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Design, synthesis, DNA-binding affinity, cytotoxicity, apoptosis, and cell cycle arrest of Ru(II) polypyridyl complexes

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ABSTRACT

A novel polypyridyl ligand CNPFIP (CNPFIP = 2-(5(4-chloro-2-nitrophenyl)furan-2-yl)-1*H*-imidazo[4,5f][1,10]phenanthroline) and its mononuclear Ru(II) polypyridyl complexes of $[Ru(phen)_2CNPFIP]^{2+}$ **(1)** (phen = 1,10-phenanthroline), $[Ru(bpy)_2CNPFIP]^{2+}$ **(2)** (bpy = 2,2′-bipyridine), and $[Ru(dmb)_2CNPFIP]^{2+}$ **(3)** (dmb = 4,4′-dimethyl-2,2′-bipyridine) have been synthesized successfully and characterized thoroughly by elemental analysis, UV/Vis, IR, NMR, and ESI-MS. The interaction of the Ru(II) complexes with calf thymus DNA (CT-DNA) was investigated by absorption titration, fluorescence, viscosity measurements. The experimental results suggest that three complexes bind to CT-DNA through an intercalative mode and the DNA-binding affinity of complex **1** is greater than that of complexes **2** and **3**. The photocleavage of plasmid pBR322 DNA by ruthenium complexes **1**, **2**, and **3** was investigated. We have also tested three complexes for their antimicrobial activity against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) bacteria. The *in vitro* cytotoxicity of these complexes was evaluated by MTT assay, and complex **1** shows higher cytotoxicity than **2** and **3** on HeLa cells. The induced apoptosis and cell cycle arrest of HeLa cells were investigated by flow cytometry for 24 h. The molecular docking of ruthenium complexes **1**, **2**, and **3** with the active site pocket residues of human DNA TOP1 was performed using LibDock.

Binding studies of small molecules to DNA are very important in the development of DNA molecular probes and new therapeutic reagents [1–5]. The interaction of transition metal complexes with DNA has long been the subject of intense investigation in relation to the development of new reagents for biotechnology and medicine [6–8]. In general, most drugs have three distinct modes of noncovalent interaction with DNA, intercalative association in which a planar, aromatic moiety slides between the DNA base pairs, DNA groove binding through a combination of hydrophobic, electrostatic, and hydrogen-binding interactions, and external binding by electrostatic attraction [9,10]. Varying substitutive group or substituent position in the intercalative ligand can create some interesting differences in the space configuration and the electron density distribution of Ru(II) polypyridyl complexes, which results in some differences in spectral properties and the DNA-binding behaviors of the complexes, and will be helpful to more clearly understand the binding mechanism of Ru(II) polypyridyl complexes to DNA [11]. In the past two decades ruthenium coordination compounds have attracted considerable interest as potential anticancer agents because of their low toxicity and their efficacy against platinum drug-resistant tumors, reflected in promising results in various stages of preclinical to early clinical studies [12–17]. Some of the mononuclear Ru(II) complexes also show promising cytotoxicity *in vitro* [18,19]. In these endeavors, ruthenium complexes are attractive alternatives to platinum-based anticancer agents because of their rich synthetic chemistry, variable oxidation states, accessibility under physiological conditions, selective antimetastatic properties, multiple mechanisms of action distinct from platinum-based drugs, and low systemic toxicity [20–25]. Ruthenium anticancer complexes have been extensively studied and two of them NAMI-A and KP1019 respectively have successfully entered clinical trials [13,16,26].

Our group has synthesized some Ru(II) mixed polypyridyl complexes, which bind to DNA through an intercalative mode

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and promote cleavage of plasmid pBR322 DNA, and they exhibited antitumor activity [27–34]. In this context, we have synthesized and characterized a new ligand CNPFIP[1] and its three complexes $[Ru(phen)_2(CNPFIP)]^{2+}$ **(1)**, $[Ru(bpy)_2(CNPFIP)]^{2+}$ **(2),** and $[Ru(dmb)_2(CNPFIP)]^{2+}$ **(3)**. Moreover, we describe the interaction of the Ru(II) polypyridyl complexes with calf thymus DNA (CT-DNA) using electronic absorption, fluorescence spectroscopy, viscosity measurements, and photoactivated cleavage. We have also tested three complexes for their antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*. The cytotoxicity of these complexes was evaluated by MTT assay. The apoptosis and cell cycle arrest of HeLa cells were investigated by flow cytometry for 24 h. The molecular docking of ruthenium complexes **1**, **2**, and **3** with the active site pocket residues of human DNA TOP1 was performed using LibDock.

