure, so we expect that in case of zinc complex binding affinity will be improved by additional interactions between Zn^{2+} ions and negatively charged oxygen atoms from phosphate residue.

2. Material and methods

2.1. Material

Two oligonucleotides, [5′-TAGGG(TTAGGG)$_3$-3′](TA-core) and [5′-AGGG(TTAGGG)$_3$-3′](A-core), and two biotinylated oligonucleotides, [Biotin-5′-TAGGG(TTAGGG)$_3$-3′](Biotin-TA-core) and [Biotin-5′-AGGG(TTAGGG)$_3$-3′](Biotin-A-core), were purchased from Genenet Co. (Fukuoka, Japan) and were used without further purification. Before using oligonucleotides were heated to 95 °C

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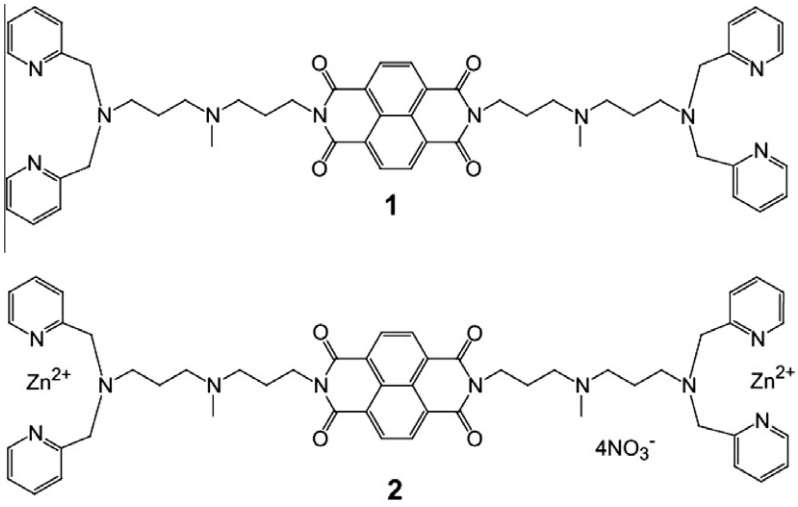

Figure 1. Chemical structure of *N*,*N*′-bis[3-[3-(2,2′-dipicolyl)methylaminopropyl]-methylaminopropyl]-naphthalene-1,4,5,8-tetracarboxylic acid diimide (**1**) and its complex with zinc ions (**2**).

and annealed by slow cooling to the room temperature. Ligand **1** and **2** were synthesized and characterized as previously described.[9] 3.0 M KCl, and 5.0 M NaCl were obtained by LifeTechnologies (Carlsbad, CA). 1.0 M Tris–HCl (pH 7.4) buffer was obtained from Sigma–Aldrich (St. Louis, MO). GoTaq Hot Start polymerase was purchased from Promega (Madison, WI). HeLa cells, and TRAPeze kit were obtained from EMD Millipore (Billeria, MA).

2.2. Methods

2.2.1. Circular dichroism titration experiments

All measurements were performed at 25 °C in a 50 mM Tris–HCl buffer (pH 7.4) containing 100 mM NaCl or KCl. Ligand **1** and **2** were diluted from stock solution to a concentration of 500 μM with water and titrated into 1.5 μM A-core or TA-core at 0.75–7.5 μM. Circular dichroism (CD) spectra were recorded using a Jasco J-820 spectropolarimeter with a 1 cm path-length quartz cell and a scan speed of 50 nm/min with a response time of 1.0 s, and over a wavelength range 220–550 nm. Each spectrum was obtained by averaging four scans and subtracting buffer baseline scan.

2.2.2. Spectrophotometric titration experiments

Absorption spectra were measured on a Hitachi U-3310 spectrophotometer with a 0.5 cm path-length quartz cell and were recorded in the 200–600 nm range at 25 °C. Spectrophotometric absorption titrations were carried out by the stepwise addition of 200 μM TA-core or A-core solution to a cell containing 5 μM solution of ligand **1** or **2**, to final concentration of DNA equal 20 μM. All measurements were performed at 25 °C in a 50 mM Tris–HCl buffer (pH 7.4) containing 100 mM NaCl or KCl. The binding constants and binding stoichiometry of the complexes were calculated by evaluation of Spectrophotometric titration using Scatchard analysis (Table 1).[10]

