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PAPER

Light-driven conformational regulation of human telomeric G-quadruplex DNA in physiological conditions†

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Human telomeric G-quadruplexes have raised broad interest not just due to their involvement in the regulation of gene expressions and telomerase activities but also because of their application in nanoarchitectures. Herein, three azobenzene derivatives **1**–**3** were synthesized with different substituent groups and their photo-isomerization properties were investigated by UV/Vis spectroscopy. Then circular dichroism spectroscopy (CD), fluorescence experiments and native-gel electrophoresis were performed to evaluate their capabilities of conformational photo-regulation both in the absence and presence of metal ions. The results suggested that the compounds synthesized can successfully regulate the conformation of human telomeric G-quadruplex DNA in K^+ conditions to some extent. This work will initiate the possibility for the design and intriguing application of light-induced switching to photoregulate the conformation of G-quadruplex DNA under physiological conditions, providing a possible pathway to control G-quadruplex conformation in biological applications and also expanding the potential use of G-quadruplexes in nanomachines.

Introduction

Broad interest has been raised in the study of G-quadruplex DNA due to the discovery of its involvement in the regulation of gene expressions and telomerase activities over the past decades.[1] Human telomeric DNA sequences, typically 5–8 kb long with a 3′ single-stranded overhang about 100–200 nt,[2] are non-coding highly repetitive sequences consisting of tandem repeats of the sequence d(TTAGGG) and have been found to form several kinds of G-quadruplex structures under different conditions *in vitro*.[3] Studies indicated that the structures of human telomeric G-quadruplexes have three conformations: parallel, anti-parallel, and hybrid-type (Fig. 1). Actually, human telomeric G-quadruplex structures are polymorphic,[4] which could definitely influence interaction between G-quadruplexes and ligands. In addition, this polymorphism might also be applied in nanomachine and nanoarchitecture. Recently, a few nanomachines have been constructed based on G-quadruplex regulation. Pioneering work by Mergny and his co-workers reported a stretch movement from G-quadruplex to duplex fueled by G-rich and C-rich oligonucleotides.[5] Ligands were also utilized to regulate G-quadruplex formation and transformation,[6a,b] and, in particular, even single wall carbon nanotubes were found to have the ability to dissociate human telomeric duplex to form i-motif and G-quadruplex structures.[6c]

Among these previous reports, external stimuli such as temperature,[7] pH value,[8] electrical-field strength,[9] and molecular recognition[10] have been used to control material responses. Regarding biological reactions, the most promising external trigger

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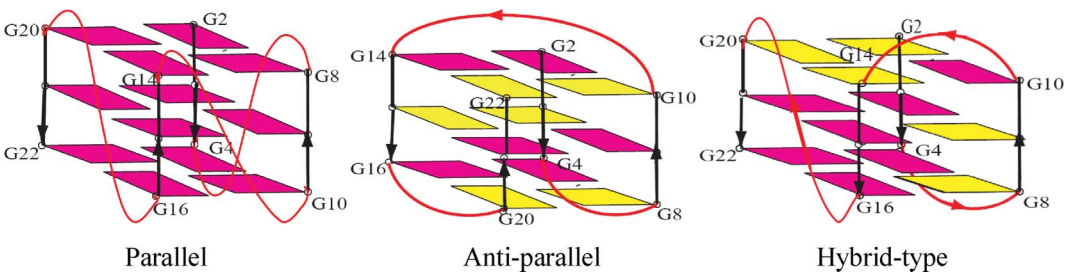
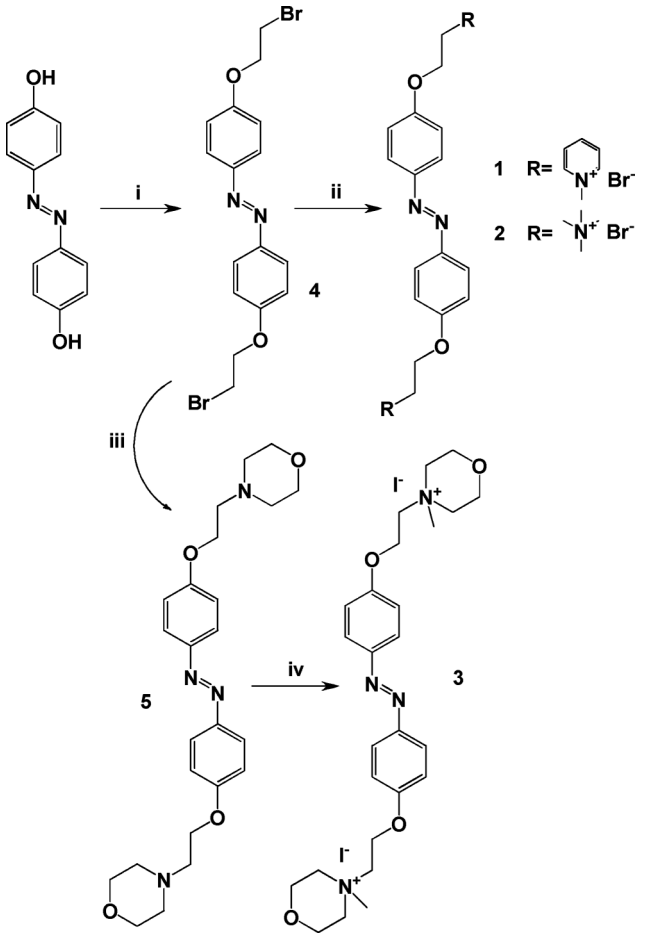


