Journal of Photochemistry & Photobiology, B: Biology 160 (2016) 142–153

Contents lists available at ScienceDirect

Journal of Photochemistry & Photobiology, B: Biology

journal homepage: www.elsevier.com/locate/jphotobiol

DNA-binding, cytotoxicity, cellular uptake, apoptosis and photocleavage studies of Ru(II) complexes

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ARTICLE INFO

Article history:
Received 9 July 2015
Received in revised form 30 March 2016
Accepted 31 March 2016
Available online 8 April 2016

Keywords:
Ruthenium(II) polypyridyl complexes
DNA binding
Photocleavage
Cytotoxicity
Apoptosis
Docking

ABSTRACT

Two Ru(II) complexes $[Ru(phen)_2bppp](ClO_4)_2$ (1) and $[Ru(phen)_27\text{-Br-dppz}](ClO_4)_2$ (2) [phen = 1,10 phenanthroline, 7-Br-dppz = 7-fluorodipyrido[3,2-a:2′,3′-c]phenazine, bppp = 11-bromo-pyrido[2′,3′:5,6] pyrazino[2,3-f] [1,10]phenanthroline] have been synthesized and characterized by elemental analysis, ES-MS, ^{1}H-NMR, ^{13}C-NMR and IR. The in vitro cytotoxicity of the complexes examined against a panel of cancer cell lines (HeLa, Du145 and A549) by MTT method, both complexes show prominent anticancer activity against various cancer cells. Live cell imaging study and flow cytometric analysis demonstrate that both the complexes 1 and 2 could cross the cell membrane accumulating in the nucleus. Further, flow cytometry experiments showed that the cytotoxic Ru(II) complexes 1 and 2 induced apoptosis of HeLa tumor cell lines. Photo induced DNA cleavage studies have been performed and results indicate that both the complexes efficiently photo cleave pBR322 DNA. The binding properties of two complexes toward CT-DNA were investigated by various optical methods and viscosity measurements. The experimental results suggested that both Ru(II) complexes can intercalate into DNA base pairs. The complexes were docked into DNA-base pairs using the GOLD docking program.

1. Introduction

Binding studies of small molecules with DNA are very important in the development of new therapeutic reagents and DNA molecular probes [1–4]. In the pursuit of tools for biomedicine and anticancer drugs, transition metal complexes that bind to DNA have been extensively studied in the last few decades [5–7]. In this frame ruthenium complex have been actively studied and are expected to have low toxicity and good selectivity for tumors [8]. This significant feature can be due to the ability of ruthenium to mimic the iron in binding to biological molecules [9–12], affording a new mechanism of action [13,14] and the potential to overcome platinum resistance [9,14]. Two Ru-based drugs are in clinical development NAMI-A is effective against lung metastases [15] and Kp1019 is active against colon carcinomas [16]. Dipyrido [3,2-a:2′,3′-c] phenazine (dppz) is a very interesting ligand and the bioactivity of ruthenium complexes containing dppz has been extensively studied. More recent studies demonstrate that the Ru(II) polypyridyl complexes have received considerable attention as DNA binding substrates and show high cytotoxicity properties in vitro and can effectively induce apoptosis, among these Ru polypyridyl complexes with dppz ligands have been studied frequently because of their strong DNA binding and their extraordinary photo physical properties. It was found that modification of the structure of the intercalating ligand caused a change in the orientation of the intercalated complex, this further effects DNA binding stability and cytotoxicity properties. Many research groups have published binding affinity and cytotoxic activity of complexes with dppz based ligands either modifying ancillary ligands or intercalative ligands [17,18]. Several research groups are reporting Ru(II) complexes with different types of ligands which show promising antitumor activity [19,20]. Recently polypyridyl Ru(II) complexes with notable antitumor activity have been reported by our group [21–28]. Ru(II) polypyridyl complexes can bind to DNA by non-covalent interactions, such as electrostatic binding, groove binding, intercalative binding and partial intercalative binding. Intercalation, which is well-known to strong influence on the properties of DNA has been reported as a preliminary step in mutagenesis [29]. In particular, metal complexes possessing planar aromatic ligands, which bind to DNA by intercalation, is receiving considerable attention [1,30]. Ruthenium complexes, owing