Table 1
Binding parameters for the interaction of ligand **1** and **2** with G-quadruplexes determined by Spectrophotometric titration method at 25 °C in 50 mM Tris–HCl buffer containing 100 mM KCl or NaCl

G-quadruplex	Ligand	Existing ion	n[b]	$10^{-6}K$[c]/M^{-1}
A-core	**1**	K^+	1.7	1.1
A-core	**2**	K^+	2.4	6.1
A-core	**1**	Na^+	—[a]	—[a]
A-core	**2**	Na^+	2.6	1.4
TA-core	**1**	K^+	1.8	2.8
TA-core	**2**	K^+	2.4	9.1
TA-core	**1**	Na^+	—[a]	—[a]
TA-core	**2**	Na^+	2.9	3.1

[a] Not determined.
[b] Binding number for strand.
[c] Binding constant.

2.2.3. Thermal melting experiments

Thermal melting experiments were performed with 1.5 μM A-core or TA-core containing 3.0 μM ligand **1** or **2** in a 50 mM Tris–HCl buffer (pH 7.4) containing 100 mM NaCl or KCl using a Jasco J-820 spectropolarimeter with a 1 cm path-length quartz cell and conducted over the temperature range of 20–95 °C, with linear temperature ramp of 0.5 °C/min.

2.2.4. Telomerase Repeat Amplification Protocol assay

Telomerase Repeat Amplification Protocol (TRAP) assay were performed using published procedure.[11] TRAPeze Telomerase Detection Kit was used. Briefly, TS forward primer was elongated by telomerase (1000 ng protein extract from HeLa) in TRAP buffer (20 mM Tris–HCl [pH 8.3], 1.5 mM $MgCl_2$, 63 mM KCl, 0.05% Tween 20, 1 mM EGTA) containing 0.05 mM dNTPs, 0.4 μM TS primer, RP primer, K1 primer, TSK1 primer and 2.0 units of GoTaq Hot Start polymerase. Mixture was added to freshly prepared ligand solutions at various concentrations (0.5–10 μM), to a positive control containing no ligand. Negative control was sample without telomerase and ligand. An internal control was provided to exclude non-specific PCR inhibition. Firstly, the elongation step was carried out for 60 min at 30 °C and followed by 5 min at 95 °C. Secondly, 35 cycles of PCR were performed (94 °C, 30 s; 62 °C, 1 min; 72 °C, 1 min). Telomerase extension products were then analyzed on a denaturing 12.5% polyacrylamide vertical gel prepared in 1.25 × TBE (89 mM Tris base, 89 mM borate, and 1 mM ethylenediaminetetraacetic acid, pH 8.0). After electrophoresis the gel was stained in 1 × GelStar Nucleic Acid Stain (Takara Bio, Japan) in 1 × TBE buffer for 30 min and photographed.

2.2.5. Surface plasmon resonance experiments

Surface plasmon resonance (SPR) measurements were performed with Biacore 3000 system (GE Healthcare Japan, Tokyo, Japan) using streptaividin-coated sensor chips (Sensor chip SA, GE Healthcare Japan) following previously published procedure.[12] Briefly, flow cells 2 and 4 were used as sample flow cells for the immobilization of the biotinylated oligonucleotides: Biotin-A-core and Biotin-TA-core. Immobilization process was done at a flow rate

of 2 μL/min, 500 RU of Biotin-A-core or Biotin-TA-core was immobilized on flow cell 2 and 4, respectively. Experiments in the presence of Na^+ were performed in HBS-P buffer (0.01 M HEPES [pH 7.4]; 0.15 M NaCl; 0.005% surfactant P20; GE Healthcare Japan), while for potassium condition buffer was freshly prepared (0.01 M HEPES [pH 7.4]; 0.15 M KCl; 0.005% surfactant P20), degassed and filtered. Flow cells 1 and 3 were blank and signal was subtracted from the signal from sample cells. All experiments were performed at 25 °C with multiple injections of different ligand concentrations (1–960 nM) over the immobilized DNA surface at flow rate of 10 μL/min, for 1200 s. Following this, dissociation from the surface was monitored for 1500 s. Regeneration was done using 10 mM glycine-hydrochloride pH 2.0.

