Inorganic Chemistry
Communication

Inorg. Chem. **2009**, *48*, 5599–5601 **5599**
DOI: 10.1021/ic900902f

In Vitro Transcription Inhibition by Ruthenium(II) Polypyridyl Complexes with Electropositive Ancillary Ligands

Feng Gao,*,† Xing Chen,† Jin-Quan Wang,‡ Yu Chen,† Hui Chao,*,† and Liang-Nian Ji*,†

†*MOE Laboratory of Bioinorganic and Synthetic Chemistry, State Key Laboratory of Optoelectronic Materials and Technologies, School of Chemistry and Chemical Engineering, Sun Yat-Sen University, Guangzhou 510275, P. R. China, and* ‡*School of Life Science and Biopharmacology, Guangdong Pharmaceutical University, Guangzhou, 510006, P. R. China*

Received May 9, 2009

Three Ru(II) polypyridyl complexes with potential high DNA-binding ability have been designed and synthesized by extending the conjugated plane of the intercalative ligand and introducing electropositive pendants to the ancillary ligand. Spectral titration, DNA thermal denaturation, viscosity experiments, and quantum chemistry calculations were performed, and the complexes were found to intercalate into DNA base pairs with very high affinity even at high salt concentrations. Benefiting from their high DNA-binding ability, the complexes can effectively inhibit the DNA transcription activity by blocking the binding of T7 RNA polymerase to the template DNA. As efficient transcription inhibitors, the complexes demonstrated high in vitro antitumor activity against four selected tumor cell lines.

Binding and cleavage of nucleic acids lies at the heart of cellular transcription and translation; therefore, these substrates are obvious targets for therapeutic intervention and the development of diagnostic probes of nucleic acid structure.[1] Many antitumor drugs and antiviral agents,[2] such as cisplatin, actinomycin D, daunorubicin, and other drugs, act as inhibitors of transcription, by inhibiting the transcription process through their interaction with the template DNA, binding to the active site of RNA polymerase, blocking the DNA/RNA channel, or targeting transcription factors.[3−6] Currently, exploration of new compounds as potential future drugs is of great urgency to overcome the drug-induced cellular resistance and the efficacy of each drug against certain cancers. Stabilization of the DNA duplex structure is one of the most important means of inhibiting transcription.[2−6] Therefore, the design of small molecules with high DNA-binding ability will be of great benefit to the exploration of novel transcription inhibitors and antitumor drugs.

Ru(II) polypyridyl complexes with dppz-type ligands (dppz = dipyrido[3,2- *a*:2′,3′-*c*]phenazine) have been found to intercalate into adjacent DNA base pairs with high affinity.[7] Numerous other structural analogues with different shapes and electronic properties have been synthesized and investigated. Although many of these complexes have been utilized as DNA sequence-specific and mismatch probes, DNA photocleavage reagents, topoisomerase inhibitors, and antitumor reagents,[8−12] their action on the transcription still remains largely untapped.

To explore the transcription inhibition activity of DNA-intercalative polypyridyl Ru(II) complexes and the relations with their DNA binding ability, we designed a series of Ru(II) complexes (Figure 1) with potential high DNA affinity, based on the classical DNA intercalator $[Ru(bpy)_2(dppz)]^{2+}$. Ligand pdppz (pdppz = phenanthro[4,5-abc]dipyrido[3,2-h:2′,3′-j]phenazine) and its complex $[Ru(bpy)_2(pdppz)]^{2+}$ (bpy = 2,2′-bipyridine) were designed to extend the conjugated plane of

*To whom correspondence should be addressed. E-mail: gaofeng9@mail.sysu.edu.cn (F.G.), ceschh@mail.sysu.edu.cn (H.C.), cesjln@mail.sysu.edu.cn (L.-N.J.).