Abbreviations: 7-AAD, 7-aminoactinomycin D; phen, 1,10-phenanthroline; CT-DNA, calf thymus DNA; DAPI, 4′, 6-diamidino-2-phenylindole dihydrochloride; DMSO, dimethyl sulfoxide; Dppca, dipyridophenazine-11-carboxylic acid; dppz, dipyridophenazine; ESI–MS, electro spray ionization mass spectrometry; 7-Br-dppz, 7-bromodipyrido [3, 2-a: 20, 30-c] phenazine; Bppp, 11-bromo-pyrido[2′,3′:5,6]pyrazino[2,3-f] [1,10]phenanthroline; GOLD, genetic optimization for Ligand Docking; IC_{50}, half-maximal inhibitory concentration; MLCT, metal-to-ligand charge transfer; MTT, 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide; PBS, phosphate-buffered saline; Tris, Tris(hydroxymethyl)amino methane.

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http://dx.doi.org/10.1016/j.jphotobiol.2016.03.061
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to possessing several favorable properties suited to rational anticancer drug design and biological applications are regarded as promising alternatives to platinum complexes in cancer therapies. Schatzschneider et al. reported that $[Ru(bpy)_2(dppz)]^{2+}$ can effectively inhibit the proliferation of HT-29 cells, and $[Ru(bpy)_2(dppn)]^{2+}$ can be up taken and had a low IC_{50} (half maximal inhibitory concentration) value against MCF-7 cells [31]. Molecular docking studies have been successfully employed in rational drug design, but they have not been widely applied to study metal complexes. Although several docking programs, such as Gold, can tackle metal centers, it usually corresponds to part of a cofactor or an enzyme active site. Only a limited number of docking studies have been performed so far where the metal center is incorporated into the ligand being docked [32,33]. Changes in the structure of intercalative ligand could be used to attain diverse DNA binding mode of Ru(II) complexes which would result in the changes in the DNA-binding behavior, photo physical properties, excited state reactivity and biological activities of the complexes. Therefore, extensive studies using different structural legends are necessary to further elucidate the DNA binding mechanism and affinities of Ru(II) complexes and discover new DNA probes, Photodynamic therapy (PDT) agents (or) complexes with other biological functions and this will result in some changes in the DNA-binding mode, affinities or photo cleavage properties of the complexes. Thus we can understand the factors that determine the DNA binding mode and affinities of Ru (II) complexes.

2. Experimental Section

All chemicals were reagent grade and were used as received from commercial suppliers unless otherwise stated. All the solvents were degassed and distilled according to standard procedures [34]. The compounds 1,10-phenanthroline-5,6 dione [35], cis-$[Ru(phen)_2Cl_2].2H_2O$ [36], were synthesized by the literature methods. 5-Bromo 1, 2-diamine benzene, 6-Bromo-3 methyl pyridine 2-amine, $RuCl_3.3H_2O$, Ethidium bromide, and MTT were purchased from Sigma-Aldrich. 1, 10-phenanthroline monohydrate was purchased from Merck. Dimethyl sulfoxide (DMSO), RPMI 1640, CT (Calf Thymus) DNA was purchased from Aldrich, super coiled pBR 322 plasmid DNA (stored at −20 °C) was obtained from Fermentas life sciences was used as received, agarose (Genie), Ultra-pure Milli-Q water (18.2 mΩ) was used in all experiments and for preparing various buffers double distilled water was used. Binding studies of the complexes with CT-DNA was studied in Tris-HCl buffer (5 mM Tris HCl, 50 mM NaCl, and pH 7.2). The cell lines BEL-7, HeLa and A549 were obtained from the American Type Culture Collection and maintained in RPMI 1640 medium. DAPI 0.1 mg/mL purchased from Sigma-Aldrich. A solution of CT-DNA in the buffer gave a ratio of 1.8–1.9:1 UV absorbance at 260 and 280 nm indicates that the DNA was sufficiently free of protein [37]. The DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6600 M^{-1} cm^{-1}) at 260 nm [38]. Stock solutions were stored at 4 °C and used after no more than 4 days. Concentrated stock solutions of metal complexes were prepared in buffer for all the experiments.