3. Results

3.1. CD spectra

CD spectra were recorded in order to characterize G-quadruplex structure as well as to monitor stability of G-quadruplex conformation upon addition of the ligands. CD data revealed that both sequence: $AGGG(TTAGGG)_3$ and $TAGGG(TTAGGG)_3$ form G-quadruplex structure in the presence of 100 mM Na^+ or K^+ ions. G4 structures formed by A-core and TA-core in K^+ solution exhibited very similar CD signature profile, in which the positive peak around 290 nm, the smaller peak around 270 nm and the smaller negative peak at 240 nm were observed (Fig. 2A and B). Only negligible difference in intensity of peak around 270 nm was seen which is consisted with the formation of mixed hybrid and chair-type G-quadruplex,[1,13] and the formation of mainly (about 70%) hybrid-type G-quadruplex by A-core and TA-core,[14,15] respectively. CD profiles in Na^+ solution suggest that both oligonucleotides adopt antiparallel stranded G-quadruplex.[2,6] Antiparallel topology is reveled with a positive peak at 245 nm, a negative peak at 265 nm, and a positive peak at 290 nm (Fig. 2C and D).

Upon the addition of ligand **1** to potassium solution of A-core or TA-core the intensity of peaks changed, but peaks position seems to be stable (Fig. 2A and B). Similar results were obtained for ligand **2** (Fig. 3A and B). While under sodium conditions, the whole profile of CD spectra of both DNA changed in complex manners. A hypsochromic shift of the main peaks is clearly observed. The CD spectra of A-core as well as TA-core showed deformation of positive and negative peaks; however, the shifts are more significant upon addition of ligand **2** (Fig. 3C and D), similarly to potassium conditions.

3.2. Spectrophotometric titration

In order to estimate binding constants and stoichiometry of investigated complexes spectrophotometric titration was done. All results have been presented in Table 1. In potassium solution ligand **2** revealed the highest affinity to TA-core with binding constant $K = 9.1 \times 10^6\ M^{-1}$. Comparable value of binding constant ($K = 6.1 \times 10^6\ M^{-1}$) was obtained for A-core, while in case of ligand **1** binding constants are substantially lower for both oligonucleotides. Ligand **1** has $K = 2.8 \times 10^6\ M^{-1}$ for TA-core and $K = 1.1 \times 10^6\ M^{-1}$ for A-core. In contrast, ligand **1** has lower affinity to both antiparallel G-quadruplexes since the average percentage of hypochromicity for ligand **1** in the presence of Na^+ was approximately around 40%,

