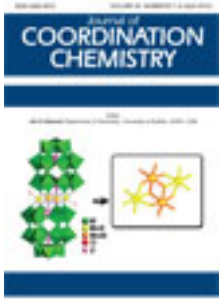


Journal of Coordination Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gcoo20

Synthesis of a ruthenium(II) polypyridine complex with 1,10-phenanthrolineselenazole as ligand and investigation of its G-quadruplex DNA-binding properties

Fang Yuan [a], Xiaojia Chen [b], Yanhui Zhou [a], Fang Yang [a], Qianling Zhang [c] & Jie Liu [a]

[a] Department of Chemistry, Jinan University, Guangzhou 510632, China

[b] National Engineering Research Center of Genetic Medicine, Guangdong Provincial Key Laboratory of Bioengineering Medicine, Bioengineering Institute of Jinan University, Guangzhou 510632, China

[c] School of Chemistry and Chemical Engineering, Shenzhen University, Shenzhen 518061, China

Published online: 21 Mar 2012.

To cite this article: Fang Yuan, Xiaojia Chen, Yanhui Zhou, Fang Yang, Qianling Zhang & Jie Liu (2012) Synthesis of a ruthenium(II) polypyridine complex with 1,10-phenanthrolineselenazole as ligand and investigation of its G-quadruplex DNA-binding properties, Journal of Coordination Chemistry, 65:7, 1246-1257, DOI: 10.1080/00958972.2012.670228

To link to this article: http://dx.doi.org/10.1080/00958972.2012.670228

Journal of Coordination Chemistry
Vol. 65, No. 7, 10 April 2012, 1246–1257

Synthesis of a ruthenium(II) polypyridine complex with 1,10-phenanthrolineselenazole as ligand and investigation of its G-quadruplex DNA-binding properties

FANG YUAN†¶, XIAOJIA CHEN‡¶, YANHUI ZHOU†, FANG YANG†, QIANLING ZHANG*§ and JIE LIU*†

†Department of Chemistry, Jinan University, Guangzhou 510632, China
‡National Engineering Research Center of Genetic Medicine, Guangdong Provincial Key Laboratory of Bioengineering Medicine, Bioengineering Institute of Jinan University, Guangzhou 510632, China
§School of Chemistry and Chemical Engineering, Shenzhen University, Shenzhen 518061, China

(*Received 9 November 2011; in final form 19 January 2012*)

A new Ru(II) complex, $[Ru(bpy)_2L](ClO_4)_2$ (bpy = 2,2′-bipyridine, L = 1,10-phenanthrolineselenazole), has been synthesized and structurally characterized by elemental analysis, ESI-MS, and ^1H NMR. The interaction of human telomeric oligomer 5′-$AG_3(T_2AG_3)_3$-3′ with the Ru(II) complex was explored by competition FRET experiment, fluorescence titration, circular dichroism spectroscopy, thermal denaturation, polymerase chain reaction stop assay, and TRAP assay. The Ru(II) complex can selectively bind to G-quadruplex DNA. The results indicated that the complex not only induces a remarkable conformational change of human telomeric DNA, but also has the ability to stabilize the G-quadruplex.

Keywords: Ru(II) complex; Telomeric oligomer; Stabilized G-quadruplex

1. Introduction

G-quadruplexes are non-canonical structures formed by certain guanine-rich sequences of DNA. The G-quadruplex formed by the human telomeric DNA sequence has been of particular interest, owing to the importance of telomere maintenance for cellular proliferation. Consequently, the G-quadruplex formed by telomeric DNA is under investigation as a potential molecular target for anticancer drugs [1, 2]. In human telomeric DNA, the G-quadruplex DNA has a short sequence 5′-$AG_3(T_2AG_3)_3$-3′ and is tandemly repeated in rich guanine residue domains. The formation of G-quadruplex complexes can prevent telomerase elongation by interrupting the interaction between the enzyme and unfolded guanine-rich single-strand [3]. Therefore, the use of Ru(II)

*Corresponding authors. Email: qlzh98@yahoo.com.cn; tliuliu@jnu.edu.cn
¶Contributed equally to this work.

ISSN 0095-8972 print/ISSN 1029-0389 online © 2012 Taylor & Francis
http://dx.doi.org/10.1080/00958972.2012.670228

complexes that promote the formation of and/or stabilize G-quadruplex structures has become attractive for anticancer drug design.

Interaction of Ru(II) complexes containing planar polycyclic heteroaromatic ligands with DNA has attracted much attention [4]. Studies on DNA-binding of these complexes are very important in development of molecular light switches, DNA footprinting agents, nucleic acid probes, chemotherapy, and photodynamic therapy [5–7]. In general the main ligands of Ru(II)-polypyridyl complexes possess extended p-aromatic and planar structures that can insert and stack between the base pairs of double helical DNA. Subtle changes in the molecular structures of Ru(II) complexes might bring about substantial effects on the binding modes, sites, and affinities, and provide the chance to explore valuable information on conformation or site-specific DNA probes. Therefore, studies on modifying the main ligand is quite significant for understanding the optical properties of DNA-binding and action mechanism of Ru(II) polypyridine complexes. However, most studies are on double helix DNA rather than the G-quadruplex DNA [8, 9]. Most of the reported complexes contain only planar aromatic ligands and investigations of Ru(II)-polypyridine complexes with Se metal ligands as DNA-binding reagents are relatively few [10]. Ru(II) complexes have prominent DNA-binding properties, especially $[Ru(bpy)_2(dppz)]^{2+}$ (dppz = dipyrido [3,2-a:2,3′-c] phenazine) known as DNA "light switch." Shi *et al*. [11] studied the action of $[Ru(bpy)_2(dppz)]^{2+}$ with 5′-$AG_3(T_2AG_3)_3$-3′ and found that the complex can serve as a prominent molecular "light switch" for both G-quadruplexes. As a star molecule, it would consist of one dppz ligand and two polycyclic ligands. However, complex combined with one 1,10-phenanthrolineselenazole ligand is rare. Herein, we studied the interaction of a complex of $[Ru(bpy)_2L](ClO_4)_2$ with G-quadruplex.

