ISBN 978-9940-611-06-4

COAST
INTERNATIONAL CONFERENCE ON ADVANCES IN SCIENCE AND TECHNOLOGY

II INTERNATIONAL CONFERENCE ON
ADVANCES IN SCIENCE AND TECHNOLOGY

PROCEEDINGS COAST 2023

FACULTY OF MANAGEMENT HERCEG NOVI

HERCEG NOVI, MONTENEGRO

31 MAY - 03 JUNE 2023

PROCEEDINGS

II INTERNATIONAL CONFERENCE ON ADVANCES IN SCIENCE AND TECHNOLOGY
COAST 2023

Book title:
Proceedings COAST 2023

Publisher:
Faculty of Management Herceg Novi

Design and Computer processing:
Sanja Samardžić, MSc, Jelena Poznanović, MSc, Zvonko Perušina, BSc

CIP - Каталогизација у публикацији
Национална библиотека Црне Горе, Цетиње

INTERNATIONAL conference on advances in science and technology (II ; 2023 ; Herceg Novi)
Proceedings/International conference on on advances in science and technology, Herceg Novi, 31 May - 03 June, 2023 = Zbornik radova / Međunarodna konferencija o savremenim dostignućima u nauci i tehnologiji, Herceg Novi, 31 maj - 03 jun 2023. godine : Fakultet za menadžment, 2023 (Herceg Novi). - 1421 стр. : илустр.

Радови на срп. и енгл. језику. - Текст ћир. и лат. - Напомене и библиографске референце уз текст. - Библиографија уз сваки рад. - Сажеци на енгл. и срп. језику уз радове.

ISBN **978-9940-611-06-4**
COBISS.CG-ID **27152388**

SYNTHESIS, CHARACTERIZATION AND HSA/DNA INTERACTIONS OF NEW $[RH_2(CH_3COO)_4L_2)]$ COMPLEX

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ABSTRACT:
In this report, we have synthesized a complex of $[Rh_2(CH_3COO)_4(H_2O)_2]$ and 1-butylimidazole ligand (L). To a solution of $[Rh_2(CH_3COO)_4(H_2O)_2]$ (0.0478 g, 0.10 mmol) in toluene (10 mL) the 1-butylimidazole ligand (L) (0.0290 mL, 0.22 mmol) was added. The resulting mixture was stirred at room temperature for 1 hour until the reagents were completely dissolved. The solution was left at room temperature to evaporate slowly to a volume of 6 to 7 mL and then stored overnight in the refrigerator. The precipitated purple crystals were filtered off under vacuum and washed with diethyl ether. Characterization of the synthesized complex $[Rh_2(CH_3COO)_4L_2)]$ was carried out by elemental microanalysis, IR, NMR and by determination of the melting point. The interactions of the new complex with molecules of human serum albumin (HSA) and calf thymus DNA (CT-DNA) were studied by fluorescence spectroscopy as well as by docking experiments on the mentioned molecules. The high value of the binding constant, Kb, and the Stern-Volmer quenching constant, K_{SV}, are the result of good binding of the complex to HSA and CT-DNA.

Keywords: rhodium(II), metal complex, CT-DNA interactions, HSA interactions, docking experiment

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1. INTRODUCTION

The success of cisplatin as an antitumor agent has encouraged many research groups worldwide to direct their efforts toward the synthesis of other clinically important metal complexes [1-4]. Indeed, besides its advantages in cancer treatment, cisplatin also shows resistance and some toxic effects after long-term use, such as cardiotoxicity, neurotoxicity, nephrotoxicity, etc. [5,6]. Due to these side effects, the efforts of many scientists are focused on the synthesis of new platinum complexes with improved properties [7]. An ideal anticancer drug must be able to destroy tumor cells while leaving adjacent healthy tissue unharmed. Great attention is paid to platinum group metals, i.e., their complexes, including rhodium complexes. The electronic configurations of the ions of rhodium(I), rhodium(II), and rhodium(III) (d^8, d^7, and d^6, respectively) suggest that they are

isoelectronic with the ions of ruthenium(II), platinum(II), and platinum(IV), whose complexes exhibit significant antitumor activity [8,9]. The dinuclear complexes of rhodium(II) have been studied in detail, and their physicochemical properties and reactivity are such that they are among the most promising antitumor complexes [10-17]. The subject of this work was the synthesis and characterization of a new dinuclear rhodium(II) complex of formula $[Rh_2(CH_3COO)_4L_2]$, where L = 1-butylimidazole (Fig. 1.). In addition, fluorescence spectroscopy was used to investigate the structural changes in the HSA molecule caused by the addition of the complex, and the binding constant and the number of binding sites were determined simultaneously. The ability of the complex to displace ethidium bromide from the EB-DNA system was tested using the same method.