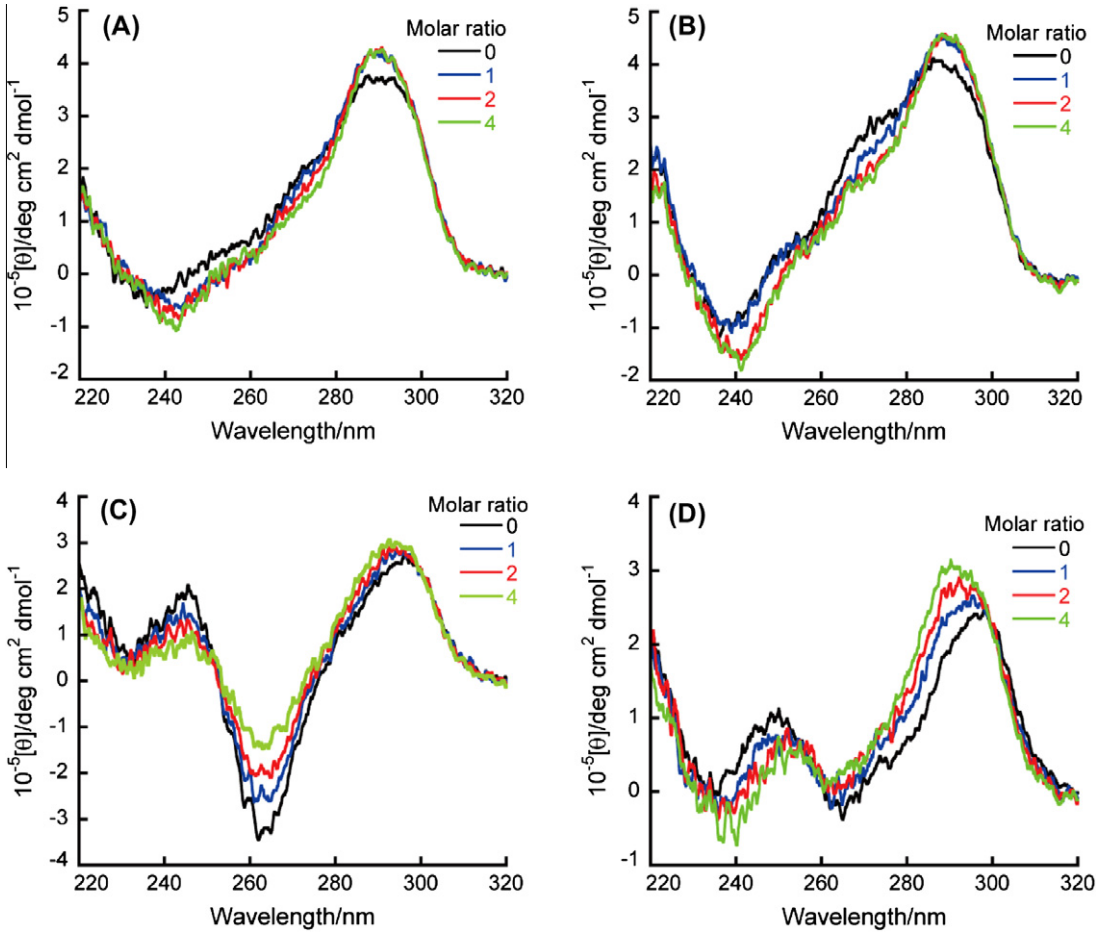

Figure 2. CD spectra of 1.5 μM of A-core (A, C) and TA-core (B, D) with various molar ratios of DNA/ligand **1**. Experiments were performed at 25 °C in 50 mM Tris–HCl buffer containing 100 mM KCl (A, B) or 100 mM NaCl (C, D).