2.1. Spectroscopic Measurements

UV–visible spectra were recorded with an Elico Biospectrophotometer, model BL198. Fluorescence spectral studies were recorded on Elico spectrofluorimeter model SL 174. IR spectra were recorded on KBr disks on a Perkin-Elmer FT-IR-1605 spectrometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker 400 MHz spectrometer with DMSO-d_6 as solvent and tetramethylsilane (TMS) as the internal standard at RT. Elemental micro analysis (C, H and N) was carried out with a Perkin-Elmer 240 elemental analyzer. ESI-MS mass spectra were recorded on ESI-MS Micro mass wuattro Lc triple quadrupole mass spectrometer with Mass Lynx software (Manchester, UK) in m/z. LeicaTCSSP5 confocal microscope (Leica Microsystems, Wetzlar, Germany). Flowcytometer Guava Easycyte 8HT (Millipore).

2.2. Synthesis

2.2.1. Synthesis of bppp

A solution of 1,10-phenanthroline-5,6-dione (0.210 g,1 mM) and 6-Bromo-3 methyl pyridine 2-amine (0.188 g,1 mM) in ethanol (20 mL) was heated at reflux for 4 h. After cooling, the precipitate was collected by filtration, washed with cold ethanol, and vacuum-dried.

Yield: 71%. Anal. Calcd for $C_{17}H_8N_5Br$ (%): C, 56.38; H, 2.23; N, 19.34. Found (%): C, 56.85; H, 2.25; N, 19.58. ESI–MS (DMSO): m/z = 362 (calcd 362). IR (KBr, cm^{-1}): 1620 (C = N), 1455 (C = C). ^1H NMR (400 MHz, DMSO-d_6): δ 9.45–9.10 (m, H_c, 2H), 9.00–8.70 (m, H_a, 2H), 8.45–8.35 (d, H_k, 1H, J = Hz), 8.25–8.05 (dd, H_j, 1H, J = Hz), 7.90–7.70 (m, H_b, 2H).

2.2.2. Synthesis of 7-Br-dppz

A solution of 1,10-phenanthroline-5,6-dione (0.210 g, 1 mM) and 4-bromo-1,2-phenylenediamine (0.187 g, 1 mM) in ethanol (20 mL) was heated at reflux for 4 h. After cooling, the precipitate was collected by filtration, washed with cold ethanol, and vacuum-dried.

Yield: 73%. Anal. Calcd for $C_{18}H_9N_4Br$ (%): C, 59.85; H, 2.51; N, 15.51. Found (%): C, 59.80; H, 2.55; N, 15.58. ESI–MS (DMSO): m/z = 360 (calcd 361). IR (KBr, cm^{-1}): 1604 (C = N), 1476 (C = C). ^1H-NMR (400 MHz, DMSO-d_6): δ 9.40–9.30 (t, H_c, 2H), 9.25–9.15 (m, H_a, 2H), 8.35–8.30 (d, H_k, 1H), 8.10–8.00 (d, H_j, 1H), 7.95–7.85 (d, H_m, 1H), 7.80–7.00 (m, H_b, 2H,).

2.2.3. Synthesis of $[Ru(phen)_2(bppp)](ClO_4)_2$ (1)

A mixture of cis-$[Ru(phen)_2Cl_2].2H_2O$ (0.5 mM), bppp (0.5 mM) was heated to reflux in 25 mL ethanol and 15 mL H_2O for 8 h under nitrogen-atmosphere to give a clear red solution. Upon cooling, the solution was treated with a saturated aqueous solution of $NaClO_4$ to give a red precipitate. The red solid was collected and washed with small amounts of water, ethanol and ether, dried under vacuum.