Experimental

Physical measurements

Electronic absorption titrations were carried out on Bio Spectrophotometer (Model BL198, Elico, Hyderabad, India) at room temperature. Fluorescence titrations were performed on a Spectrofluorometer Model SL174 (Elico, Hyderabad, India) at room temperature. Infrared (IR) spectra were recorded in KBr discs on a Perkin–Elmer FTIR-1605 spectrometer. NMR spectra were recorded on a Bruker 400 MHz spectrometer with DMSO-d_6 as solvent and TMS as an internal standard. Electrospray ionization mass spectra (ESI-MS) were recorded on a LQC system (Finnigan MAT, USA) using CH_3CN as mobile phase. Viscosity experiments were carried out with an Ostwald Viscometer immersed in a thermostated water bath maintained at 30 ± 0.1 °C. Gels were photographed in a Gel doc system (Alpha InfoTech Corporation). Bright Line Heamocytometer (Sigma Ltd.) and 96-well plates (Orange Scientific) (Thermo Scientific Multi Skan EX Elisa reader) were used for MTT assay. A flow cytometer (Guava Easycyte 8HT (Millipore)) was used to study apoptotic-inducing activities and cell cycle analysis.

Materials

All materials and solvents were purchased commercially and used without further purification unless otherwise noted. 1,10-Phenanthroline monohydrate, 2,2′-bipyridine, 4,4′-dimethyl-2,2′-bipyridine, CT-DNA, $RuCl_3{\cdot}3H_2O$, 5-(4-chloro-2-nitrophenyl)furan-2-carbaldehyde, dimethyl sulfoxide, and RPMI 1640 were purchased from Sigma Chemical Company. MTT and cisplatin (Sigma Aldrich) were used as received. Supercoiled pBR322 DNA was obtained from Bangalore Genie. Doubly distilled water was used for preparing various buffers. Interaction of the complexes with DNA was studied in Tris buffer (5 mM Tris–HCl, 10 mM NaCl, pH 7.1). The CT-DNA had a ratio of UV absorbance at 260 and 280 nm of 1.8–1.9:1, indicating that the DNA was sufficiently free from protein [35]. The concentration of CT-DNA was determined spectrophotometrically using the molar absorption coefficient 6600 M^{-1} cm^{-1} (260 nm) [36,37].

[1] *Abbreviations used:* CT-DNA, calf thymus DNA; CNPFIP, 2-(5(4-chloro-2-nitrophenyl)furan-2-yl)-1*H*-imidazo[4,5f][1,10]phenanthroline; phen, 1,10-phenanthroline; bpy, 2,2′-bipyridine; dmb, 4,4′-dimethyl-2,2′-bipyridine; IR, infrared; ESI-MS, electrospray ionization mass spectrometry; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMSO, dimethyl sulfoxide; Tris, tris(hydroxymethyl)amino methane; RPMI, Roswell Park Memorial Institute medium; MLCT, metal-to-ligand charge transfer; HeLa, Henrietta Lacks (uterine cell variety; named for deceased patient; Hepes, (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid); Na_2EDTA, ethylenediaminetetraacetic acid disodium salt; IC_{50}, inhibitory concentration of 50%.

Synthesis and characterization

1,10-Phenanthroline-5,6-dione [38], *cis*-$[Ru(phen)_2Cl_2]$ $2H_2O$, *cis*-$[Ru(bpy)_2Cl_2]$ $2H_2O$, and *cis*-$[Ru(dmb)_2Cl_2]$ $2H_2O$ were synthesized [39]. The syntheses of ligand and its Ru(II) complexes are shown in the scheme.

Synthesis of CNPFIP

CNPFIP was synthesized [40] with a mixture of 5-(4-chloro-2-nitrophenyl)furan-2-carbaldehyde (0.5 mM) and 1,10-phenanthroline-5,6-dione (0.5 mM) Yield: 76%. ^1H NMR data (400 MHz, ppm, DMSO-d_6 and TMS): δ 8.97 (d, 2H), 8.27 (S, 1H), 7.99 (d, 2H), 8.09(d, 2H), 8.40 (d, 2H), 6.59 (d, 1H), 6.58 (d, 1H). IR (KBr, cm^{-1}): 1479 (C═C), 3116 (C–H), 3421 (N–H), 1528 (C═N). ES-MS (*m/z*): [M+1]: 442. Anal calc for $C_{23}H_{12}Cl\,N_5O_3$: C, 62.52; H, 2.74; N, 15.85. Found: C, 62.48; H, 2.70; N, 15.82.