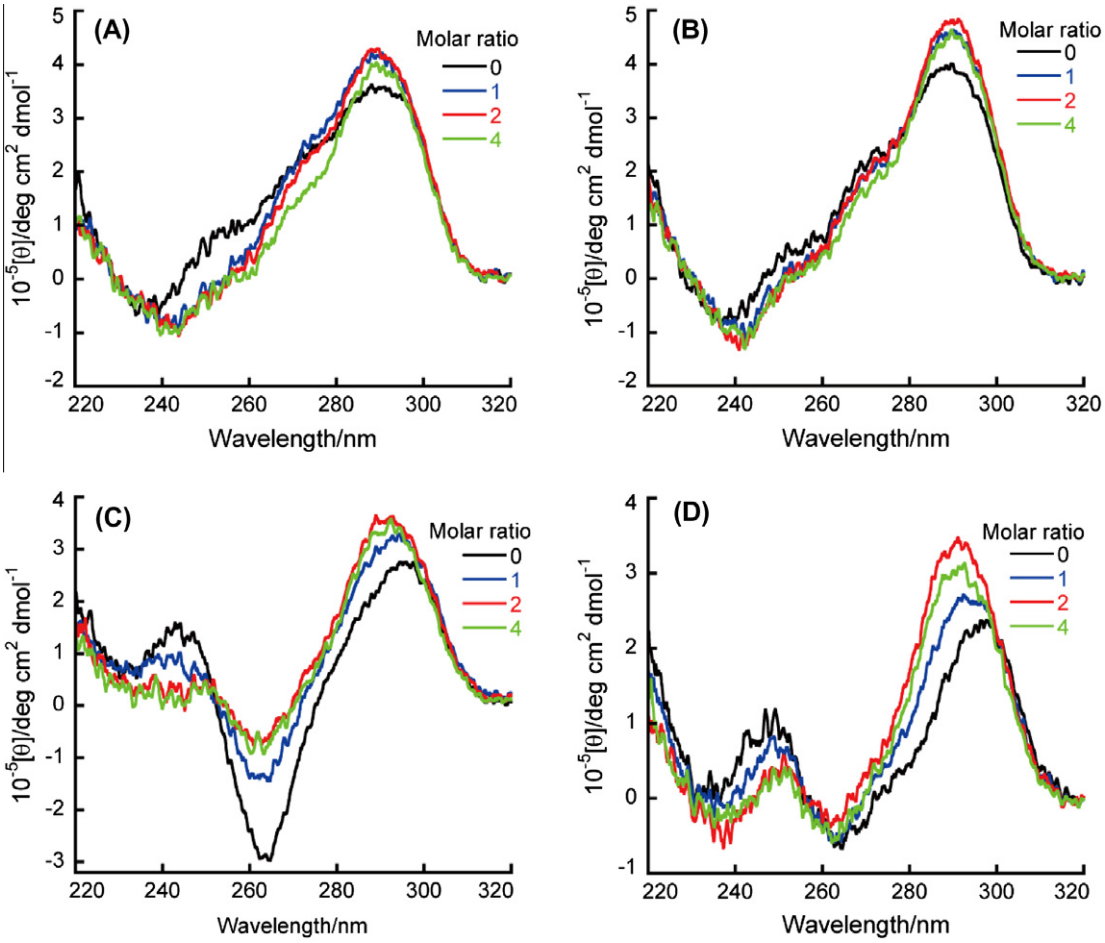

Figure 3. CD spectra of 1.5 μM of A-core (A, C) and TA-core (B, D) with various molar ratios of DNA/ligand **2**. Experiments were performed at 25 °C in 50 mM Tris–HCl buffer containing 100 mM KCl (A, B) or 100 mM NaCl (C, D).

while larger hypochromicity (around 50%) was observed under the same conditions for ligand **2**. The stoichiometry is higher for the complexes with ligand **2** than for ligand **1**.

3.3. CD melting temperature

The thermal stability of the G-quadruplex structures might be investigated by the temperature dependence of the positive CD peak at 290 nm. Figure 4 represents typical melting curves showing normalized ellipticity changes against temperature. In the presence of K^+ ions T_m increased in A-core/ligand **1** complex relative to that of free A-core (ΔT_m = 12 °C), while in the presence of ligand **2** ΔT_m value increased to 19 °C. TA-core is also stabilized more by ligand **2**. ΔT_m value is determined as 13 °C and 20 °C for TA-core/ligand **1** and TA-core/ligand **2** complex, respectively. Similar results are observed in sodium solution; however, ΔT_m values are significantly lower comparing with potassium one. A-core has ΔT_m = 1 °C for ligand **1**, while for ligand **2** has ΔT_m = 6 °C. In case of TA-core ΔT_m is estimated as 1 °C and 8 °C for complex with ligand **1** and **2**, respectively. The melting temperature (T_m) of free tetraplex is the highest for oligonucleotides in potassium solution (68 °C) and the lowest for oligonucleotides in sodium solution (57 °C).

3.4. TRAP assay

TRAP assay has been used to provide quantitative estimate of telomerase inhibition.[11] Using this assay, both ligands showed high activity with half maximal effective concentration (EC_{50}) values 4 μM and 3 μM for ligands **1** and **2**, respectively. Gels from TRAP assay for ligand **1** and **2** are presented on Figure 1S and 2S, respectively (Supplementary data).

3.5. SPR experiments

Biotin-functionalized oligonucleotides (Biotin-A-core and Biotin-TA-core) were immobilized on streptavidin-coated chips to study binding interactions with ligands **1** and **2**. A shape of obtained sensorgrams indicates that both ligands revealed higher affinity to the oligonucleotides formed in the presence of K^+ ions (Fig. 3SA and 3SB). In the presence of Na^+ ions, ligands showed lower affinity to both oligonucleotides (Fig. 4SA and 4SB). Moreover, binding stoichiometry is higher in case of ligand **2** since response value is higher in sensorgrams for this ligand. For ligand **1** RU value is lower in the presence of Na^+ ions or K^+ ions, indicating lower binding ratio.

4. Discussion

4.1. Quadruplex affinity

The G-quadruplex binders, including naphthalene diimide derivatives, stabilize G-quadruplex structure, in most cases, via π–π stacking and electrostatic interactions resulting in binding of ligand on the external tetrad of guanines. However, the side chains are also important structural elements in optimizing ligand-G4 DNA binding mode.[3,16] We assumed that complex of naphthalene diimide derivative with Zn^{2+} (ligand **2**), comparing to ligands **1**,