(1) Boerner, L. J. K.; Zaleski, J. M. *Curr. Opin. Chem. Biol.* **2005**, *9*, 135–144.
(2) (a) Darnell, J. E.Jr. *Nat. Rev. Cancer* **2002**, *2*, 740–749. (b) Zhou, M. *Curr. Cancer Therapy Rev.* **2006**, *2*, 331–339. (c) Jamieson, E. R.; Lippard, S. J. *Chem. Rev.* **1999**, *99*, 2467–2498. (d) Portugal, J.; Martin, B.; Vaquero, A.; Ferrer, N.; Villamarin, S.; Priebe, W. *Curr. Med. Chem.* **2001**, *8*, 1–8.
(3) Campbell, E. A.; Korzheva, N.; Mustaev, A.; Murakami, K.; Nair, S.; Goldfarb, A.; Darst, S. A. *Cell* **2001**, *104*, 901–912.
(4) Mansilla, S.; Rojas, M.; Bataller, M.; Priebe, W.; Portugal *J. Biochem. Pharmacol.* **2007**, *73*, 934–942.
(5) (a) Fu, P. K.-L.; Bradley, P. M.; Turro, C. *Inorg. Chem.* **2003**, *42*, 878–884. (b) Aguirre, J. D.; Lutterman, D. A.; Angeles-Boza, A. M.; Dunbar, K. R.; Turro, C. *Inorg. Chem.* **2007**, *46*, 7494–7502.
(6) Pauly, M.; Kayser, I.; Schmitz, M.; Dicato, M.; Del Guerzo, A.; Kolber, I.; Moucheron, C.; Kirsch-De Mesmaeker, A. *Chem. Commun.* **2002**, 1086–1087.
(7) (a) Moucheron, C.; Kirsch-De Mesmaeker, A.; Choua, S. *Inorg. Chem.* **1997**, *36*, 584–592. (b) Arounaguiri, S.; Maiya, B. G. *Inorg. Chem.* **1999**, *38*, 842–843. (c) Gao, F.; Chao, H.; Zhou, F.; Yuan, Y. X.; Peng, B.; Ji, L. N. *J. Inorg. Biochem.* **2006**, *100*, 1487–1494. (d) Liu, Y.; Hammitt, R.; Lutterman, D. A.; Thummel, R. P.; Turro, C. *Inorg. Chem.* **2007**, *46*, 6011–6021.
(8) Erkkila, K. E.; Odom, D. T.; Barton, J. K. *Chem. Rev.* **1999**, *99*, 2777–2795.
(9) Elias, B.; Kirsch-De Mesmaeker, A. *Coord. Chem. Rev.* **2006**, *250*, 1627–1641.
(10) Clarke, M. J. *Coord. Chem. Rev.* **2003**, *236*, 209–233.
(11) Gao, F.; Chao, H.; Wang, J. Q.; Yuan, Y. X.; Sun, B.; Wei, Y. F.; Peng, B.; Ji, L. N. *J. Biol. Inorg. Chem.* **2007**, *12*, 1015–1027.
(12) (a) Puckett, C. A.; Barton, J. K. *J. Am. Chem. Soc.* **2007**, *129*, 46–47. (b) Zeglis, B. M.; Barton, J. K. *Inorg. Chem.* **2008**, *47*, 6452–6457.

Published on Web 06/08/2009

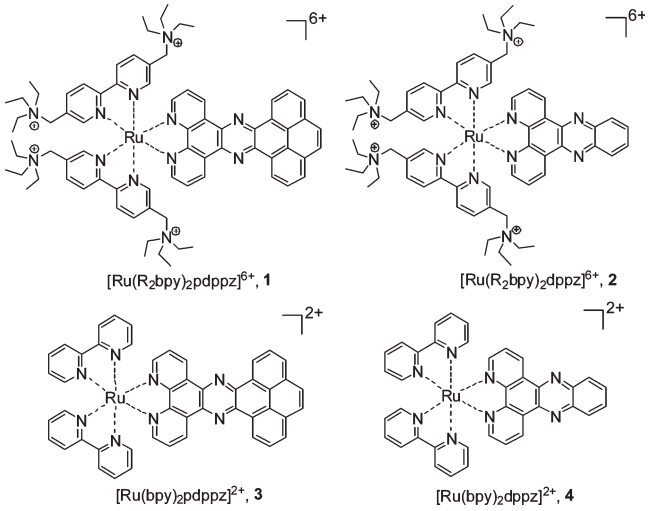

Figure 1. Structures of the Ru(II)complexes.