Fig. 1 The structures of human telomeric G-quadruplexes (G4A).

is photo-irradiation.[11] Recently, Ogasawara and Maeda[12] successfully demonstrated for the first time that G-quadruplex formation can be controlled reversibly by light, but the photo-control process cannot be accomplished without photochromic modification of nucleobase. Therefore, a new photoresponsive molecule is required in the field of conformational regulation. Among the candidates, the azobenzene moiety has received considerable attention due to its nearly reversible photo-isomerization property,[13] as the azobenzene group can undergo *cis*-to-*trans* isomerization under reversible irradiation upon UV/Vis photo-illumination[14] This excellent photochemical characteristic of azobenzene has been widely used in a variety of biomolecules,[15] such as oligonucleotides, peptides, phospholipids and sugar scaffolds, to modify and regulate the corresponding biological processes.

In view of the above findings, we conceive that the light-induced azobenzene moiety can be used as a photo-switch to regulate the conformation of G-quadruplex DNA. In the recent research of our group,[16] we designed an azobenzene derivative with piperidine parts that was the first small molecule to induce reversible stretching and folding of G-quadruplex on the basis of photo-irradiation. However, in further research, it has been found that this designed azobenzene derivative can only be used in the conditions without metal ions which limited its wide application. Consequently, designing a light-induced conformational switching of G-quadruplex DNA under physiological conditions has become a big challenge in this field. In the present work, we have synthesized the azobenzene derivatives **1–3** (Scheme 1) and demonstrated their photoisomerization by UV/Vis spectroscopy. Their capabilities of conformational regulation of the G-quadruplex DNA upon irradiation in the non-cations, K^+, and Li^+ conditions were all studied by CD spectroscopy. Furthermore, the fluorescence spectra and native-gel electrophoresis experiments gave an auxiliary proof to the interaction between the G-quadruplex and different configurations of these compounds. The regulation of human telomeric G-quadruplex by this azobenzene scaffold not only provided a possible pathway to control G-quadruplex conformation in biological applications but also expanded the potential for G-quadruplex to be involved in nanomachines.



Scheme 1 Reagents, conditions, and yields. i) 1,2-dibromoethane, K_2CO_3, dry acetone, Ar, reflux, 16.1%; ii) pyridine for compound **1** and trimethylamine for compound **2**, CH_3CH_2OH, Ar, reflux, 66%. iii) morpholine, K_2CO_3, dry acetone, Ar, reflux, 89.3%; iv) CH_3I, $CHCl_3$, 45 °C, 61.2%.

