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DNA binding, photocleavage and topoisomerase inhibitory activity of polypyridyl ruthenium(II) complexes containing the same ancillary ligand and different main ligands

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ABSTRACT

The binding and photocleavage properties of $[Ru(phen)_2L]^{2+}$ {where phen is 1,10-phenanthroline and L is 2-(4-formylphenyl)imidazo[4,5-*f*][1,10]phenanthroline (fmp) or 2-(2′,3-*dibromo*-4-formyl-phenyl)imidazo[4,5-*f*][1,10]-phenanthroline (fmp-2Br)} with regard to DNA were investigated using various biophysical techniques. Results suggest that the two complexes can bind to DNA via an intercalative mode with different affinities and efficiently photocleavage pBR322 DNA in vitro under irradiation. Furthermore, the results of topoisomerase inhibition and DNA strand passage assay reflect that both complexes are efficient dual topoisomerase I and IIα inhibitors.

1. Introduction

DNA topoisomerases are ubiquitous enzymes that alter the configuration or topology of duplex DNA during cellular processes [1,2]. Topoisomerase I catalyzes a transient single-stranded break of the DNA double helix during DNA relaxation whereas topoisomerase II catalyzes transient double-stranded breaks [3–5]. Under normal conditions, the step of DNA religation is much faster than that of DNA cleavage, which may be tolerated by the cell. However, conditions that significantly change either the physiological concentration or the lifetime of the breaks are responsible for DNA alterations, playing a crucial role in inhibiting cell cycle progression [6]. They are essential for many vital DNA functions during cell growth, such as replication, transcription, recombination, and chromatin remodeling [7,8]. Therefore, it has long been accepted that the ability to interfere with enzymes or generate enzyme-mediated damage is an effective strategy for cancer therapy and, in this connection, DNA topos (I and II) proved to be the excellent targets of clinically significant classes of anticancer [6].

In recent years, numerous natural, modified, and synthetic compounds have been chosen to detect the inhibition of topoisomerase activity [3,9–14]. However, a great number of such studies at present mainly focus on organic compounds and, to a far lesser extent, on metal complexes [10,15–19]. In comparison with organic molecules, metal complexes can have a larger variety of structures with comparable or, in some cases, higher environmental stability and a much greater diversity of tunable electronic properties by virtue of the coordination metal center. In this regard, polypyridyl ruthenium(II) complexes, due to a ch photophysical and photochemical properties, strong DNA-binding ability and promising biological activity, have attracted considerable attentions in recent years [20–22]. Surprisingly and in contrast to studies on the interaction between polypyridyl Ru(II) complexes and DNA, investigations of the inhibition of topoisomerase activity by polypyridyl Ru(II) complexes are very scarce [10]. Therefore, studies on the relationship between the structures of polypyridyl Ru(II) complexes and their potential of inhibition of DNA topoisomerases are very important for developing novel antitumor drugs and elucidating the underlying molecular mechanism.

Note that, the emergence of resistance phenomena to Topo I inhibitors is often accompanied by a concomitant rise at the level of Topo II expression and viceversa, resulting in the failure of clinical therapies [6]. In this regard, a single compound able to inhibit both Topo I and II may present the advantage of improving antitopoisomerase activity, with reduced toxic side effects, with respect to the combination of two inhibitors [23–26]. More recently, although some DNA-intercalating polypyridyl Ru(II) complexes exhibited inhibition activities on Topo II [17,19,27–30], studies involving the dual Topo I and II inhibitors based on polypyridyl Ru(II) complexes are very scarce [22,31]. Thus, it is of interest

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and importance to develop dual Topo I and II inhibitors as novel anti-cancer agents.