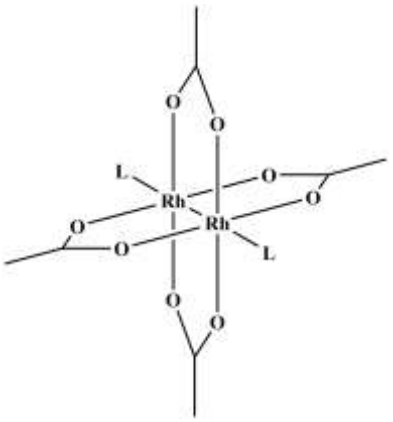

Fig.1. The structural formula of the investigated complex

2. EXPERIMENTAL

2.1. Materials and physical measurements

1-Butylimidazole, toluene, CH_3OH, $CDCl_3$, human serum albumin (HSA), highly polymerized calf thymus DNA (CT-DNA), phosphate-buffered saline (PBS) and ethidium bromide were purchased from Sigma-Aldrich, and used as received. $[Rh_2(CH_3COO)_4(H_2O)_2]$ was purchased from Acros Organics and used as received.
Elemental microanalyses for C, H, N were performed in the Microanalytical laboratory, of the Faculty of Chemistry, University of Belgrade, Serbia. IR spectra in the range 400-4000 cm^{-1} were recorded on a Perkin Elmer FT-IR spectrophotometer Spectrum Two, using the KBr pellet technique. ^1H and ^{13}C NMR spectra were recorded using a Varian Gemini 2000 spectrometer (200 MHz). Chemical shifts are reported as δ values (ppm) relative to TMS as an internal standard. Fluorescence spectra were performed using a RF-

1501 PC spectrofluorometer (Shimadzu, Japan). Melting point was measured on the Stuart melter with an accuracy of ±1 °C.

2.2. Synthesis of the complex

The $[Rh_2(CH_3COO)_4L_2]$ was synthesized following by the method described elsewhere [18]. To a solution of $[Rh_2(CH_3COO)_4(H_2O)_2]$ (0.0478 g, 0.10 mmol) in toluene (10 mL) the 1-butylimidazole ligand (L) (0.0290 mL, 0.22 mmol) was added. The resulting mixture was stirred at room temperature for 1 hour until the reagents were completely dissolved. The solution was left at room temperature to evaporate slowly to a volume of 6 to 7 mL and then stored overnight in the refrigerator. The precipitated purple crystals were filtered off under vacuum and washed with diethyl ether (45 mg, 65%). Anal. Calcd. for ($C_{22}H_{36}O_8N_4Rh_2$) C: 38.28; H: 5.26; N: 8.12. Found: C: 38.20; H: 5.23; N: 18.48. ^{1}HNMR (200 MHz, $CDCl_3$): δ = 8.41 (m, Ar-CH, 2H), 7.91 (m, Ar-CH, 2H), 7.25 (m, Ar-CH, 2H), 4.15 (t, N-CH_2-, 4H), 1.94 (m, -CH_2-, 15H), 1.91 (s, CCH_3,12H), 1.50 (m, -CH_2-, 20H), 1.02 (t, -CH_3, 5H). ^{13}CNMR (50 MHz, $CDCl_3$): 191.80 (COO^-), 138.21 (Ar-CH), 129.99 (Ar-CH), 119.29 (Ar-CH), 47.57 (N-CH_2), 33.05 (-CH_2-), 23.91 (-CH_2-), 20.03 (C-CH_3), 13.66 (-CH_3). IR(KBr, 4000-400 cm^{-1}): 1597 ($\nu_{as}COO^-$), 1518 ($\nu_{as}COO^-$), 1426 (ν_sCOO^-), 1410 (ν_sCOO^-). Melting point: 203 °C.

2.3. Interactions of the complex with HSA and DNA by fluorescence spectroscopy

Double distilled water was used to prepare all solutions. The solutions of HSA and EB were prepared by dissolving in a phosphate buffer (PBS, 5×10^{-2} mol dm^{-3}, pH 7.4). The stock solution of CT-DNA was prepared in 5×10^{-2} M PBS buffer (pH 7.24), stored in the dark at 4 °C, and used within four days. The purity of DNA was checked by monitoring the ratio of absorbance at 260 nm to that at 280 nm. The solution gave a ratio of 1.8-1.9 at A260/A280, indicating that the DNA was sufficiently free of proteins [19]. The concentration of CT-DNA in the stock solution was determined by UV absorbance at 260 nm using a molar absorption coefficient (ε_{260} = 6600 M^{-1} cm^{-1}) [20].