Results and discussion

Synthesis

The synthesis of compounds started with the nucleophilic substitutions of 4,4′-dihydroxyazobenzene,[17] with six equivalents of 1,2-dibromoethane in dry acetone under reflux conditions. All the target compounds were simply synthesized by treating compound **4** according to the procedures below under Ar atmosphere (Scheme 1). Different substituted quaternary ammoniums including both aliphatic and aromatic species were selected to explore their distinction. The compounds synthesized were fully characterized by NMR spectroscopy and HRMS methods (see Supplementary Information†).

UV/Vis spectral study

The isomerization processes of the designed compounds were confirmed from the alterability of UV-visible absorption spectra (Fig. 2). Upon photo-irradiation in a solution with compound **1** (25 μM in 10 mM Tris/HCl buffer with 1 mM EDTA, pH 7.4) at 350 nm for approximately 120 s, the π–π* transition at 355 nm decreased, along with an obvious blue shift; meanwhile, the intensity of the absorption band centered at 430 nm owing to the n–π* transition increased (Fig. 2-1a), implying the compound had converted into the *cis*-form.[18] Under the same conditions, it took approximately 4 min for the 25 μM solution of compound **2** to reach the photostationary state (Fig. 2-2a). Illumination of the 25 μM solution of compound **3** for 90 s causes isomerization, with the *cis*-form separating itself from the *trans*-form (Fig. 2-3a). These changes represent the *trans*-to-*cis* isomerization of azobenzene derivatives.[19] On the other hand, visible light irradiation after UV exposure reisomerizes the *cis*- to the *trans*-form to some extent, but not completely.[20] Under irradiation of the solution of compound **1** for 120 s with visible light, the reaction system attained a photostationary state (Fig. 2-1b). Under the same experimental conditions, it required about 200 s for compound **2** (Fig. 2-2b) and 230 s for **3** (Fig. 2-3b) to reach the corresponding photostationary state. These results were consistent with those of previous studies[21] These observations implied that alternate irradiation with visible and UV light (350 nm) could induce repeated photoconversion between the *cis* form and the photostationary state of the synthesized compounds **1–3**.