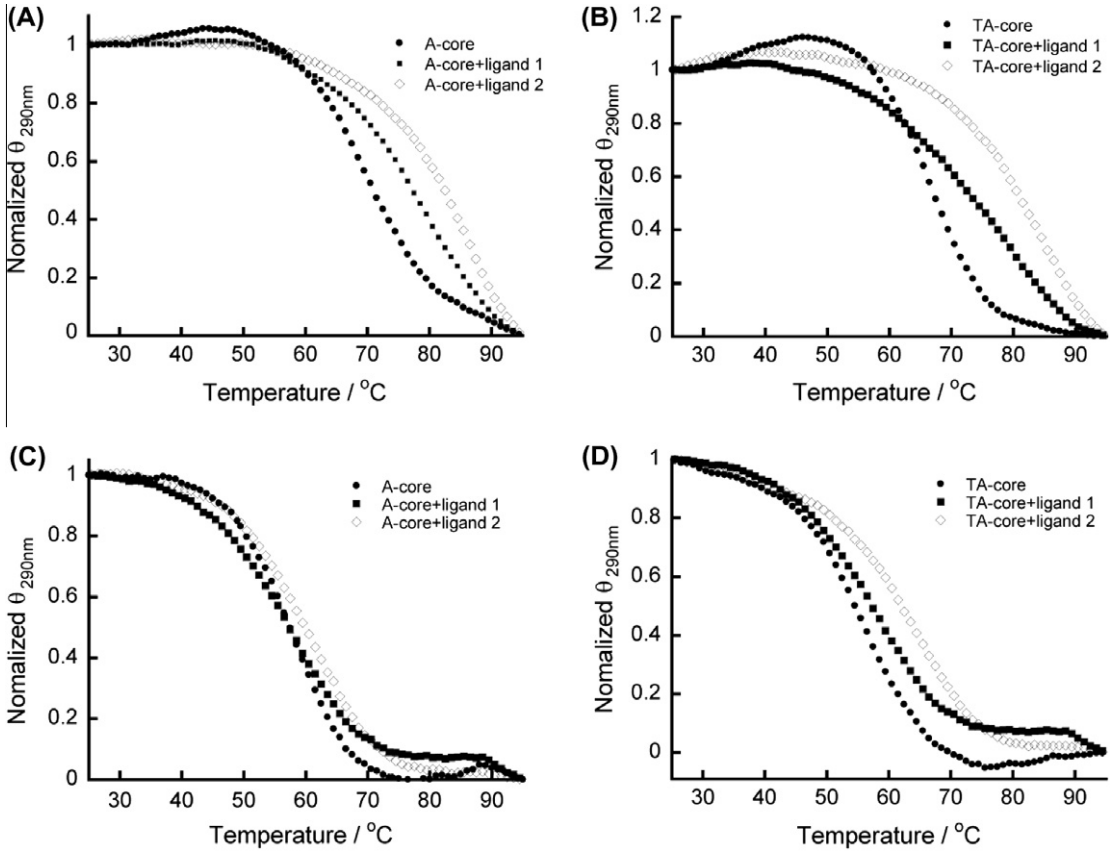

Figure 4. CD melting curves of 1.5 μM of A-core (A, C) and TA-core (B, D) at a molar ratio of 1:2 DNA/ligand for ligand **1** and **2**. Experiments were performed at 25 °C in 50 mM Tris–HCl buffer containing 100 mM KCl (A, B) or 100 mM NaCl (C, D).

will provide additional electrostatic interactions between positively charged atom of zinc and negatively charged G4 DNA. In order to evaluate binding affinity of ligands **1** and **2** to telomeric DNA, spectrophotometric titration was done (Fig. 5S and 6S). Experiments were performed in the presence of sodium or K^+ ions in order to compare affinity under different conditions. In potassium solution ligand **2** revealed the highest affinity to TA-core, while in case of ligand **1** binding constants are substantially lower for both oligonucleotides. Metal moiety grafted in the periphery of the aromatic core of ligand **2** causes the association with the negatively charged G-quadruplex-DNA effecting in higher affinity. Confirmation on additional anchorage of a G-quadruplex binding motif provided by Zn(II) complex is also observed in sodium solution. Unfortunately, binding constants for ligand **1** in the presence of Na^+ ions were not estimated, but taking into account the average percentage of hypochromicity it can be concluded ligand **1** has lower affinity to both oligonucleotides than ligand **2**. Binding constant values obtained using Spectrophotometric spectroscopy are similar to that from SPR results observed for tri- and tetra-substituted naphthalene diimides, for which the *K* values are in the range of $\sim 10^6$–10^7 M^{-1}.[3] We conclude that investigated ligands are good G-quadruplex structures stabilizers since binding constants are comparable to multi-substituted derivatives. Still binding affinity of naphthalene diimide derivatives could be enhanced by designing ligand with four dipicolylamine moieties.