2.3.1. HSA binding experiments

For all experiments, the complex was dissolved in PBS buffer at room temperature. Fluorescence spectra were measured to investigate the structural changes in HSA caused by the addition of the complex and to determine the binding constant (*K*) and the number of binding sites (n) for the compound formed between the complex and HSA. The HSA concentration was fixed at 2.0 μM and the concentration of the compound was varied from 0 to 50.0 μM. Fluorescence quenching spectra were measured at an excitation wavelength of 295 nm between 310 and 460 nm. The fluorescence quenching is described by the Stern-Volmer equation [21]:

$$\frac{F_0}{F} = 1\ K_q\tau_0[\text{complex}] = 1 + Ksv[\text{complex}] \quad (1)$$

where F_0 is the emission intensity in the absence of the compound, F is the emission intensity in the presence of the compound, K_{SV} is the Stern-Volmer quenching constant, k_q is the bimolecular quenching constant, τ_0 (10^{-8} s) is the lifetime of the fluorophore in the absence of the quencher, and [complex] is the concentration of the compound. The K_{SV} value is determined as the slope from the plot of F_0/F versus [complex]. The binding constant (K) and binding stoichiometry (n) of the HSA compound system can be estimated from the following equation (2) [21] using the fluorescence intensity data:

$$\log\frac{F_0-F}{F} = \log K + \text{n}\log[Q] \quad (2)$$

The values of K and n were obtained from the intercept and slope of the plots of log (F_0-F)/F versus log [Q].

2.3.2. DNA binding experiments

EB competition studies of the complex were performed using fluorescence emission spectroscopy. To study the interactions of the rhodium(II) complex with DNA-EB and the possibility of displacement of EB, solutions were prepared with the same equilibrium concentration of EB/DNA (0.0241 mM) and different equilibrium concentrations of the complexes (between 0.023 and 0.162 mM). DNA-EB Solutions were prepared by mixing 31 μL CT DNA (concentration 1.95 mM) and 61 μL EB (concentration 1 mM) in PBS buffer (pH = 7.4). The excitation wavelength was 527 nm, while the emission range was 550-750 nm. The Stern-Volmer constant K_{SV} is described by the Stern-Volmer relationship (equation (1)), similar to that described above for HSA binding studies.

2.4. Docking experiment

Structure of rhodium(II) complex for docking experiments was prepared using Amsterdam density functional (ADF) program [22] to obtain optimized geometry and atomic partial charges. Starting geometry was taken from x-ray structure that was optimized using unrestricted B3LYP [23] functional with Slater type TZP basis set. The calculations were done with conductor-like screening model (COSMO) [24], with water as a solvent.
Target structure of HSA was obtained from RCSB protein data bank site [25] with code 1HK1. Docking experiment and target molecule were prepared using AutoDockTools. Docking processes were run using AutoDock 4.2 [26] with extended parameter library for Rh atoms. A total of 10 Lamarckian hybrid genetic algorithm-local search runs and maximum of 2.5×10^7 energy evaluations per run were performed.

3. RESULTS AND DISCUSSION

In this work, we synthesised a new dinuclear rhodium(II) complex with the formula $[Rh_2(CH_3COO)_4L_2]$, where L = 1-butylimidazole (Fig. 2). The complex was obtained in

good yield as a purple crystals and is stable both in the solid state and in solution in air. It is soluble in methanol, ethanol, DMSO, DMF, slightly soluble in acetone and acetonitrile and insoluble in diethyl ether and water. The IR and NMR spectra are consistent with the complex structure.