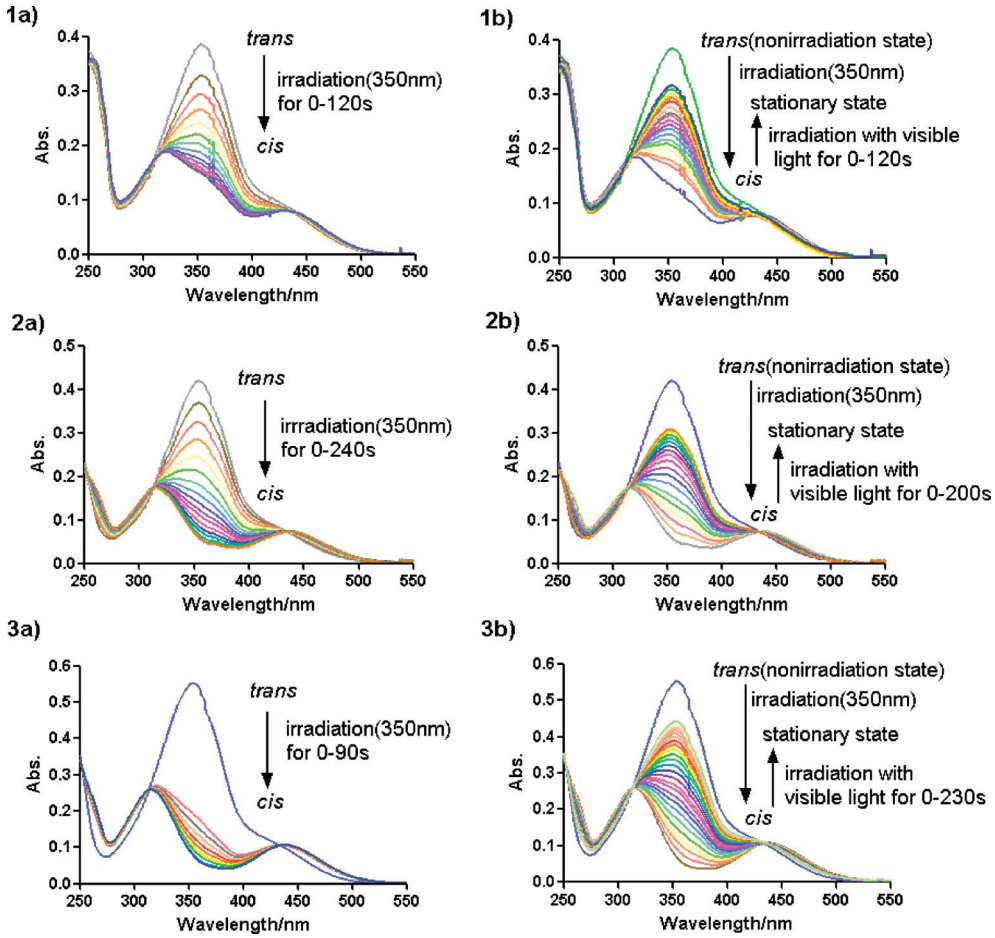


Fig. 2 Changes in the absorption spectra of synthesized compounds (25 μM) in 10mM Tris/HCl buffer with EDTA (1 mM) at pH 7.4. 1a) UV/Vis spectra of compound **1** upon UV irradiation at 350 nm at various reaction times (0–240 s). 1b) UV/Vis spectra of compound **1** prior to irradiation (top line) and under visible light at various reaction times (0–120 s) followed by the UV irradiation at 350 nm. 2a) UV/Vis spectra of compound **2** upon UV irradiation at 350 nm at various reaction times (0–240 s). 2b) UV/Vis spectra of compound **2** prior to irradiation (top line) and under visible light at various reaction times (0–200 s) followed by the UV irradiation at 350 nm. 3a) UV/Vis spectra of compound **3** upon UV irradiation at 350 nm at various reaction times (0–90 s). 3b) UV/Vis spectra of compound **3** prior to irradiation (top line) and under visible light at various reaction times (0–230 s) followed by the UV irradiation at 350 nm. EDTA: ethylenediaminetetraacetic acid, Tris: 2-amino-2-hydroxymethylpropane-1,3-diol.

CD titration studies with telomere DNA (d(TTAGGG)$_4$)

As various topologies of quadruplex DNA are associated with specific CD signatures, [22] CD offers a simple means for distinguishing DNA secondary structures, although it is not always reliable as several exceptions have been revealed up to now.[23] It has been proved that the human telomeric quadruplex is polymorphic.[4] Depending on strand orientation, parallel G-quadruplex structure is generally characterized by a positive peak at 265 nm and a negative peak at 240 nm. In contrast, the antiparallel structure has a positive peak at 295 nm and a negative peak at 265 nm, and the hybrid-type G-quadruplex structure has a strong positive peak at 290 with a shoulder at 270 nm. Overall, CD is a still convenient method for preliminarily determining the conversion of the quadruplex from the unfolded conformation.

First, the folding and stretching motions of the G-quadruplex by the proposed photocontrollers were evaluated in the condition without metal ions. The CD signal of these compounds were found to be absent between 220 nm and 320 nm as shown in Fig. S1. However, the telomere DNA (d(TTAGGG)$_4$) exhibited a typical parallel quadruplex CD signature in the presence of the *trans* form of compound **2** with a positive peak at 265 nm and a negative peak at 240 nm (Fig. 3-2a). The signal at 265 nm had disappeared after photoisomerization to the *cis* form upon irradiation with UV light at 350 nm for 5 min, which suggested that d(TTAGGG)$_4$ cannot form a quadruplex when compound **2** adopts the *cis* form. This result suggested that the open and closed forms of the d(TTAGGG)$_4$ DNA were interconverted by UV/Vis photoregulation (Fig. 3-2b). Moreover, during UV/Vis photoillumination for 3 continuous cycles, from the CD spectra of d(TTAGGG)$_4$, no changes in absorptivity were observed for the signal at 265 nm, indicating that the exchange process was totally reversible. Similarly, in the presence of *trans*-form compound **3**, the d(TTAGGG)$_4$ DNA showed a parallel quadruplex CD signature (Fig. 3-3a). These peaks vanished after photoisomerization to the *cis* form by irradiation at 350 nm for 5 min (Fig. 3-3b) and this exchange process could also be reversible, even after