Herein, two polypyridyl Ru(II) complexes—$[Ru(phen)_2(fmp)]^{2+}$ (Ru**1**) {phen is 1,10-phenanthroline and fmp is 2-(4-formylphenyl)imidazo[4,5-f] [1,10] phenanthroline} and $[Ru(phen)_2(fmp\text{-}2Br)]^{2+}$ (Ru**2**) {fmp-2Br is 2-(2′,3-dibromo-4-formyl-phenyl)-imidazo[4,5-*f*] [1,10]-phenanthroline}, which contain the same ancillary ligand and different main ligands—were chosen and designed (Fig. 1). The detailed profiles of DNA binding, DNA photocleavage and topoisomerase inhibitory activity of Ru**1** and Ru**2** were demonstrated by using various biophysical techniques. We hope that this work will aid in advancing our knowledge of the interaction between polypyridyl-based Ru(II) complexes and DNA, as well as laying the foundation for the rational design of dual Topo I and II inhibitors as novel anti-cancer agents.

2. Materials and methods

2.1. Materials

All chemicals used were obtained from commercial sources and directly used without additional purification. 1,10-phenanthroline-5,6-dione [32] and *cis*-$[Ru(phen)_2Cl_2]\cdot 2H_2O$ [33] were prepared according to literature procedures. 1,4-benzenedicarboxaldehyde and 2,5-dibromo-1,4-benzenedicarboxaldehyde were purchased from Sigma Chemical Company (St. Louis, MO, USA). Doubly distilled water was used to prepare buffers. Double stranded calf thymus DNA (CT-DNA) and pBR322 DNA were obtained from the Sino-American Biotechnology Company. The DNA concentration per nucleotide in Tris–HCl buffer was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [34,35]. DNA topoisomerase I (Topo I) from calf thymus together with human topoisomerase II (Topo IIα) were purchased from TopoGen Inc.

2.2. Physical measurement

Microanalyses (C, H and N) were carried out on a Perkin–Elmer 240Q elemental analyzer. UV–Vis spectra were recorded with a PerkinElmer Lambda 25 spectrophotometer, and emission spectra were recorded with a PerkinElmer LS 55 luminescence spectrometer at room temperature. ^{1}H NMR spectra were recorded on an Avance-400 spectrometer with d_6-DMSO as solvent at room temperature and tetramethylsilane as the internal standard. Mass Spectrometer was performed on an Autoflex III™ Maldi-Tof mass spectrometer (Bruker) using DMSO as the mobile phase.

2.3. Synthesis

2.3.1. Synthesis of the ligand fmp

The procedure for the ligand 2-(4-formylphenyl)imidazo[4,5-*f*] [1,10]phenanthroline (fmp) was carried out as below. A mixture of 1,10-phenanthroline-5,6-dione (420 mg, 2 mM), 1,4-benzenedicarbroxadehyde (270 mg, 2.0 mM), ammonium acetate (2.3 g, 30 mM), and glacial acetic acid (30 cm^3) was refluxed with stirring for 2 h, then cooled to room temperature and diluted with water (60 mL). Dropwise addition of concentrated aqueous ammonia gave an orange-yellow precipitate, which was collected and washed with distilled water. The crude product was completely dissolved in ethanol and purified by recrystallization. The pure and yellow crystalline solid was filtered from the solution, which was dried in vacuo. Yield: 580 mg, 90%. *Anal.* Calc. for $C_{20}H_{14}N_4O_2$: C, 69.95; H, 4.28; N, 16.41. Found: C, 70.17; H, 4.12; N, 16.37%. Maldi-Tof-MS (*m*/*z*): 325.13 ($[M+H]^+$).

2.3.2. Synthesis of the ligand fmp-2Br

The procedure for ligand 2-(2′,3-dibromo-4-formyl-phenyl)imidazo[4,5-*f*] [1,10]-phenanthroline (fmp-2Br) was similar to that for the preparation of fmp, with 2,5-dibromo-1,4-benzenedicarbroxadehyde (440 mg, 1.5 mM) in place of 1,4-benzenedicarbroxadehyde. Yield: 610 mg, 85%. *Anal.* Calc. for $C_{20}H_{14}Br_2N_4O_2$: C, 47.72; H, 2.97; N, 11.01. Found: C, 47.84; H, 2.81; N, 11.16%. Maldi-TOF-MS (*m*/*z*): 383.00 ($[M+H]^+$).