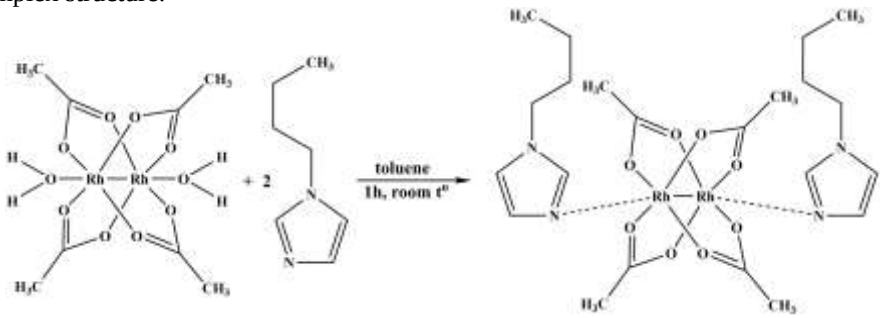

Fig. 2. Synthesis of a dinuclear rhodium(II) complex

3.1. Interaction of the complex with HSA

It is known that the most important task of serum albumin is the transport of metal ions and metal complexes and other biologically active compounds in the blood. To study the structural changes in HSA caused by the addition of ligands or complexes and to determine the quenching constants (k_q), binding constant (K), and number of binding sites (n) for the complex formed between the rhodium(II) complex and HSA, fluorescence spectra were measured. HSA solutions exhibit strong fluorescence emission with a peak at about 350 nm, which is due to the tryptophan residues when excited at 295 nm [27]. The fluorescence spectra of HSA with different concentrations of the new rhodium(II) complex were recorded and are shown in Figure 3.

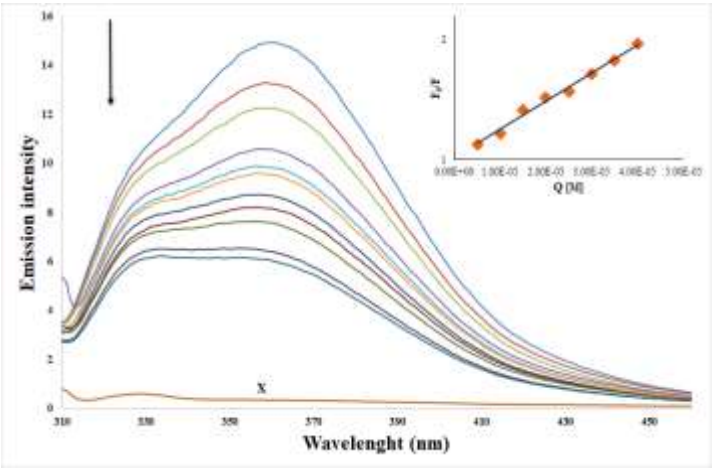

Fig. 3. Fluorescence emission spectra of HSA in the presence of different concentrations of the complex (T = 298 K, pH = 7.4). [HSA] = 2.0 μM. [Complex] = 0-50 μM. Curve x shows the emission spectrum of the complex only. The arrow shows the change in intensity as the complex concentration is increased. Inset: plot of F_0/F versus [complex].

The K_{SV} and quenching constants (k_q) of the interactions of the complex with albumin were calculated using the Stern-Volmer quenching equation (Eq. (1)) (Table 1), where the fluorescence lifetime of tryptophan in HSA was assumed to be $\tau_0 = 10^{-8}$ s. As can be seen from Table 1, the quenching constants ($>10^{12}$ M^{-1} s^{-1}) are higher than the different quenching types for biopolymer fluorescence (10^{10} M^{-1} s^{-1}), indicating that a new conjugate was formed between the complex and HSA and that the interaction of the complex with albumins occurs by a static quenching mechanism. Using the equation (Eq. (1)), the values of *K* (association binding constant) and *n* (number of binding sites per albumin) for the complex were determined from the intercept and slope of the plots of log $(F_0 - F)/F$ versus log [Q]. The values for the binding constant *K* and for *n* are given in Table 1. The calculated value for *n* is one, indicating the presence of only one binding site in HSA.

Table 1. The binding constants and parameters (K_{sv}, k_q, K_b, n) derived for complex

biomolecule	K_{SV} (M^{-1})	k_q $(M^{-1}\ s^{-1})$	R^{2a}	K_b (M^{-1})	*n*	R^{2a}
HSA	2.35×10^4	2.35×10^{12}	0.9891	1.79×10^4	1.02	0.9900
DNA	7.51×10^4	7.51×10^{12}	0.9964	5.49×10^3	0.97	0.9971
[a] R is the correlation coefficient						

3.2. Interaction of the complex with DNA

Determination of the interactions between the small molecules and DNA is important in pharmacology when evaluating the potential of new antitumor complexes [28], and therefore the interactions between the DNA and the synthesized complexes must be studied. The mode and strength of binding of the synthesized complex with rhodium(II) to CT-DNA were studied by fluorescence spectroscopic methods.

To investigate the ability of the complex to displace EB from the EB-DNA complex, competitive EB binding studies were performed with fluorescence measurements. The compounds competing with EB for intercalation with DNA result in displacement of the bound EB, causing a decrease in fluorescence intensity. EB is a typical indicator of intercalation because it forms soluble complexes with nucleic acids and emits intense fluorescence in the presence of CT DNA [29]. The emission spectra of EB bound to CT DNA in the presence of complex were recorded and are shown in Fig. 4.