In this article, a new Ru(II) complex, $[Ru(bpy)_2L](ClO_4)_2$ (bpy = 2,2′-bipyridine, L = 1,10-phenanthrolineselenazole), was synthesized and characterized and the interaction of this compound with DNA as well as related properties were experimentally explored.

2. Experimental

2.1. *Material*

All reagents and solvents were purchased commercially and used without purification unless otherwise noted. Human telomeric DNA oligomers 5′-AGGGTTAGGGTTAGGGTTAGG G-3′(5′-$AG_3(T_2AG_3)_3$-3′) (HTG22), the labeled oligonucleotides F22T: 5′-FAM-d($AG_3(T_2AG_3)_3$)-TAMRA-3′, TS (5′-$A_2TC_2GTCGAGC(AG)_2T_2$-3′), CXext (5′-$GTG(C_3T_2-A)_4$-3′), NT (5′-$ATCGCT_2CTCG_2C_2T_4$-3′), and TSNT (5′-$AT_2C_2GTCGAGC(AG)_2T_2A_4G_2C_2(GA)_2$AGCGAT-3′) were purchased from Shanghai Sangon Biological Engineering Technology & Services (Shanghai, China) and used without purification. Concentrations of calf-thymus DNA (CT-DNA) (highly polymerized stored at 4°C) (Sigma, stored at −20°C) oligomers were determined by measuring the absorbance at 260 nm after melting. Single-strand extinction coefficients were calculated from mononucleotide data using a nearest-neighbor approximation [12]. The formations of intramolecular G-quadruplexes was carried out as follows: the oligonucleotide samples,

dissolved in different buffers, were heated to 90°C for 5 min, gently cooled to room temperature, and then incubated at 4°C overnight. Buffer A: 10 mmol L^{-1} Tris-HCl, pH = 7.2; Buffer B: 10 mmol L^{-1} Tris-HCl, 100 mmol L^{-1} NaCl, pH = 7.2; Buffer C: 10 mmol L^{-1} Tris-HCl, 100 mmol L^{-1} KCl, pH = 7.2; Buffer D: 10 mmol L^{-1} Tris-HCl, 60 mmol L^{-1} NaCl, pH = 7.4. Solutions of CT-DNA in the buffer 5 mmol L^{-1} Tris HCl/50 mmol L^{-1} NaCl in water gave a ratio of 1 : 9 of UV absorbance at 260 and 280 nm, A260/A280 [13], indicating that the DNA was sufficiently free of protein. Concentrated stock solutions of DNA (10 mmol L^{-1}) were prepared in buffer and sonicated for 25 cycles, where each cycle consisted of 30 s with 1 min intervals. The concentration of DNA in nucleotide phosphate was determined by UV absorbance at 260 nm after 1 : 100 dilutions. The extinction coefficient, $\varepsilon_{260\,nm}$, was taken as 6600 $(mol\,L^{-1})^{-1}\,cm^{-1}$. Stock solutions were stored at 4°C and used after no more than 4 days.

2.2. *Physical measurement*

Microanalyses (C, H, and N) were carried out with a Perkin-Elmer 240 C elemental analyzer. Electrospray ionization mass spectrometry (ESI-MS) was recorded on a LQC system (Finnigan MAT, USA) using CH_3CN as mobile phase. ^1H NMR spectra were recorded on a Varian Mercury-plus 300 NMR spectrometer with DMF-d_6 as solvent and $SiMe_4$ as an internal standard at 300 MHz at room temperature. All chemical shifts relative to TMS (tetramethylsilane) were given. UV-Vis and emission spectra were measured on a Perkin-Elmer Lambda-850 spectrophotometer. Competition FRET experiment was measured on a RT-PCR. Circular dichroism (CD) spectra were recorded on a Jasco J-810 spectropolarimeter.

2.3. *Synthesis and characteristics*

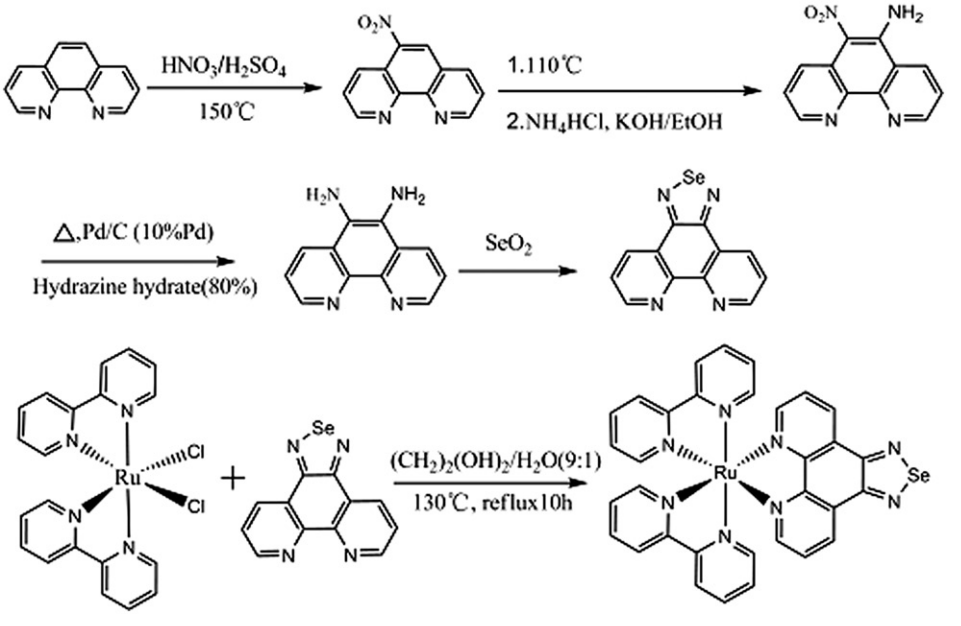
2.3.1. 5-nitrophenanthroline. A mixture of phenanthroline (5 g, 28 mmol) and concentrated sulfuric acid (30 mL) was heated at 150°C. After being added to an amount of nitric acid, the solution was refluxed for 2 h. The mixture was cooled to room temperature and then put into ice water (500 mL) and sodium hydroxide added (10 mol L^{-1}) to adjust the pH to 7. After filtration and washing with distilled water three times, a yellow precipitate resulted. Yield: 3 g (50%).