We also used SPR in order to investigate binding affinity of ligands **1** and **2**.Though SPR is a powerful tool to study interaction of small molecules and G-quadruplexes, this method has some limitations. The most troublesome is mass transport effect, which might be observed at high analyte concentrations.[17] High concentration can be also response for not good shape of sensorgram. Unfortunately, as shown on Figures 3SA–4SB we recorded non-specific shape of sensorgrams. As Rich et al. suggested,[18] we tried to decrease concentration, but we could not have observed good signal since the ligand is a small molecule and the instrument response from small-molecule binding is low. However taking into account information included in the same article we conclude that a shape of sensorgrams suggests higher affinity of ligands **1** and **2** to both oligonucleotides in potassium solution (Fig. 3SA and 3SB, inserts). Moreover, ligand **2** reached higher RU value at saturation than ligand **1** indicating that more molecules bind to the TAGGG oligomer. Similar results were obtained for AGGG oligomer. Concluding, SPR revealed higher affinity of ligands **1** and **2** to both oligonucleotides in the presence of K^+ ions. Moreover, ligand **2** binds to both sequences at the higher ratio. It is worth mentioning that higher than 2:1 drug/G4 DNA stoichiometry have been observed for several ligands,[19,20] including TMPyP4.[21]

These encouraging results prompted us to investigate if ligands **1** and **2** also show telomerase inhibition. Effective concentration for telomerase inhibition, obtained by TRAP method, was 4 μM and 3 μM for ligands **1** and **2**, respectively. The presence of Zn^{2+} ions appears to have a slight effect on biological activity of telomerase, probably connected with quadruplex binding affinity. Higher affinity of ligand **2** to G4 DNA effects in lower EC_{50} value. The values obtained from TRAP assay are comparable to previously observed for naphthalene diimide derivatives (ca. 5 μM).[4]

4.2. Quadruplex formation

As mentioned before, the investigated oligonucleotides form a mixture of multiply G-quadruplex conformations in potassium solution. After addition of ligand **1** the positive peak around 290 nm increased and those at 270 nm and 240 nm decreased, indicating a binding event that probably induced stabilization of

one of the hybrid structure, which is energetically favorable (Fig. 2A and B). This structure is stable even if molar ratio of ligand **1**/DNA reached 4:1. Initially, similar changes connected with favorable topology formation are observed for ligand **2**. However, CD spectra of both oligonucleotides upon interaction with ligand **2** at their molar ratio 4:1 showed different changes comparing to ligand **1** (Fig. 3 A and B). The spectral differences between the molar ratio 2:1 and 4:1 could be attributed to contribution from some minor binding sites with ligand **2**. This contribution is more noticeable in sodium solution, especially for TA-core (Fig. 3D). These data are consisted with stoichiometry obtained from spectrophotometric titration experiments (Table 1, n). Our results reveal approximate 2:1 binding ratio for ligand **1** to both G-quadruplexes in K^+ solution, implying that both ends of the G-quartet are binding sites. Similar results were obtained from CD spectra, which are shown on Figures 7S and 8S. For ligand **2** the stoichiometry is higher than for ligand **1**, so probably additional molecule is responsible for groove binding or different type of interaction, which is made possible due to quadruplex rearrangement caused by ligand **2**. Interestingly, under Na^+ conditions, in all cases, antiparallel structure is probably converted into hybrid one, which maximum of main peak is located at 290 nm, while many G-quadruplex ligands may induce alteration of G4 structures.[20,22] Moreover, CD patterns for complexes at 1:2 ratio of ligand/DNA in Na^+ and in K^+ solution are similar implying that similar binding sites of DNA are involved in both solutions. Another possibility is that spectral conversion of A-core or TA-core is not due to structural conversion via the switch of the orientation of GGG strand from antiparallel to parallel form. Changes might be contributed to fast binding resulting in different loop arrangement as Chang et al.[23] suggested for example of carbazole derivative, where observed spectral conversion of DNA is very unlikely due to conversion between different types of G4 structures. However, these results do not give definite answer, so the conversion between different topological types of A-core and TA-core deserves more study.

