Biomolecular Interactions of Novel Ruthenium(II) complexes with human serum albumin

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Abstract: Ruthenium complexes occupied an important place in a group of transition metal complexes that were synthesized to replace platinum-based complexes and provide better selectivity and lower toxicity. This study involves creating three new ruthenium (η^6-*p*-cymene) complexes with the formula [(η^6-*p*-cym)Ru(*O*–*O*)Cl] using *O*,*O*-diketo ester ligands called ethyl 2-hydroxy-4-aryl-4-oxobut-2-enoate (**1–3**). The interactions with the blood transport protein HSA were studied to see how well the complexes could reach their target. The mentioned interactions were monitored by fluorescence spectroscopy with HSA alone and with the addition of the site markers Eosin Y (a marker for site I of subdomain IIA) and Ibuprofen (a marker for site II of subdomain IIIA). The site markers were used to precisely localize the binding site in the transported blood protein. The influence of bidentate ligands on binding efficacy was assessed by examining the calculated binding constants. Based on *in vitro* HSA experiments, complex **1** exhibited the highest HSA activity, suggesting that the presence of an alkene chain contributes to increased activity.

Keywords: Ruthenium(II), HSA, site markers, interactions.

1. Introduction

Although cisplatin is an effective chemotherapeutic agent against various cancers, it has several side effects that diminish its importance [1]. The mentioned side effects restrict its effectiveness as an antitumor agent. Among the transition metal complexes synthesized to replace cisplatin, ruthenium complexes have gained attention for their promising anticancer properties, which arise from their ability to enhance these effects by selecting the appropriate ligand during their synthesis. Numerous research groups have synthesized and studied organoruthenium complexes with *N*,*N*-, *N*,*O*-, and *O*,*O*-chelating ligands over the past two decades. In J. Sadler's group, the complex of the general formula [(η^6-biphenyl)RuCl(en)]PF_6, en = ethylendiamine (RM175) emerged as a

particularly promising candidate [2]. Structural analogs with symmetric alkyl- and aryl-substituted diketonates as ligands were reported to exhibit high cytotoxicity [3,4].
In this study, the synthesis of three novel ruthenium (η^6-*p*-cymene) complexes of the general formula [(η^6-*p*-cym)Ru(*O*–*O*)Cl] with *O*,*O*-diketo ester ligands ethyl 2-hydroxy-4-aryl-4-oxobut-2-enoate (**1–3**) was presented. The interactions of the studied complexes with the blood transport protein HSA were evaluated to determine their potential to reach their target. These interactions were monitored using fluorescence spectroscopy, both with HSA alone and with the addition of site markers. This approach aimed to localize the binding site precisely within the transport protein. The influence of ligand's structures on the binding activity was monitored by comparing the calculated binding constants.

2. Methodology

2.1. Chemical and solutions

Studied complexes **1-3** were previously synthesized and characterized. Human serum albumin (HSA), Eosin Y, and ibuprofen were commercially available and were used as received. Phosphate buffer (PBS, 10 mM, C_{NaCl} = 137 mM, C_{KCl}= 2.7 mM, pH=7.4) was purchased from Sigma Aldrich.

2.2. Instrumental methods

Fluorescence measurements were carried out by RF-1501 PC spectrofluorometer (Shimadzu, Japan). The excitation and emission bandwidths were both 10 nm.

2.3. HSA binding studies

The protein binding study was performed by tryptophan fluorescence quenching experiments using human serum albumin (HSA, 2 μM) in 10 mM PBS buffer (pH = 7.4). Using complexes **1-3** as quenchers with increasing concentrations (2 μM to 20 μM), the emission intensity of human serum albumin HSA (2 μM) in 10 mM PBS buffer (pH = 7.4) was observed. Fluorescence emission spectra were recorded from 300 to 500 nm at an excitation wavelength of 285 nm. For precise determination of the binding sites in human serum albumin, the markers Eosin Y, as a marker for site I of subdomain IIA, and ibuprofen, as a marker for site II of subdomain IIIA, were used. Following the addition of increasing quantities of complexes **1-3** (up to 2×10^{-5} M), the emission intensity was observed. The equimolar concentrations of HSA and marker were added (2 μM).

3. Results and discussion

The mechanism of action of Ru(II)-based metallodrugs in chemotherapy is a debated topic, requiring a comprehensive study of their pharmacological profile via interaction with metabolic byproducts.