2.3.2. 5-amino-6-nitrophenanthroline. A mixture of 5-nitrophenanthroline (2 g, 9 mmol) and absolute ethyl alcohol was heated at 110°C. After the solid was dissolved and resulted in a yellow precipitate, hydroxylamine hydrochloride (4 g) was added to the mixture. Upon dropwise addition of a potassium hydroxide absolute ethyl alcohol solution, a reddish brown solution formed. After being cooled to room temperature, the solution was refluxed 30 min and added into ice water (600 mL), and let stand overnight. The mixture was filtered and washed with distilled water, methanol, and chloroform, resulting in a yellow precipitate. Yield: 0.8 g (35%).

2.3.3. 5,6-diaminophenanthroline. A mixture of 5-amino-6-nitrophenanthroline (1 g, 4 mmol) and absolute ethyl alcohol (1 L) was heated at 90°C for 8 h. After being added to two reagents (Pd/C (10%) (0.5 g) catalyst and hydrazine hydrate (80%) (20 mL)), 5 h later the hot solution was filtered and concentrated to saturation. Petroleum ether was added to the saturated filtrate to form a yellow cotton-like precipitate. Precipitate was collected by filtration. Yield: 0.6 g (75%).

2.3.4. 1,10-phenanthrolineselenazole [14]. A mixture of 5,6-diaminophenanthroline (0.1 g, 0.5 mmol) and selenium dioxide was ground to red powder in the same mole ratio. The powder was washed with distilled water (50 mL), which resulted in a pink product.

2.3.5. $[Ru(bpy)_2L](ClO_4)_2$. The synthetic routes for $[Ru(bpy)_2L](ClO_4)_2$ were showed in Scheme 1. A mixture of 1,10-phenanthrolineselenazole (0.2 g, 0.7 mmol), $Ru(bpy)_2Cl_2 \cdot 2H_2O$ (0.3 g, 0.6 mmol) and $(CH)_2(OH)_2/H_2O$ (9 : 1 v/v) was heated at 130°C for 10 h [15]. The solution was cooled to room temperature. Upon filtration and dropwise addition of saturated $NaClO_4$, a red-orange precipitate formed. The precipitated complex was dried, then dissolved in a small amount of acetonitrile, and purified with MeCN by chromatography over alumina. Yield: 0.15 g (15%). ^{1}H NMR [CD_3CN] (figure 1): δ 9.11 (d, 2 H), 8.57 (t, 4 H), 8.15–8.11 (multiplet, 4 H), 8.05 (t, 2 H), 7.84 (d, 2 H), 7.80 (q, 2 H), 7.74 (d, 2 H), 7.48 (t, 2 H), 7.31 (t, 2 H). ESI-MS [CH_3CN] (figure 2): m/z 801.0(M-ClO_4), 350.3(M-2ClO_4/2). Calcd for $RuC_{32}H_{22}N_8Se$: C, 55.01; H, 3.15; N, 16.05. Found: C, 55.04; H, 3.13; N, 16.04. UV-Vis (λ (nm),



Scheme 1. Synthetic routes for $[Ru(bpy)_2L](ClO_4)_2$.