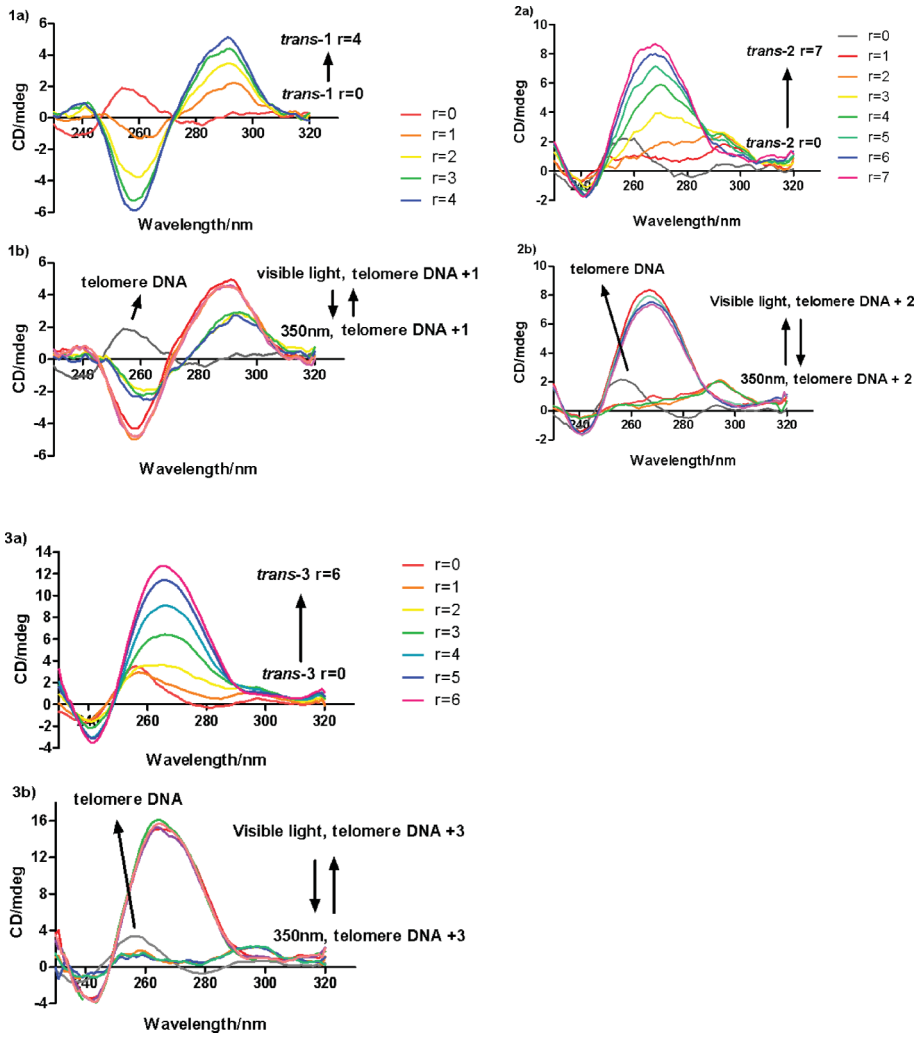


Fig. 3 CD studies of telomere DNA (20 μM) in 10 mM Tris/HCl buffer with EDTA (1 mM) at pH 7.4 in the absence of cation. a) Titration of *trans*-**1** (1a), *trans*-**2** (2a), *trans*-**3** (3a); b) CD Spectra of telomere DNA in the absence and presence of these synthesized compounds and the corresponding reversible G-quadruplex conformations upon photoirradiation (3 cycles). 1b for the situation of compound **1** ($r = 7$, r is the ratio of compound to DNA), 2b for compound **2** (r = 9), 3b for compound **3** ($r = 6$).

