Biological activity of thienyl-terpyridine Ru(II) complex in the presence of biocompatible ionic liquids

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Abstract: New mononuclear Ru(II)-thienyl-terpyridine complex [Ru(thienyl-tpy)(bpy)Cl]Cl (where thienyl-tpy = 4′-(2-thienyl)-2,2′:6′,2′′-terpyridine, bpy = 2,2′-bipyridine) has been synthesized and then characterized through elemental analysis, followed by various spectroscopic (IR, UV−vis, 1H and 13C NMR) and mass spectrometric technique (ESI Q-TOF MS). Furthermore, the interactions with CT DNA were performed both in the absence and presence of biocompatible ionic liquids 1-amino-2-propanol-lactate (IL1), 1-amino-2-propanol-oxalate (IL2) and 1-amino-2-propanol-citrate (IL3).

Keywords: Ruthenium(II), thienyl-terpyridine, ionic liquids, DNA

1. Introduction

The primary purpose of discovering new metallodrugs is to create and implement novel medicines with better efficacy and tolerance. Scientists are still working on developing new transition metal ion complexes for cancer therapy that have fewer side effects, are more resistant to drugs, and are more biocompatible than the compounds used so far [1]. Ruthenium-based pharmaceuticals have shown encouraging results as potential

anticancer agents due to their unique features such as biological, photophysical, optical, electronic, and catalytic properties, which provide advantageous therapeutic application opportunities [2]. Among the several reported Ru complexes with anticancer therapeutic potential, the Ru(II) polypyridyl complex (TLD1433) and Ru(III) complexes (NAMI-A and KP1019) have advanced to clinical trials [3-5]. The medicinal chemistry community has not yet identified all pharmacological targets (intracellular or extracellular) of anticancer ruthenium compounds. Most of the ruthenium complexes appear to be multitarget compounds capable of binding to several biological molecules, mainly DNA and proteins, but determining which target is the most relevant for biological activity is difficult and requires extensive studies of their interactions with different biomolecules.
In this work, we present the synthesis and characterization of the new thienyl-terpyridine Ru(II) complex [Ru(thienyl-tpy)(bpy)Cl]Cl (where thienyl-tpy = 4′-(2-thienyl)-2,2′:6′,2′′-terpyridine, bpy = 2,2′-bipyridine) and its biological activity with calf-thymus DNA (CT-DNA) in the presence of biocompatible ionic liquids 1-amino-2-propanol-lactate (IL1), 1-amino-2-propanol-oxalate (IL2) and 1-amino-2-propanol-citrate (IL3).

2. Biological activity of thienyl-terpyridine Ru(II) complex

The synthesis of the Ru complex was performed by reacting the neutral Ru(III) precursors *mer*-[Ru(thienyl-tpy)Cl_3] with the bpy chelating ligand under the reflux. The complex was characterized by elemental analysis and by various spectroscopic techniques, such as IR, UV-Vis, ^{1}H and ^{13}C NMR, and ESI-MS. Before examining the biological properties of the mononuclear Ru (II) complex, we studied its stability in an aqueous solution. The intensity of the absorption maxima of the Ru(II) complex decreased over 24 h, which was ascribed to the hydrolysis of Ru(II)-bound chloride.

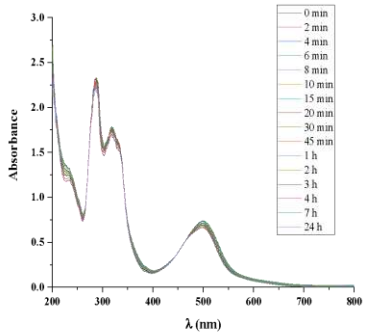

Figure 1. UV–Vis spectra of Ru(II) complex in water over a 24 h period.

2.1 DNA binding investigation

DNA–compound interactions can be established via different modes, including covalent binding, minor groove binding, major groove binding, intercalation and electrostatic interactions [6]. UV–Vis absorption spectroscopy can assess the general interactions (overall binding affinity) between metal compounds and DNA. The binding affinity of

the complex was evaluated by following the changes in the spectra of the compound upon increasing the concentration of the CT-DNA. In the present study, the absorption titration studies were conducted at room temperature using fixed concentration of complex (10 μM) in PBS and varying amounts of CT-DNA (2–20 μM).
The intrinsic binding constant, K_b, was determined by monitoring the change in the absorbance at the MLCT band after the addition of growing concentration of the DNA solution, based on the following eqn (1)

$$DNA]/(\varepsilon_A - \varepsilon_f) = [DNA]/(\varepsilon_b - \varepsilon_f) + 1/[K_b(\varepsilon_b - \varepsilon_f)] \quad (1)$$

K_b is given by the ratio of the slope to the *y* intercept in plots [DNA]/($\varepsilon_A - \varepsilon_f$) *vs.* [DNA], where [DNA] is the concentration of DNA in base pairs, $\varepsilon_A = A_{obsd}/[complex]$, ε_f is the extinction coefficient for the unbound complex and ε_b is the extinction coefficient for the complex in the fully bound form.

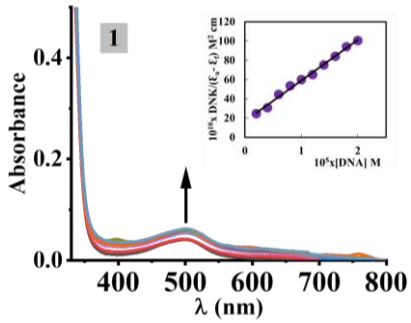

Figure 2. Absorption spectra of complex in 10 mM PBS upon addition of CT DNA.

Interactions of Ru complex and CT-DNA were also examined in the presence of ionic IL1, IL2 and IL3. This was done in order to see if the ionic liquids used as cosolvents have an impact on the interactions between the examined complex and the DNA molecule. Ionic liquids were added in the same amount as the examined complex, e.g., the concentrations were kept the same as the concentration of the complex. Based on the results shown in Table 1, it can be noted that the presence of ionic liquids affects the values of K_b. The highest value was noted in the presence of IL3, while the lowest was in the presence of IL1. In the presence of all three ILs, the values are higher compared to the value obtained in the absence of ILs, implying that the presence of ionic liquids enhances the reactivity of the examined complex with the DNA molecule. Furthermore, it was concluded that applied IL can be used as cosolvents, increasing the solubility of Ru(II)-thienyl-tpy complex. For comparison, ruthenium(II)-terpyridine complexes of the general formula $[Ru(Cl\text{-}tpy/Cl\text{-}Ph\text{-}tpy)(N\text{-}N)Cl]^+$ that we previously obtained had similar K_b values of order 10^5 M^{-1} for the interaction with CT DNA [6].

Table 1. The DNA-binding constants (K_b) for the Ru complex.

	PBS	IL1	IL2	IL3
		K_b [M^{-1}]		

Ru complex	2×10^5	2.22×10^5	2.50×10^5	3.33×10^5

3. Conclusions

Here, we described the interactions of Ru(II)-thienyl-tpy complex with CT DNA in the absence and presence of ILs (IL1–IL3), indicating that the IL used as cosolvents significantly affect the binding of the complex to the CT DNA molecule. From the obtained results, it was concluded that applied ILs could be used as cosolvents for the examined complex with the most promising being IL3 (1-amino-2-propanol-citrate).

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