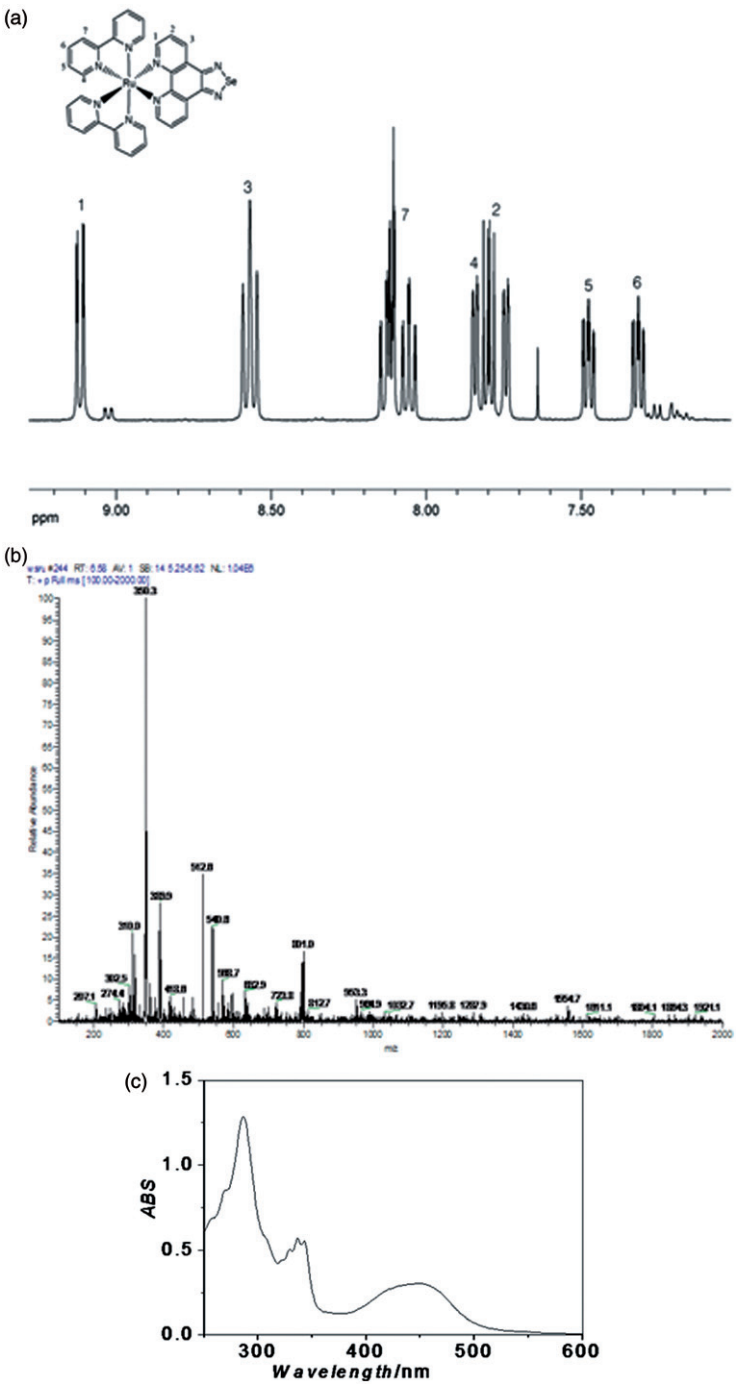


Figure 1. (a) ^{1}H NMR of $[Ru(bpy)_2L](ClO_4)_2$; (b) ESI-MS of $[Ru(bpy)_2L](ClO_4)_2$; (c) absorption spectra of $[Ru(Phense)(bpy)_2](ClO_4)_2$, ($[Ru] = 20\,mmol\,L^{-1}$) in CH_3CN.

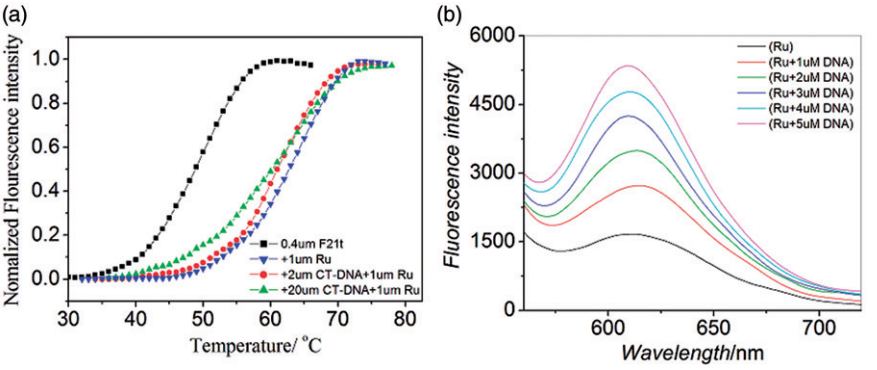


Figure 2. (a) The competition FRET experiment of $[Ru(bpy)_2L](ClO_4)_2$ between quadruplex DNA and CT-DNA in Tris buffer D in the presence of G-quadruplex DNA and CT-DNA. $[Ru] = 1\,\mu mol\,L^{-1}$, [G-quadruplex DNA] = [CT-DNA] = $0.4\,\mu mol\,L^{-1}$; (b) Emission spectra of $[Ru(bpy)_2L](ClO_4)_2$ in Tris buffer (pH 7.2) in the presence of increasing amounts of G-quadruplex DNA. $[Ru] = 10\,\mu mol\,L^{-1}$, [DNA] = 0–5 $\mu mol\,L^{-1}$ from bottom to top. Figure 2(b) show the emission intensity changes upon increasing the DNA concentration.

ε ($(mol\,L^{-1})^{-1}\,cm^{-1}$)) ($CH_3CN$) (Supplementary material): MLCT 447 (15,100), phense 336 (28,600), phen 285 (63,500).

2.4. *DNA-binding experiments*

Buffer B was used for emission titration. Buffers A–C were used for CD spectral measurements. Buffer C was used for thermal DNA denaturation experiments. Buffer D was used for the competition FRET experiment.

The competition FRET experiment was measured on a RT-PCR (MJ Research, Waltham, MA). The following oligonucleotide sequences were used: F22T: 5′-FAM-d($AG_3(T_2AG_3)_3$)-TAMRA-3′ and CT-DNA. TAMRA (6-carboxytetramethylrhodamine) is the acceptor fluorophore and FAM (6-carboxyfluorescein) is the donor fluorophore. From 50 $\mu mol\,L^{-1}$ stock solutions, 400 $nmol\,L^{-1}$ solutions in buffer D were prepared. The nucleotides were annealed by heating the samples to 95°C for 5 min and allowing them to cool within 1 h. one $mmol\,L^{-1}$ solutions of the compound in deionized water were prepared and diluted to double of the required concentrations with buffer D. In RT-PCR 96 well plates, the fluorescence was read at intervals of 1°C from 30°C to 90°C. Before each reading the temperature was held constant for 60 s. The competition FRET assay was performed similarly to the FRET assay, but in addition to the tagged G-quadruplex sequence F22T and the drug, CT-DNA solution was added. The duplex DNA was added in ratios quadruplex/duplex = 1 : 5 and 1 : 50. Concentration in the wells were 400 $nmol\,L^{-1}$ of G-quartets for F22T, 1 $\mu mol\,L^{-1}$ for the compound and 0, 2 $\mu mol\,L^{-1}$, and 20 $\mu mol\,L^{-1}$ of CT-DNA to be tested. The test was repeated three times.

The emission titrations of the Ru(II) complex in buffer were performed using a fixed concentration (10 $\mu mol\,L^{-1}$) for $[Ru(bpy)_2L](ClO_4)_2$ to which increments of the DNA stock solution were added. Ru-DNA solutions were allowed to incubate for 5 min

before the absorption spectra were recorded. Titrations for each sample were repeated at least three times.