5 cycles. However, the CD spectrum of $d(TTAGGG)_4$ in the presence of *trans*-form compound **1**, dramatically different from the others, indicated an antiparallel G-quadruplex structure, which is characterized by a negative peak at 260 nm and a positive peak at 295 nm (Fig. 3-1a). Upon irradiation with UV light at 350 nm for 5 min, the intensity of the signals was somewhat altered, but the overall shape of the typical antiparallel quadruplex CD signal (positive signal at 295 nm and negative at 265 nm) was unchanged, and the process was reversible under UV/Vis photoillumination for 3 continuous cycles (Fig. 3-1b). Herein, it must be noted that although induced CD signals of these compounds in the DNA solutions could be observed between 320 nm and 500 nm (Fig. S1), few signals could influence the DNA spectra below 320 nm, which thus demonstrated these spectra were generated by G-quadruplex DNA.

In K^+-rich conditions, the CD spectra of telomere DNA ($d(TTAGGG)_4$) (Fig. 4, black curves) corresponds to a mixed-hybrid type G-quadruplex structure, which is characterized by two positive peaks at 290 and 270 nm (a positive maximum at 295 nm and a shoulder at 270 nm). The spectrum was obviously altered by the addition of compound **3**, the intensity of the signal at 268 nm was reduced and a negative peak at nearly 260 nm appeared (Fig. 4-3a), indicating that an antiparallel G-quadruplex structure was formed under these conditions. After irradiation with UV light at 350 nm, the newly formed antiparallel G-quadruplex structure had turned back to the former mixed-hybrid type. After irradiation by visible light to get the *iso*-form of compound **3**, the $d(TTAGGG)_4$ showed the corresponding CD signal of the antiparallel G-quadruplex structure again, which suggested that the conformation of the G-quadruplex of telomere DNA ($d(TTAGGG)_4$) alternated between the antiparallel and mixed-hybrid forms by photoregulation with UV/Vis light (Fig. 4-3b). These results revealed that the conformation of $d(TTAGGG)_4$ could be regulated by compound **3** upon photo-irradition in the presence of K^+. The situations were quite similar in the presence of compounds **1** (Fig. 4-1a, 1b) and **2** (Fig. 4-2a,