Yield: 69%. Anal. Calcd for $C_{41}H_{24}N_9O_8Cl_2BrRu$ (%): C, 46.51; H, 2.22; N, 11.90. Found (%): C, 46.62; H, 2.24; N, 11.93. IR (KBr, cm^{-1}): 1627 (C = N), 1421 (C = C), 624 (Ru–N). ^1H NMR (400 MHz, DMSO-d_6): δ 9.45 (d, H_a, 2H, J = 8.2 Hz), 9.38 (d, H_1, 4H, J = Hz), 9.36 (d, H_k, 1H, J = 7.5 Hz), 9.22–9.11 (d, H_3, 4H, J = 7.4 Hz), 9.10–9.00 (t, H_c, 6H), 8.90 (d, H_j,1H, J = 7.2 Hz), 8.40 (m, H_b, 2H), 8.1 (d, H_2, 4H, J = 8.0 Hz), 7.90–7.80 (m, H_6, 4H). ^{13}C [^1H] NMR(100 MHz DMSO-d_6): δ 156.20 (C_1, 4C), 153.11(Ca, 2C), 152.41 (C_l, 1C), 151.17 (C_f, 1C), 150.78 (C_g, 1C),147.21 (C_i, 1C), 146.75 (C_h, 1C), 143.23(C_5, 4C), 141.07(C_3, C_c, 6C), 139.55 (Cj, 1C), 138.13 (C_2, C_b, 6C), 137.54(C_e, 2C), 133.16 (C_d, 2C), 131.51 (C_4, 4C), 130.18(C6, 4C), 129.03 (C_k, 1C).

2.2.4. Synthesis of $[Ru(phen)_2(7\text{-}Br\text{-}dppz)](ClO_4)_2$ (2)

A mixture of cis-$[Ru(phen)_2Cl_2].2H_2O$ (0.5 mM), 7-Br-dppz (0.5 mM) was heated to reflux in 25 mL ethanol and 15 mL H_2O for 8 h under nitrogen-atmosphere to give a clear red solution. Upon cooling, the solution was treated with a saturated aqueous solution of $NaClO_4$ to give a red precipitate. The red solid was collected and washed with small amounts of water, ethanol and ether, dried under vacuum.

Yield: 70%. Anal. Calcd for $C_{42}H_{25}N_8O_8Cl_2BrRu$ (%): C, 47.4; H, 2.23; N, 10.59. Found (%): C, 52.12; H, 2.72; N, 11.83. IR (KBr, cm^{-1}): 1615 (C = N), 1409 (C = C), 622 (Ru–N). ^1H NMR (400 MHz, DMSO-d_6): δ 9.54–9.45 (m, H_a, 2H), 8.84–8.70 (m, H_1, 4H), 8.64–8.60 (t, H_k, 1H, J = 6.8 Hz) 8.44–8.40 (m, H_3, 4H), 8.38–8.36 (t, H_c, 6H, J = 8.0 Hz), 8.34–8.30 (d, H_j, 1H, J = 7.5 Hz), 8.29–8.19 (m, H_b, 2H), 8.09–7.81 (m, H_m, 1H), 7.93–7.90 (m, H_2, 4H), 7.88–7.86 (m, H_6, 4H). ^{13}C [^1H] NMR(100 MHz DMSO-d_6): δ 154.30 (C_1, 4C), 153.27(C_a, 2C), 152.67 (C_l, 1C), 150.88 (C_f, 1C), 147.09 (C_g, 1C),142.15 (C_i, 1C), 140.72 (C_h, 1C), 137.14(C_5, 4C), 136.97(C_3, C_c, 6C), 135.57 (C_j, 1C), 133.36 (C_2, C_b, 6C),