Thermal denaturation studies were carried out with a Jasco J-810 spectrophotometer in buffer C. With the use of the thermal melting program, the temperature of the cell containing the cuvette was run from 30°C to 100°C. The absorbance at 260 nm was monitored every 2°C for solutions of G-quadruplex DNA (2 μmol L^{-1}) in the absence and presence of Ru(II) complex (4 μmol L^{-1}). The melting temperature $T_{m,}$ which was defined as the temperature where half of the total base pairs was unbonded, was determined from the midpoint of the melting curves [16]. DT_m values were calculated by subtracting T_m of the free nucleic acid from that of DNA-complex adduct.

Preparation of Ru complex promoted by G-quadruplex-hemin DNAzyme is as follows: an equal volume of Ru complex solution (in water) was added to the DNA solutions (20 mmol L^{-1} DNA, 10 mmol L^{-1} Tris-HCl, 100 mmol L^{-1} EDTA, pH = 8.00), allowing the DNA strands to form the G-quadruplex structure in 40 min. Then an equal volume of hemin (in DMSO) was dissolved in the above G-quadruplex solutions and kept for 2 h at room temperature to form the DNAzymes. Subsequently, 180 μL of 296 μmol L^{-1} TMB-1 was added. 76 mmol L^{-1} H_2O_2 solution was added as the substrate to 20 μL of the above peroxidatic DNAzyme system. The mixture was kept for 1.5 h at room temperature, different colors were observed with the naked eye and the photograph of the mixture was taken with a digital camera.

CD spectra were measured on a Jasco J-810 spectropolarimeter at room temperature. The region between 220 and 320 nm was scanned for each sample in buffers A–C. 1.0 μL Ru(II) complex (1 mmol L^{-1}) was added sequentially to solutions containing G-quadruplex (2.0 μmol L^{-1}). All solutions were mixed thoroughly and allowed to equilibrate for 5 min before data collection. The CD spectral studies for each sample were repeated at least three times. The scan of the buffer alone was subtracted from the average scan for each sample.

In the telomeric repeat amplification protocol (TRAP) assay, telomerase extract was prepared from HepG-2 cells. TRAP assay was performed by using a modification of the TRAP assay. Polymerase chain reaction (PCR) was performed in a final 50 μL reaction volume composed of a 45 μL reaction mixture containing 20 mmol L^{-1} Tris-HCl (pH 8.0), 50 μmol L^{-1} deoxynucleotide triphosphates, 1.5 mmol L^{-1} $MgCl_2$, 63 mmol L^{-1} KCl, 1 mmol L^{-1} EGTA, 0.005% Tween 20, 20 μg mL^{-1} BSA, 3.5 pmol of primer HTG22 (5′-$AG_3(T_2AG_3)_3$-3′), 18 pmol of primer TS (5′-$A_2TC_2GTCGAGC(AG)_2T_2$-3′), 22.5 pmol of primer CXext (5′-$GTG(C_3T_2A)_4$-3′), 7.5 pmol of primer NT (5′-$ATCGCT_2CTCG_2C_2T_4$-3′), 0.01 amol of TSNT internal control (5′-$AT_2C_2GTCGAGC(AG)_2T_2A_4G_2C_2(GA)_2AGCGAT$-3′), 2.5 U of Taq DNA polymerase, and 100 ng of telomerase. Compounds or distilled water was added under a volume of 5 μL. PCR was performed in an Eppendorf Master cycler equipped with a hot lid and incubated for 30 min at 30°C, followed by 92°C 30 s, 52°C 30 s, and 72°C 30 s for 30 cycles. After amplification, 8 μL of loading buffer (containing 5 × Tris-Borate-EDTA buffer (TBE buffer), 0.2% bromophenol blue, and 0.2% xylene cyanol) was added to the reaction. A 15 μL aliquot was loaded onto a 16% non-denaturing acrylamide gel (19 : 1) in 1 × TBE buffer and electrophoresed at 200 V for 1 h. Gels were fixed and then stained with $AgNO_3$.

In PCR stop assay, the oligonucleotide HTG22 (5′-$AG_3(T_2AG_3)_3$-3′) and the corresponding complementary sequence (HTG22rev, $ATCGCT_2CTCGTC_3TA_2C_2$) were used. The reactions were performed in 1 × PCR buffer, containing 10 pmol of

each oligonucleotide, 0.16 mmol L^{-1} dNTP, 2.5 U Taq polymerase, and different concentrations of complex. Reaction mixtures were incubated in a thermocycler with the following cycling conditions: 94°C for 3 min, followed by 30 cycles of 94°C for 30 s, 58°C for 30 s, and 72°C for 30 s. PCR products were then analyzed on 15% non-denaturing polyacrylamide gels in 1 × TBE and silver stained.

3. Results and discussion

3.1. *FRET and fluorescence titration studies*

Here we chose high ionic strength systems to approximate physiological conditions. Two different DNA sequences, including the labeled oligonucleotides F22T(5′-FAM-d(5′-$AG_3(T_2AG_3)_3$-3′)–TAMRA-3′) and CT-DNA. Furthermore, the G-quadruplex selectivity of $[Ru(bpy)_2L](ClO_4)2$ was assessed by a FRET-based competition assay where the ability of ligand to retain G-quadruplex stabilizing affinity was challenged by non-fluorescent duplex DNA (CT-DNA) [17]. In the presence of CT-DNA, the thermal stabilization of F22T enhanced by $[Ru(bpy)_2L](ClO_4)2$ was slightly affected (figure 2a). From the figure we found that a duplex : quadruplex = 5 : 1 ratio does not interfere with the stabilization of the G-quadruplex by $[Ru(bpy)_2L](ClO_4)2$, and even at a 50-fold excess of duplex DNA, G-quadruplex stabilization is almost unchanged. The results demonstrate that the compounds have high selectivity for G-quadruplex DNA over CT-DNA.