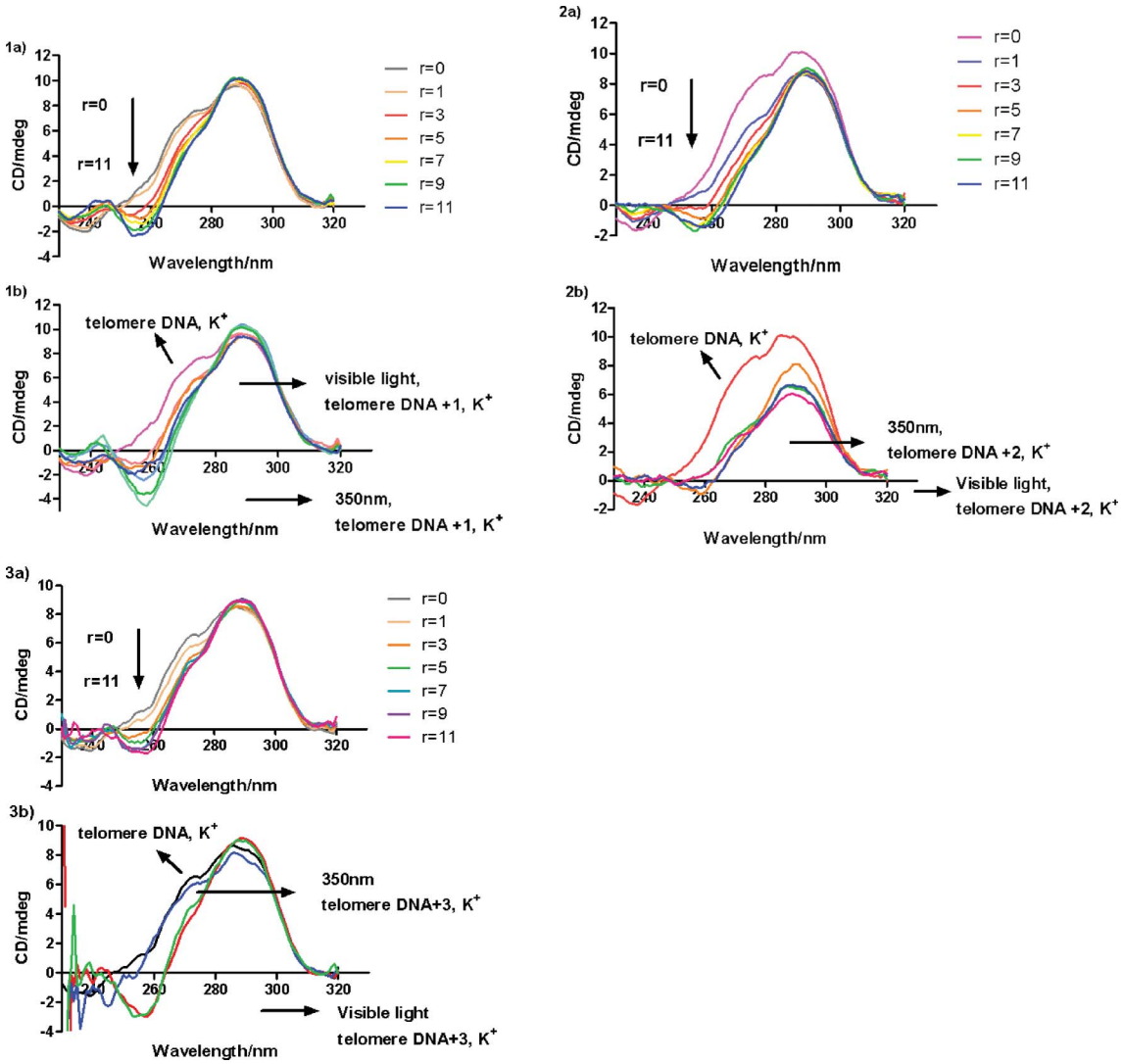


Fig. 4 CD studies of telomere DNA (20 μM) in 10 mm Tris/HCl buffer with EDTA (1 mM) at pH 7.4 in the presence of K^+ (50 mM). a) Titration of *trans*-**1** (1a), *trans*-**2** (2a), *trans*-**3** (3a); b) CD Spectra of telomere DNA in the absence and presence of these synthesized compounds and the corresponding reversible G-quadruplex conformations upon photoirradiation (3 cycles). 1b for the situation of compound **1** ($r = 11$), 2b for compound **2** ($r = 11$), 3b for compound **3** ($r = 12$).

2b), which both interconvert between the mixed-hybrid type G-quadruplex structure of $d(TTAGGG)_4$ in K^+-rich conditions and the antiparallel form, and the exchange processes were reversible, although not so totally as for compound **3**. Besides, it is necessary to mention that the spectrum of the K^+-quadruplex only was unchanged between the photoillumination condition and UV irradiation at 350 nm (Fig. S2 in Supplementary Information†).

Considering the affect of ionic strength, comparable studies were then performed in the presence of Li^+. As expected, the CD spectra of DNA ($d(TTAGGG)_4$) were quite similar as for the situation in the absence of any metal ions when the three synthesized compounds were added into the systems (Fig. S3). These observations strongly suggested that the presence of Li^+ had no effect on the photoregulation of the structures of telomere DNA ($d(TTAGGG)_4$) and further implied that the affect of ionic strength could be ignored.