The addition of complexes **1-3** (up to 2.0×10^{-5} M) to a human serum albumin solution caused significant emission intensity changes at λ = 322 nm, indicating protein quench (Figure 1). The fluorescence maxima showed a modest red shift, indicating the development of ruthenated protein adducts.

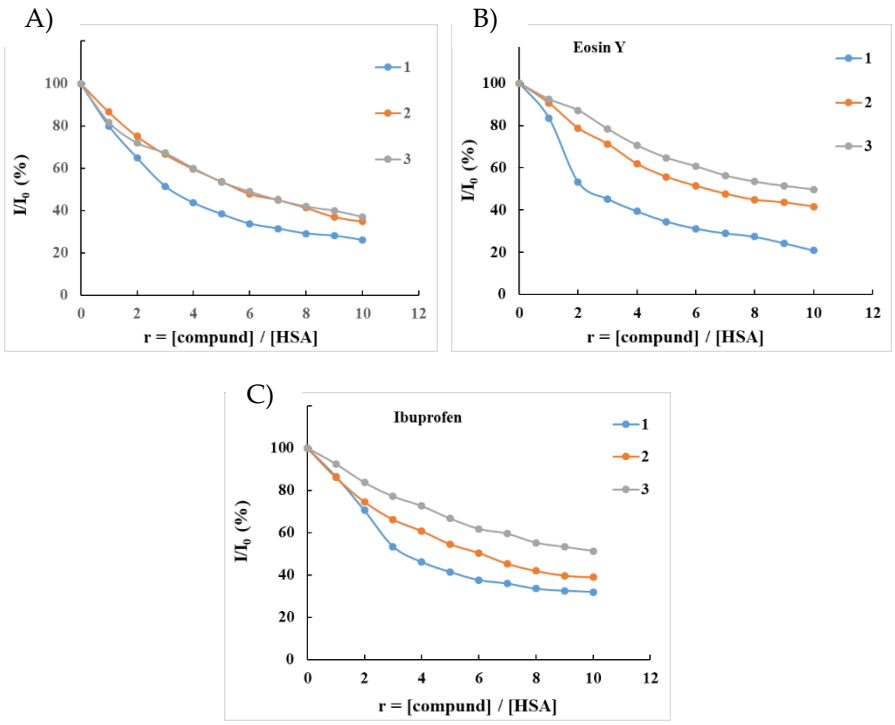

Figure 1. Plot of A) % relative fluorescence intensity at λ_{em} = 322 nm (%) *vs* r (where r = [complex]/[HSA]) for complexes **1 – 3** (26% of the initial fluorescence intensity for **1**, 34% for **2**, and 37% for **3**) in buffer solution (10 mM PBS at pH = 7.4), B) in the presence of Eosin Y (21.0% of the initial fluorescence intensity for **1**, 42% for **2**, and 50% for **3**), and C) in the presence of Ibuprofen (32.0% of the initial fluorescence intensity for **1**, 39% for **2**, and 52% for **3**).

The Stern-Volmer constants were calculated from the Stern-Volmer equation and the plots, while the binding constants (K_b) and the number of binding sites per albumin (n) were determined from double logarithmic Scatchard equation and the Scatchard plot. The measured HSA-binding affinity exhibited a trend of **1** > **2** > **3**. Fluorescence titration methods utilizing site markers demonstrated that the analyzed complexes exhibit a strong affinity for both IIA and IIIA sites, no preference for any protein binding site.

4. Conclusions

Here we described three new ruthenium (η^6-*p*-cymene) complexes with the formula [(η^6-*p*-cym)Ru(*O*–*O*)Cl] using *O*,*O*-diketo ester ligands called ethyl 2-hydroxy-4-aryl-4-oxobut-2-enoate (**1–3**).

Monitoring of the interaction of studied complexes with the most abundant protein of blood serum, HSA, confirmed a strong binding to the protein. The confirmed binding potential of **1**-**3** is sufficient to be transported to the site of the disease, which is of signifycant importance for its use as an anticancer agent.

Competitive experiments with HSA and site markers, Eosin Y and ibuprofen, showed that complexes **1-3** bind to both sites (site I of subdomain IIA and site II of subdomain IIIA) with great affinity ($K_b = 10^5$ M^{-1}). No preference for either binding site was observed, indicating a minor influence of the differences in the structures of the *O*,*O*-diketo ester on the binding event.

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