In the absence of G-quadruplex DNA, the complex can emit luminescence in buffer C at ambient temperature, with a maximum appearing at 607 nm for the complex. As shown in figure 2(b), an obvious increase in emission intensity was observed for the complex. As increasing the concentration of G-quadruplex DNA, when the ratio of [DNA]/[Ru] reached 5 : 1, the emission intensities of the complex grew to about 3.5 times larger than in the absence of DNA. The enhancement of emission intensity was an indication of binding of the complex to the hydrophobic pocket of DNA, since the hydrophobic environment inside the DNA helix reduced the accessibility of water molecules to the complex and the complex mobility was restricted at the binding site [18, 19].

3.2. *Visual detection of G-quadruplex structures by Ru complex*

Although certain kinds of G-rich sequences have been demonstrated to form G-quadruplex structures readily under physiological concentrations of Na^+ and K^+ *in vitro* [20, 21], the existence of G-quadruplex structures *in vivo* is still controversial [22, 23]. Here we report a facile and visual approach to detect G-quadruplex with the naked eye. It is well known that most G-quadruplex DNAs can be effectively formed by K^+, and G-quadruplexes have the ability to bind with hemin to form the peroxidase-like DNAzymes. It is proven that in the presence of the DNAzymes, H_2O_2-mediated oxidation of TMB (3,3′,5,5′-tetramethylbenzidine) could be sharply accelerated and the color change is very sensitive and easy to identify. The design is based on this principle. As shown in "Supplementary material," in the presence of $[Ru(bpy)_2L](ClO_4)2$, 5′-$AG_3(T_2AG_3)_3$-3′ can also fold into G-quadruplex, and such quadruplex structure is able to bind hemin to form the hemin-G-quadruplex DNAzyme that catalyzes the

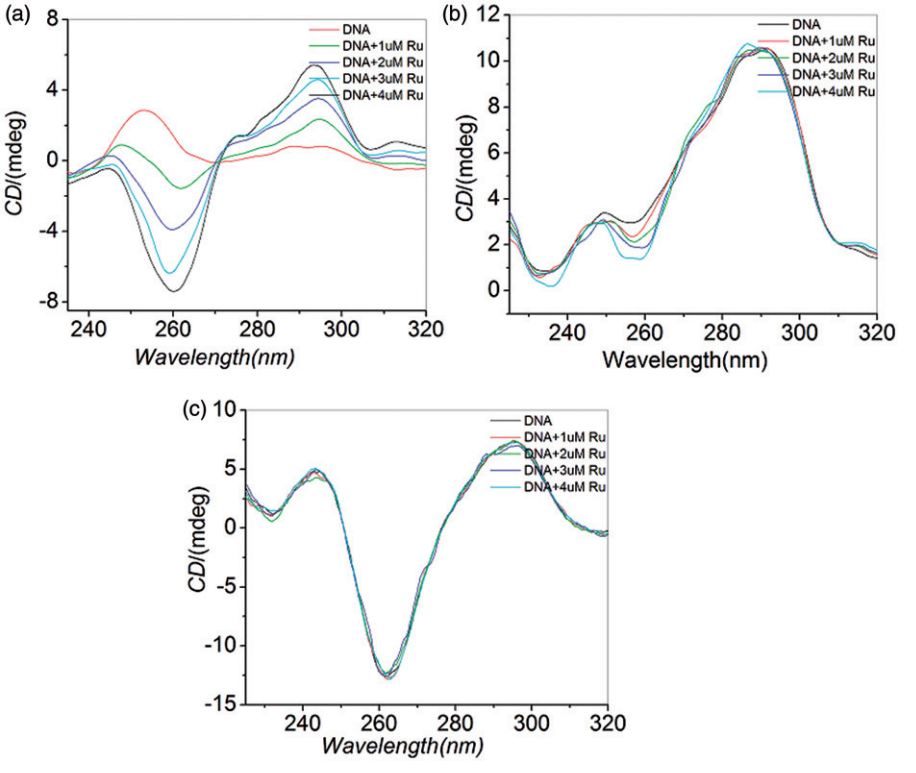


Figure 3. (a) CD spectra of G-quadruplex DNA in Tris buffer (pH 7.2) in the presence of increasing amounts of $[Ru(bpy)_2L](ClO_4)_2$. [Ru] = 0.9 μmol L^{-1}, [DNA] = 2 μmol L^{-1}; (b) CD spectra of G-quadruplex DNA in 10 mmol L^{-1} Tris-HCl and 100 mmol L^{-1} KCl (pH 7.2) in the presence of increasing amounts of $[Ru(bpy)_2L](ClO_4)_2$. [Ru] = 0–4 μmol L^{-1}, [DNA] = 2 μmol L^{-1}; (c) CD spectra of G-quadruplex DNA in 10 mmol L^{-1} Tris-HCl and 100 mmol L^{-1} NaCl (pH 7.2) in the presence of increasing amounts of $[Ru(bpy)_2L](ClO_4)_2$. [Ru] = 0–4 μmol L^{-1}, [DNA] = 2 μmol L^{-1}.

H_2O_2-mediated oxidation of colorless TMB to the blue product, as well as control K^+. But for complex with double strands of CT-DNA, the solution is still colorless. The reason is obvious, because CT-DNA cannot form the G-quadruplex structure.