the intercalative ligand, which would allow the complex to stack with the adjacent DNA base pairs more efficiently. To further enhance the DNA binding ability, an ancillary ligand with electropositive pendants (R_2bpy, 5,5′-di[1-(triethylammonio)methyl]-2,2′-dipyridine) was also used to provide additional electrostatic interaction between the complex and electronegative DNA backbone. The DNA binding, transcription inhibition, and in vitro cytotoxicity of these complexes were examined.

Binding of the complexes with calf thymus DNA (CT-DNA) was examined by absorption spectra titration, DNA thermal denaturation, and viscosity experiments (Figure S4−S6, Supporting Information. The representing data are listed in Table 1. As we expected, intrinsic binding constants K_b of the modified complexes with CT-DNA are higher than those of the parent complex **4** and increase in an order of **4** < **3** ∼ **2** < **1**. The denaturation temperatures (T_m) of CT-DNA increased significantly in the presence of the complexes and followed an order of **4** < **3** < **2** < **1**. The large increase in T_m, especially for **1** and **2**, indicates that the complexes bind tightly to DNA, and the DNA double strands can hardly dissociate to single strands. By lengthening the DNA double helix, all complexes significantly increased the relative specific viscosity of CT-DNA, following the order of **4** < **3** < **2** < **1**. It is well-known that **4** binds DNA via an intercalative mode; therefore, **1**, **2**, and **3** may intercalate into DNA base pairs with even higher affinity.

Calculations on density function theory (DFT) gave further meaningful explanations for the DNA binding behavior of the complexes. In the $\pi-\pi$ interaction between DNA and the complex, DNA base pairs are electron donors, and the complex is an electron acceptor because the highest occupied molecular obital energies (E_{HOMO}) of DNA are relatively high (the CG/CG stacking calculated with the DFT method is −1.27 eV[13]). The calculated lowest occupied molecular obital energies (E_{LUMO}) of **3** and **4** (Table 1) are comparable to those of many DNA intercalative Ru(II) complexes.[14] Interestingly, the E_{LUMO} values of **1** and **2** are significantly lower than those of **3** and **4** and, consequently, are very advantageous to their $\pi-\pi$ interactions with DNA. In addition, the natural charge populated on the Ru(II) atom (C_{Ru}, Table 1) increased apparently upon substitution with electropositive pendants. The higher values of C_{Ru} facilitate the DNA binding of **1** and **2**, because an increase in C_{Ru} may educe an enhancement of the electron withdrawing of the Ru(II) center from the intercalative ligand and the electron accepting of the intercalative ligand from DNA base pairs.

A certain ionic strength is usually required during transcription; therefore, it is necessary for the inhibitors to be able to bind DNA under a high salt concentration. The dependence of the binding constants on the concentrations of Na^+ was studied. The binding constants of **3** and **4** decrease with increasing salt concentrations due to a stoichiometric amount of counterion release that accompanies the binding to a positively charged Ru(II) complex (Figure S7, Supporting Information). However, the increase in salt concentrations can hardly affect the DNA binding of **1** and **2**. It is suggested that the electrostatic attraction between the electropositive pendants and electronegative DNA backbone significantly contributes to the DNA binding of **1** and **2** under high ionic strength.