2.3.3. Synthesis of $[Ru(phen)_2(fmp)](ClO_4)_2\cdot H_2O$ (Ru**1**)

A mixture of *cis*-$[Ru(phen)_2Cl_2]\cdot 2H_2O$ (100 mg, 0.18 mM) and fmp (57 mg, 0.18 mM) in 10 mL of glycol was thoroughly deoxygenated. The dark purple mixture was refluxed for 8 h at 120 °C with stirring under argon atmosphere to give a red-brown solution finally. An equal volume of saturated aqueous $NaClO_4$ was added to the solution under vigorous stirring when the solution was cooled to room temperature. The red solid was collected through filtration and washed with small amounts of water, ethanol and diethyl ether, respectively. The crude product was purified on a neutral alumina column with MeCN–toluene (1:4, v/v) as eluant. The principal dark red band was collected. Red microcrystals were obtained after vacuum distillation. Yield: 150 mg, 85%. Elemental *Anal.* Calc. for $C_{44}H_{30}N_8Cl_2O_{10}Ru$: C, 52.70; H, 3.02; N, 11.17. Found: C, 52.37; H, 3.29; N, 11.24%. λ_{max}/nm (ε/M^{-1} cm^{-1}, MeCN): 455 (22 950), 264 (94 500). ^{1}H NMR (400 MHz, dimethyl-d_6 sulfoxide, ppm): δ 10.12 (s, 1H), 9.07 (d, 2H, J = 8.1), 8.78 (d, 4H, J = 8.0), 8.55 (d, 2H, J = 8.1), 8.40 (s, 4H), 8.15 (dd, 4H, J = 13.3, 6.5), 8.09 (d, 2H, J = 4.8), 8.00 (d, 2H, J = 4.3), 7.86–7.65 (m, 6H). Maldi-Tof-MS (*m*/*z*): 755.05 ($[M-2ClO_4-H]^+$).

2.3.4. Synthesis of $[Ru(phen)_2(fmp\text{-}2Br)](ClO_4)_2\cdot H_2O$ (Ru**2**)

The procedure for Ru**2** was similar to that for the preparation of Ru**1**, with fmp-2Br (0.12 g, 0.25 mmol) in place of fmp. Yield: 170 mg, 60%. Elemental *Anal.*Calc. for $C_{44}H_{28}N_8Cl_2Br_2O_{10}Ru$: C, 45.54; H, 2.43; N, 9.66%. Found: C, 45.41; H, 2.64; N, 9.90%. λ_{max}/nm (ε/M^{-1} cm^{-1}, CH_3CN): 455 (33 000), 419 (35 450), 264 (109 500). ^{1}H NMR (400 MHz, dimethyl-d_6 sulfoxide, ppm): δ 10.12 (s, 1H), 8.98 (d, J = 8.1 Hz, 2H), 8.77 (d, J = 8.1 Hz, 4H), 8.47 (s, 1H), 8.40 (s, 4H), 8.17 (s, 1H), 8.15–8.13 (d, 2H, J = 8.0),

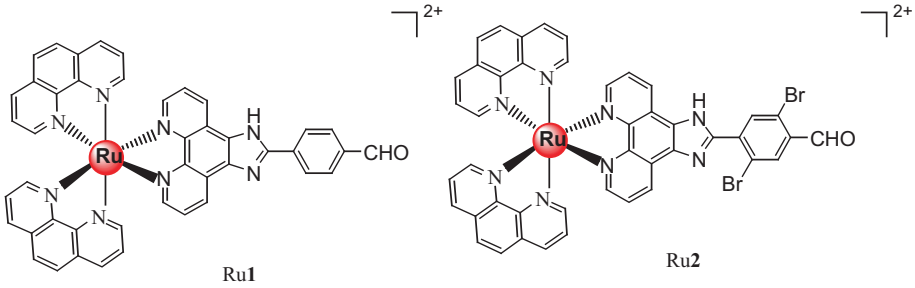

Fig. 1. Chemical structures of Ru**1** and Ru**2**.