3.3. *CD spectral studies*

CD spectra can provide information about the chirality of spectroscopically active species in solution. The rac-metal complexes give a zero CD but show induced CD (ICD) signals on enantiopreferential binding to DNA, providing further and definitive confirmation for their DNA binding [24]. Thus, the CD spectral technique has been used to study the enantiopreferential DNA binding of rac-metal complexes. Figure 3(a) shows the CD spectra for the titration of $AG_3(T_2AG_3)_3$ with increasing amounts of complexes. Without any metal cations, the CD spectra of the human telomeric 5′-$AG_3(T_2AG_3)_3$-3′ at room temperature exhibited a major positive band at 252 nm and a rather broad positive signal around 290 nm [25]. These results suggest that telomeric 5′-$AG_3(T_2AG_3)_3$-3′ might coexist as a single-strand and two types of quadruplex DNA structures: parallel and antiparallel [26]. However, upon addition of the complex to the

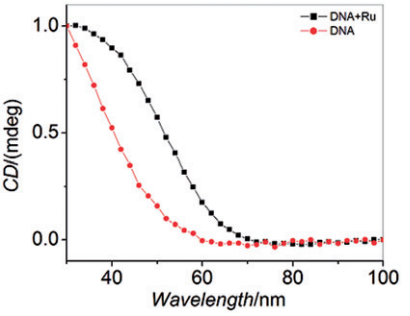


Figure 4. CD spectra of $[Ru(bpy)_2L](ClO_4)_2$ and G-quadruplex DNA in 5 mmol L^{-1} Tris-HCl and 50 mmol L^{-1} KCl (pH 7.2) were heated from 30°C to 100°C, recording data every 2°C. [DNA] = 2 μmol L^{-1}, [Ru] = 4 μmol L^{-1}.

oligonucleotide, a dramatic change in the CD spectrum was observed. The maximum positive band at 252 nm was gradually changed to a negative band at 260 nm and a new positive band at 290 nm appeared. As the complex was added, the band of the CD spectrum became stronger. These results indicate that the complex induced a remarkable conformational change of human telomeric DNA. The conformation of DNA was turned from simple chain to the anti-parallel G-quadruplex form due to binding of the rac-complex and/or ligand–ligand interactions among the DNA bound/unbound complex [27].

It has been reported that the human telomeric sequence forms a typical anti-parallel G-quadruplex structure in the presence of Na^+ ions in the CD spectra [28]. On the other hand, in the presence of K^+ ions, it may form a hybrid of parallel/anti-parallel G-quadruplex structures [29]. However, upon addition of the complex to 5′-$AG_3(T_2AG_3)_3$-3′ in 100 mmol L^{-1} KCl or NaCl buffer, the CD spectrum exhibited a maxima-minima pattern similar to, but not identical with, the spectrum in K^+ or Na^+ without the addition of complex (figure 3b and c), which implied that the conformation of the G-quadruplex was stabilized by K^+ or Na^+ and the complex could not change the conformation of the G-quadruplex at high ionic strength.

3.4. *Thermal denaturation study*

CD spectroscopy was also used to measure the thermodynamic stability profile of the G-quadruplex oligomer incubated with the complex in the presence of K^+ [30]. The melting curves of human telomeric sequence in the absence and presence of the complex are presented in figure 4. In this experiment, the thermal CD transition was monitored at 295 nm. A T_m of G-quadruplex oligomer in K^+ was determined as 42°C. On addition of the complex, the T_m of the DNA increased to 53°C at a concentration ratio [Ru]/[DNA] = 2 : 1. The complex showed T_m values of 11°C, indicating that the complex possesses the ability to stabilize the G-quadruplex structure in the presence of K^+.

3.5. *Inhibition of amplification in HTG22 by Ru complex*

In order to further evaluate the ability of $[Ru(bpy)_2L](ClO_4)2$ to stabilize G-quadruplex DNA, a PCR stop assay was used to ascertain whether the complex was bound to the

test oligomer (5′-AG3(T2AG3)3-3′) and stabilized the G-quadruplex structure [31–33]. In the presence of the complex, the template sequence HTG22 was induced into a G-quadruplex structure that blocked the hybridization with a complementary primer sequence. In that case, 5′ to 3′ primer extension by DNA Taq polymerase was arrested and the final double-stranded DNA PCR product could not be detected [34]. The inhibitory effect of the complex was clearly enhanced as the concentration increased from 0.5 to 20 μmol L^{-1}, with no PCR product detected at 20 μmol L^{-1} (Supplementary material). The experiment indicates that $[Ru(bpy)_2L](ClO_4)_2$ was a better G-quadruplex binder.

3.6. *TRAP assay*

In this TRAP assay experiment [35], a solution of $[Ru(bpy)_2L](ClO_4)_2$ was added to a telomerase reaction mixture containing the extract from HepG2 cells, which express that it had the ability to inhibit telomerase activity. ''Supplementary material'' (figure S3) showed the *in vitro* inhibitory effect of the complex toward the process of telomerase was studied in a dose-dependent manner and the number of bands clearly decreased with respect to the control, at a drug concentration in the range of 2.5–20 μmol L^{-1}. The result showed that $[Ru(bpy)_2L](ClO_4)2$ inhibited telomere elongation in a concentration-dependent manner.

4. Conclusion

The new Ru(II) complex $[Ru(bpy)_2L](ClO_4)2$ has been synthesized and characterized. G-quadruplex DNA-binding affinity of this complex is stronger than that of CT-DNA. The experimental studies show that $[Ru(bpy)_2L](ClO_4)_2$ can selectively bind to G-quadruplex DNA and change the steric configuration of G-quadruplex DNA. The complex can also inhibit telomerase's activity and stabilize G-quadruplex DNA that can increase the T_m value of G quadruplex by 11°C. More importantly, $[Ru(bpy)_2L](ClO_4)2$ can stabilize and induce intramolecular G-quadruplex structure transition and has made a suggestive choice of G-quadruplex DNA. It implies that it may be used as potential anticancer drug after further research. However, the conformation of the G-quadruplex is not changed by the complex at a high ionic strength in K^+ buffer. The details of the binding modes of this complex with G-quadruplex and the structure of G-quadruplex are not clear and further studies are needed.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (20871056, 21171070), the Planned Item of Science and Technology of Guangdong Province (c1011220800060), and the Planned Item of Science and The Fundamental Research Foundation of ShenZhen City (Major Project) (JC201005250058A).