The inhibition of transcription by each complex was determined by recording the imaged mRNA produced during the transcription reaction as a function of the complex concentration, while keeping the concentrations of all other components constant. As shown in the imaged gels in Figure 2, the produced mRNA decreases relative to the control lane (the concentration of complex is 0) as the complex concentration is increased. The concentration of each complex required to inhibit 50% of the transcription, C_{inh}^{50}, is listed in Table 1. The measured C_{inh}^{50} value for activated cisplatin is 3.8 μM under similar experimental conditions. It is evident that the C_{inh}^{50} values for **3** and **4** are comparable to that of activated cisplatin. In contrast, the C_{inh}^{50} values of **1** and **2** are quite lower than that of cisplatin by a factor of 40 and 10, respectively, indicating that **1** and **2** have higher inhibition activity than cisplatin. It is evident that there is a correlation between the transcription inhibition activity of polypyridyl Ru(II) complexes and their DNA binding strength. A similar observation has recently been reported for some Cr(III) complexes.[15]

Unlike dirhodium(II,II) complexes,[5] Ru(II) polypyridyl complexes are coordinatively inert and cannot bind with NTP and T7 RNA polymerase through axial coordination. Similar to cisplatin, the inhibition of transcription by each Ru(II) complex in this study is observed to be independent of the concentration of both the enzyme and Mg^{2+}. The transcription inhibition activities of the Ru(II) complexes only have relation to their DNA binding abilities. Instead of the product of some shorter mRNA, the result of the transcription inhibition is a decrease in the amount of the mRNA with a normal length, indicating that the mechanism of the inhibition is that the DNA-intercalating Ru(II) complexes block the binding of T7 RNA polymerase to the template DNA, rather than hinder the movement of the DNA-bound RNA polymerase along the template DNA.

Some Ru(II) complexes containing highly π-deficient polyazaaromatic ligands, such as TAP (1,4,5,8-tetraazaphenanthrene) or HAT (1,4,5,8,9,12-hexaazatri-phenylene), were found to form adducts with DNA upon visible irradiation.[6,9] However, in our case, the ligands pdppz are poorer π-acceptors.

(13) Kurita, N.; Kobayashi, K. *Comput. Chem.* **2000**, *24*, 351–357.
(14) Li, J.; Xu, L. C.; Chen, J. C.; Zheng, K. C.; Ji, L. N. *J. Phys. Chem. A* **2006**, *110*, 8174–8180.
(15) Raja, N. S.; Nair, B. U. *Toxicology* **2008**, *251*, 61–65.

Table 1. Intrinsic Binding Constants K_b with CT-DNA of the Ru(II) Complexes, the Increase in the Denaturation Temperatures (ΔT_m) of CT-DNA upon Binding by the Complex, Frontier Molecular Orbital Energies of the Complexes, Natural Charge Populated on the Ru(II) Atom Calculated at the Level of B3LYP/LanL2DZ, and Concentrations Required to Inhibit 50% of the Transcription (C_{inh}^{50})

complex	$K_b(\times 10^6\ M^{-1})$	ΔT_m (°)	E_{HOMO} (eV)	E_{LUMO} (eV)	C_{Ru} (e)	C_{inh}^{50} (μM)
1	2.5 ± 0.4	64.5[a]	−14.75	−14.48	0.645	0.1
2	1.7 ± 0.2	35.8	−16.54	−14.88	0.644	0.4
3	2.1 ± 0.3	19.7	−9.28	−7.40	0.628	1.6
4	1.2 ± 0.2	13.9	−10.86	−7.51	0.627	4.2
Rh-1[b]	0.56	10.3				3.4
Rh-2[b]	0.0033	4.2				> 600

[a] The T_m of CT-DNA bound with **1** is found to be higher than 100 °C and cannot be measured exactly by the experiment. The ΔT_m has been extrapolated by the data obtained under 95 °C. [b] From ref 5. Rh-1 = *cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(np)_2]^{2+}$; Rh-2 = *cis*-$[Rh_2(\mu\text{-}O_2CCH_3)_2(pynp)_2]^{2+}$; np = 1,8-naphthyridine; pynp = 2-(2-pyridyl)-1,8-naphthyridine.