*Synthesis of $[Ru(phen)_2(CNPFIP)](ClO_4)_2{\cdot}2H_2O$ (**1**)*

$[Ru(phen)_2(CNPFIP)]$ was synthesized using a mixture of *cis*-$[Ru(phen)_2Cl_2]$ $2H_2O$ (0.5 mM) and CNPFIP (0.5 mM) and refluxed in 25 mL ethanol and 15 mL water for 8 h under nitrogen atmosphere to give a clear red solution. On 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 a small amount of water, ethanol, and ether and then dried under vacuum. Yield: 72%. ^1H NMR data (400 MHz, ppm, DMSO-d_6 and TMS): δ 9.09 (d, 4H), 8.97 (d, 2H), 8.85 (S, 1H), 8.17 (d, 4H), 8.15 (d, 2H), 8.04 (S, 4H), 7.85 (d, 1H), 7.83 (d, 1H), 7.74 (t, 4H), 7.30 (t, 2H), 6.94 (d, 1H), 6.90 (d, 1H). IR (KBr, cm^{-1}): 1478 (C═C), 3076 (C–H), 3410 (N–H), 1534 (C═N), 696 (M–N). ES-MS (*m/z*): $[M–2(ClO_4)–H]^+$: 902, $[M–2(ClO_4)]^{2+}$: 451.5. Anal calc for $C_{47}H_{32}Cl_3N_9O_{13}Ru$: C, 49.59; H, 2.83; N, 11.08. Found: C, 49.54; H, 2.80; N, 11.05.

*Synthesis of $[Ru(bpy)_2(CNPFIP)](ClO_4)_2{\cdot}2H_2O$ (**2**)*

This complex was obtained by a procedure similar to that described under *Synthesis of $[Ru(phen)_2(CNPFIP)](ClO_4)_2{\cdot}2H_2O$ (**1**),* with a mixture of $[Ru(bpy)_2Cl_2]$ $2H_2O$ (0.5 mM) and CNPFIP (0.5 mM); yield: 71%. ^1H NMR data (400 MHz, ppm, DMSO-d_6 and TMS): δ 9.07 (d, 2H), 8.87 (d, 4H), 8.84 (d, 4H), 8.27 (S, 1H), 8.01 (d, 2H), 7.85 (d, 1H), 7.76 (d, 1H), 7.61 (t, 4H), 7.50 (t, 2H), 7.36 (t, 4H), 6.91 (d, 1H), 6.93 (d, 1H). IR (KBr, cm^{-1}): 1466 (C═C), 3078 (C–H), 3406 (N–H), 1568 (C═N), 689 (M–N). ES-MS (*m/z*): $[M–2(ClO_4)–H]^+$: 854.5, $[M–2(ClO_4)]^{2+}$: 427.5. Anal calc for $C_{43}H_{32}Cl_3N_9O_{13}Ru$: C, 47.37; H, 2.96; N, 11.56. Found: C, 47.36; H, 2.91; N, 11.54.

*Synthesis of $[Ru(dmb)_2(CNPFIP)](ClO_4)_2{\cdot}2H_2O$ (**3**)*

This complex was obtained by a procedure similar to that described under *Synthesis of $[Ru(phen)_2(CNPFIP)](ClO_4)_2.\ 2H_2O$ (**1**),* with a mixture of $[Ru(dmb)_2Cl_2]$ $2H_2O$ (0.5 mM) and CNPFIP (0.5 mM); yield: 68%. ^1H NMR data (400 MHz, ppm, DMSO-d_6 and TMS): δ 9.01 (d, 2H), 9.05 (d, 4H), 8.96 (d, 4H), 8.17 (S, 1H), 8.08 (d, 2H), 7.81 (d, 1H), 7.77 (d, 1H), 7.50 (t, 2H), 7.08 (t, 4H), 6.74 (d, 1H), 6.73 (d, 1H), 2.33 (s, 12H). IR (KBr, cm^{-1}): 1465 (C═C), 3088 (C–H), 3411 (N–H), 1542 (C═N), 683 (M–N). ES-MS (*m/z*): $[M–2(ClO_4)–H]^+$: 910, $[M–2(ClO_4)]^{2+}$: 455. Anal calc for $C_{47}H_{40}Cl_3N_9O_{13}Ru$: C, 49.25; H, 3.52; N, 11.00. Found: C, 49.23; H, 3.51; N, 10.94.