References

[1] D.J. Patel, A.T. Phan, V. Kuryavyi. *Nucleic Acids Res.*, **35**, 7429 (2007).
[2] K.E. Erkkila, D.T. Odom, J.K. Barton. *Chem. Rev.*, **99**, 2777 (1999).
[3] A.M. Zahler, J.R. Williamson, T.R. Cech, D.M. Prescott. *Nature*, **350**, 718 (1991).
[4] L.N. Ji, X.H. Zou, J.G. Liu. *Coord. Chem. Rev.*, **216**, 513 (2001).
[5] J.L. Huppert. *Chem. Soc. Rev.*, **37**, 1375 (2008).
[6] S. Balasubramanian, S. Neidle. *Curr. Opin. Chem. Biol.*, **13**, 345 (2009).
[7] S.N. Georgiades, N.H. Abd Karim, K. Suntharalingam, R. Vilar. *Angew. Chem. Int. Ed.*, **49**, 4020 (2010).
[8] C. Tan, S. Hu, J. Liu, L.N. Ji. *Eur. J. Med. Chem.*, **46**, 1555 (2011).
[9] X.X. Yang, L.M. Chen, Y.N. Liu, Y.G. Yang, T.F. Chen, W.J. Zheng, J. Liu, Q.Y. He. *Biochimie*, **64**, 983 (2011).
[10] R.M. Hartshorn, J.K. Barton. *J. Am. Chem. Soc.*, **114**, 5919 (1992).
[11] S. Shi, X.T. Geng, J. Zhao, T.M. Yao, C.R. Wang, D.J. Yang, L.F. Zheng, L.N. Ji. *Biochimie*, **92**, 370 (2010).
[12] C.R. Cantor, M.M. Warshaw, H. Shapiro. *Biopolymers*, **9**, 1059 (1970).
[13] X.L. Hong, Z.H. Liang, M.H. Zeng. *J. Coord. Chem.*, **64**, 3792 (2011).
[14] J. Liu, X.N. Zhang, X.C. Yang, W.J. Zheng. CN20101259488[P], 2011-01-19.
[15] J. Liu, W.J. Zheng, S. Shi, C.P. Tan, J.C. Chen, K.C. Zheng, L.N. Ji. *J. Inorg. Chem.*, **102**, 193 (2008).
[16] L.M. Chen, J. Liu, J.C. Chen, S. Shi, C.P. Tan, K.C. Zheng, L.N. Ji. *J. Mol. Struct.*, **881**, 156 (2008).
[17] D. Chatterjee, A. Sengupta, R.V. Eldlk. *J. Coord. Chem.*, **64**, 1 (2011).
[18] X.C. Yang, Y.N. Liu, S.T. Yao, Y. Xia, Q. Li, W.J. Zheng, L.M. Chen, J. Liu. *J. Coord. Chem.*, **64**, 1491 (2011).
[19] Z.H. Liang, Z.Z. Li, H.L. Huang, Y.J. Liu. *J. Coord. Chem.*, **64**, 3342 (2011).
[20] Y.J. Lu, T.M. Ou, J.H. Tan, J.Q. Hou, W.Y. Shao, D. Peng, N. Sun, X.D. Wang, W.B. Wu, X.Z. Bu, Z.S. Huang, D.L. Ma, K.Y. Wong, L.Q. Gu. *J. Med. Chem.*, **51**, 6381 (2008).
[21] H.Q. Yu, D. Miyoshi, N. Sugimoto. *J. Am. Chem. Soc.*, **128**, 15461 (2006).
[22] K. Paeschke, S. Juranek, T. Simonsson, A. Hempel, D. Rhodes, H.J. Lipps. *Nat. Struct. Mol. Biol.*, **15**, 598 (2008).
[23] K. Paeschke, T. Simonsson, J. Postberg, D. Rhodes, H.J. Lipps. *Nat. Struct. Mol. Biol.*, **12**, 847 (2005).
[24] S. Delaney, M. Pascaly, P.K. Bhattacharya, K. Han, J.K. Barton. *Inorg. Chem.*, **41**, 1966 (2002).
[25] S. Shi, J. Liu, T.M. Yao, X.T. Geng, L.F. Jiang, Q.Y. Yang, L. Cheng, L.N. Ji. *Inorg. Chem.*, **47**, 4910 (2008).
[26] T.M. Ou, Y.J. Lu, J.H. Tan, Z.S. Huang, K.Y. Wong, L.Q. Gu. *ChemMedChem*, **3**, 690 (2008).
[27] P.U. Maheswari, V. Rajendiran, H.S. Evans, M. Palaniandavar. *Inorg. Chem.*, **45**, 37 (2006).
[28] R. Giraldo, M. Suzuki, L. Chapman, D. Rhodes. *Proc. Natl. Acad. Sci. USA*, **91**, 7658 (1994).
[29] D.P.N. Gonçalves, R. Rodriguez, S. Balasubramanian, J.K.M. Sanders. *Chem. Commun.*, 4685 (2006).
[30] B.Q. Fu, J. Huang, L.G. Ren, X.C. Weng, Y.Y. Zhou, Y.H. Du, X.J. Wu, X. Zhou, G.F. Yang. *Chem. Commun.*, 3264 (2007).
[31] H. Han, C.L. Cliff, L.H. Hurley. *Nucleic Acids Res.*, **27**, 537 (1999).
[32] T. Lemarteleur, D. Gomez, R. Paterski, E. Mandine, P. Mailliet, J.F. Riou. *Biochem. Biophys. Res. Commun.*, **323**, 802 (2004).
[33] J.T. Wang, X.H. Zheng, Q. Xia, Z.W. Mao, L.N. Ji, K. Wang. *Dalton Trans.*, **39**, 7214 (2010).
[34] X.D. Wang, W.B. Wu, X.Z. Bu, Z.S. Huang, D.L. Ma, K.Y. Wong, L.Q. Gu. *J. Med. Chem.*, **51**, 6381 (2008).
[35] J.F. Riou, L. Guittat, P. Mailliet, A. Laoui, E. Renou, O. Petitgenet, F. Mégnin-Chanet, C. Hélène, J.L. Mergny. *Proc. Natl. Acad. Sci. USA*, **99**, 2672 (2002).