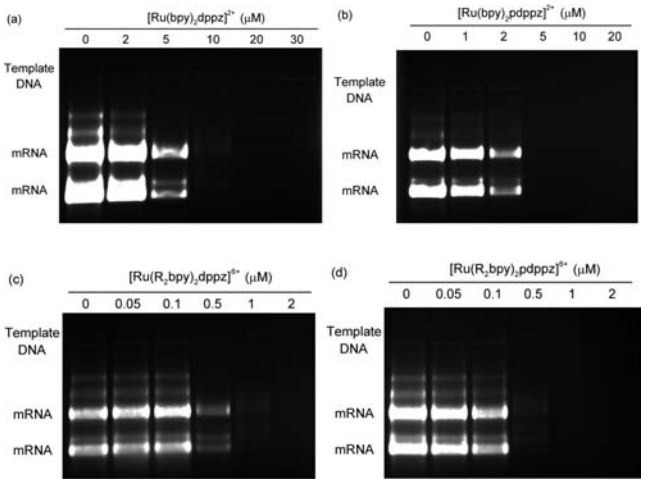

Figure 2. Inhibition on the mRNA production in the transcription reaction by the Ru(II) complexes at different concentrations. (a) $[Ru(bpy)_2dppz]^{2+}$ (**4**), (b) $[Ru(R_2bpy)_2dppz]^{6+}$ (**3**), (c) $[Ru(bpy)_2pdppz]^{2+}$ (**2**), and (d) $[Ru(R_2bpy)_2pdppz]^{6+}$ (**1**).

Similar to $[Ru(bpy)_2(dppz)]^{2+}$, despite their very high affinity for DNA, complexes **1**, **2**, and **3** display no photoreactivity toward DNA since it is not sufficiently photooxidizing to produce the guanine radical cation. This was supported by continuous irradiation experiments that illustrated that no change was observed in the absorption spectra of Ru(II) complexes under visible irradiation in the presence of CT-DNA (Figure S8−S10, Supporting Information).

The in vitro cytotoxic activities of the Ru(II) complexes were evaluated against HELA, HepG2, BEL-7402, and MCF-7 tumor cell lines. As shown in Table 2, all of the complexes demonstrate higher in vitro cytotoxicity against selected tumor cell lines than 5-fluorouracil, a widely used clinical antitumor drug, but a relatively lower cytotoxicity against cisplatin. There is no clear trend for the antitumor activity of the Ru(II) complexes, as shown in their DNA binding and transcription inhibition ability, indicating that the transcription inhibition is not the unique mechanism of antitumor Ru(II) complexes.[11]

Table 2. IC_{50} (mM) of Ru(II) Complex and Drugs against Different Tumor Cell Lines

compound	Hela	Hep-G2	BEL-7402	MCF-7
1	0.49	0.34	0.38	0.29
2	0.18	0.37	0.38	0.44
3	0.31	0.30	1.47	0.60
4	1.12	0.67	1.20	0.78
5-fluorouracil	1.23	0.88	2.43	0.31
cisplatin	0.014	0.026	0.020	0.040

In conclusion, by extending the conjugated plane of the intercalative ligand and introducing electropositive pendants to the ancillary ligand, we successfully improved the DNA binding ability of Ru(II) polypyridyl complexes, which is further found to have a profound effect on their DNA transcription inhibition activity and in vitro antitumor activity. The extremely high transcription inhibition activity makes this a potentially attractive method in the development of transcription inhibitors, antitumor drugs, and other biological reagents, which are related to DNA binding.

Acknowledgment. We thank the 973 Program of China (2007CB815306), NSFC(20771105, 20871122, 20801060), NCET (06-0718), and the Key Project (108103) of Ministry of Education and Foundation of Sun Yat-Sen University for financial support.

Supporting Information Available: Synthesis and characterization of the complexes, experimental and calculation conditions, Figures S1−S10. This material is available free of charge via the Internet at http://pubs.acs.org.