Fluorescence spectra

To further check the DNA conformation photo-regulation of these compounds, fluorescence spectroscopy was carried out. Ap (2-aminopurine) is a fluorescent adenine isomer that has been widely utilized to probe local conformational changes in DNA.[24] It is a sensitive tool for probing the loop environment without complex processing or altering the overall quadruplex. In this study, the fluorescence intensities of the Aps before and after addition of the compound were definitely different (Fig. S4), indicating different circumstances for the adenine residues. However, it must be noted that the sharp decrease of fluorescence intensities was probably due to the quenching effect of the ligand. The environments of Aps were also assessed by fluorescence quenching with ascorbic acid, which has been used to probe solvent accessibility of Ap in the quadruplex.[25] The distinct quenching efficiencies shown in Fig. S5 further revealed the different circumstances for these residues.

Although these Ap experiments could not suggest an exact G-quadruplex conformation they still revealed the conformation variation to some extent.

Native-gel electrophoresis

To investigate the binding affinity of these compounds, a native-gel electrophoresis[26] was performed by using the single-stranded G4TTA DNA sequence labeled with FAM (5′-FAM-TTAGGGTTAGGGTTAGGGTTAGGG-3′) and imaged under irradiation of UV light. The specific binding of the compound with intramolecular G-quadruplex structures which was expected to be serviceable under irradiation of visible light, would become invalid after UV irradiation at 350 nm for 300 s (Fig. 5). The mixture with compound **2** which had a *trans*-form after the visible light irradiation has a slower migrating band, referring to the complex of the oligonucleotide interacting with the compound. After the compound have been irradiated at 350 nm for more than 300 s, only one bright band could be detected, just as for the mixture without this small molecule. When the *cis*-form of the compound turned back to the *trans*-form after exposure to visible light, the upper bands had emerged again. From the result of gel, we can also infer that the affinity of the compound for DNA can be regulated by the light, revealing this to be a controllable process.

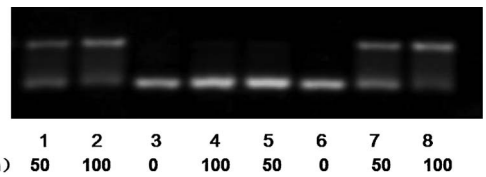


Fig. 5 Native gel electrophoresis: the native gel electrophoresis was run on 20% polyacrylamide gel containing 50 mM KCl at 4 °C, 27 V cm^{-1} in 1×TBE buffer containing 50 mM KCl. The mixture containing 1 μM FAM labeled DNA, 50 mM KCl, 0.01 M Tris-HCl buffer (1 mM EDTA, pH 7.4) were heated to 94 °C, and then slowly cooled down to 4 °C with different concentrations of compound **2**. Lane 1 and lane 2 are mixtures with compounds irradiated with visible light. Lane 4 and lane 5 were mixtures with compounds irradiate at 350 nm. Lane 7 and lane 8 were mixtures with compounds irradiate by visible light again after UV irradiation.

Conclusion

In summary, we have successfully designed and synthesized three azobenzene derivatives **1**–**3** which can photoregulate the conformation of G-quadruplex DNA, and further studied the situation in the presence of metal ions. These results suggest that: 1) Without metal ions, all the three synthesized compounds can regulate the dissociation and formation of G-quadruplex DNA upon reversible irradiation by UV/Vis photoillumination accompanied with the corresponding interconversion of the *cis*- and *trans*-forms. Due to the different substituents of the compounds, the conformations of G-quadruplexes formed were different under visible light. 2) In K^+-rich conditions, the three designed compounds can reversibly interconvert the original mixed-hybrid type G-quadruplex structure of $d(TTAGGG)_4$ to the antiparallel form reversibly upon irradiation by UV/Vis photoillumination.

These results provide possible potential applications both in biological and bionanotechnological fields. In living cells, G-quadruplex formation and dissociation have been widely considered as having an important role in gene transcription and expression. The light-induced conformational switching of human telomeric G-quadruplex DNA under physiological conditions provided a promising step to realize the G-quadruplex conformation control in living biological environments, which might result in regulation of certain cellular behavior. Besides, G-quadruplex structures have been utilized in several nanomachines and nanoarchitectures up to now. G-quadruplex conformation variation by light is definitely a convenient pathway to realize G-quadruplex stretch movement, further expanding the application of G-quadruplex structures in nanoarchitectures.

Acknowledgements

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