4.3. Quadruplex stability

Additional information about investigated complexes might be obtained using melting temperature experiments. As observed from absorption spectra ligand **1** has the lowest binding affinity to A-core and TA-core in sodium solution. This data are consisted with the lowest ΔT_m value for ligand **1**/A-core or ligand **1**/TA-core complex in the presence of Na^+ ions (Table 2). Other values are also in excellent agreement with the order of binding constants. For example ΔT_m is the highest for ligand **2**/TA-core complex under K^+ conditions concurrently this complex display the highest binding constant. Interestingly, T_m values for the all complexes in sodium solution are definitely lower than for the complexes in potassium solution. T_m Value for these complexes in the presence of Na^+ is in the range of 57–65 °C. Maximum value is about 3 degree lower than for free hybrid DNA in K^+, so probably antiparallel structure is preserved and CD spectral changes observed both in ligands **1** and **2** complexes are attributed to different loop arrangement in the most stable energetically structure formed by DNA-ligand interactions. However, we cannot exclude existing hybrid structure in Na^+ solution, because sodium ions affected very strongly on stability of quadruplex structure,[24–26] so hybrid form in Na^+ may exhibits lower melting point than in the presence of K^+ ions.

Table 2
Melting temperature for investigated samples

G-quadruplex	Ligand	Existing ion	T_m/°C
A-core	None	K^+	68
A-core	**1**	K^+	80
A-core	**2**	K^+	87
A-core	None	Na^+	57
A-core	**1**	Na^+	58
A-core	**2**	Na^+	63
TA-core	None	K^+	68
TA-core	**1**	K^+	81
TA-core	**2**	K^+	88
TA-core	None	Na^+	57
TA-core	**1**	Na^+	58
TA-core	**2**	Na^+	65

We conclude that investigated ligands stabilize efficiently G-quadruplex structures under biologically relevant conditions since T_m values are similar to previously observed. In fact, stabilization process has been improved, because previously investigated di-substituted ligands revealed lower ΔT_m value (ca. 5°C),[3] while ligands **1** and **2**, also possessing two side-chains, shown higher ΔT_m value. Taking into account our results we strongly believe that stability of the complexes of naphthalene diimide derivatives and G-quadruplexes might be improved by incorporating four dipicolylamine moieties into ligand molecule.

5. Conclusions

Binding affinity of two naphthalene diimide derivatives to different G-quadruplex structures formed by TA-core and A-core was studied using several methods. All methods showed that investigated ligands have higher affinity to telomeric DNA in the presence of K^+ ions. Moreover, ligand **1** binds weaker than ligand **2** to the both oligonucleotides. Higher binding constants for ligand **2** obtained under biologically relevant conditions are connected with additional binding sites occurred in the complexes. We also observed similar ability of investigated ligands in the presence of sodium ions since experiments revealed higher affinity of G-quadruplexes to ligand **2** than to ligand **1**. As we expected incorporation of Zn^{2+} ions into dipicolylamine moieties increased affinity of naphthalene diimide ligand to G-quadruplex telomeric DNA. DNA-ligand interactions have been also monitored using CD spectroscopy. CD spectra showed more noticeable changes in case of ligand **2** especially at 4:1 molar ratio of ligand/DNA. We suspect that this observation is related to higher binding affinity and stoichiometry of ligand **2**.

Interestingly, higher affinity of the complex of naphthalene derivative with Zn^{2+} to telomeric DNA effected stronger in stability of G-quadruplex structures than ligand **1**. Stabilization was monitored in thermal melting experiments and the presence of ligand **2** increased significantly melting point of A-core and TA-core, confirming strong DNA–ligand interactions. Undeniable impact on binding affinity might have coordination binding between condensed anion sides from G4 DNA and Zn^{2+} ions incorporated into dipicolylamine moieties of naphthalene diimide derivative. Thus, the results highlight the importance of specific structural elements of the side-chain terminal groups of naphthalene derivative in interactions with telomeric DNA.

We also estimated EC_{50} values, which indicate that investigated ligands are good telomerase inhibitors. These abilities make the investigated ligands good candidates as anticancer drugs.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.08.055.

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