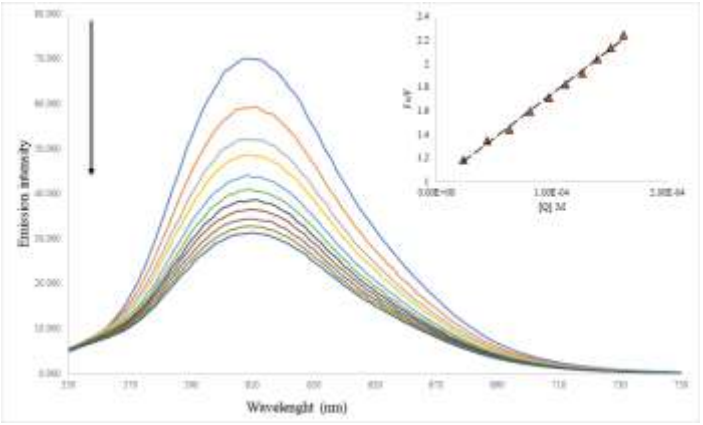

Fig. 4. Fluorescence emission spectra (λ_{ex} = 520 nm) of ethidium bromide bound to DNA. [DNA] = 24.1 μM, [EB] = 24.1 μM, and [compound] = 0-162 μM. The arrow shows the change in emission intensity with increasing complex concentration. Inset: plot of F_0/F versus [compound].

The fluorescence intensity of EB bound to DNA at 613 nm shows a decreasing trend with increasing concentration of compound. The k_q values reported in Table 1 were greater than 10^{10} M^{-1} s^{-1}, indicating that the quenching mechanism is a static quenching process due to the formation of the CT DNA-EB complex.

3.3. Docking

Docking experiments complement *in vitro* studies of interactions by providing means to calculate energy of binding between studied compound and target molecules and allowing visualization for study of different binding modes.

After screening solvent accessible surface of HSA it was determined that best binding pocket for our Rh(II) complex was in vicinity of TRP214. Actual binding mode derived from experiment does not include interaction with that residue. Insetead, acetate oxigen atoms act as electron donors for hydrogen bonds with ARG218 and ARG222 residues. Alyphatic butyl chains interact with hydrophobic parts of LYS444 and PRO447 on one side and LYS195 and PHE157 on the other side of the molecule. These, and other important interactions are shown in Figure 5. Energy of binding was -3.23 kcal mol^{-1}.

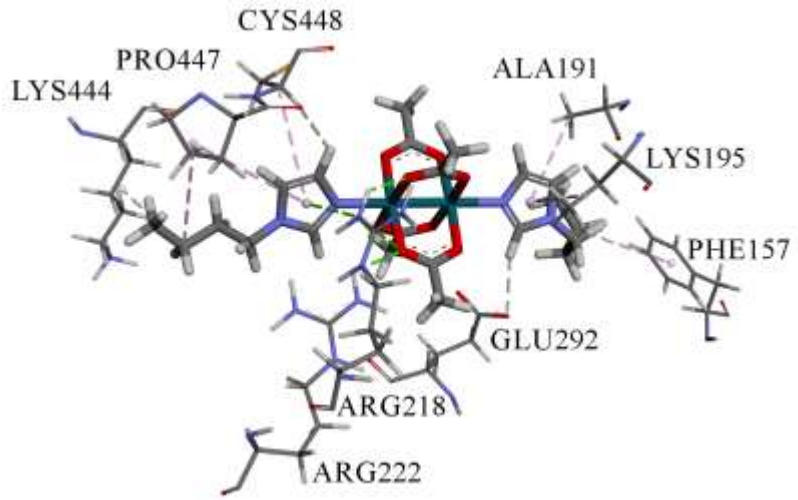

Fig. 5. Crucial interactions of Rh(II) complex with residues of HSA derived from docking experiment.

3. CONCLUSION

In the frame of this work, we have described the synthesis and characterization of a new dinuclear Rh(II) complex, $[Rh_2(CH_3COO)_4L_2]$, (L = 1-butylimidazole). The results of elemental microanalysis, melting point as well as IR and NMR spectra are in agreement with the proposed structure of the complex. The complex showed moderate binding ability for CT DNA with $K_b = 5.49 \times 10^3$. The fluorescence intensity of EB bound to DNA at 613 nm showed a decreasing trend with increasing concentration of the compound. This may

indicate that the complex can replace EB from EB-DNA and interact with CT-DNA via intercalation. The results of the complex-HSA interaction study showed good binding of the complex to protein, which means that this complex could be transported by the bloodstream via protein to the target cells. Docking study showed that the alyphatic butyl chains interact with hydrophobic parts of LYS444 and PRO447 on one side and LYS195 and PHE157 on the other side of the HSA.

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