ISBN 978-9940-611-08-8

COAST
INTERNATIONAL CONFERENCE ON ADVANCES IN SCIENCE AND TECHNOLOGY

III INTERNATIONAL CONFERENCE ON
ADVANCES IN SCIENCE AND TECHNOLOGY

PROCEEDINGS COAST 2024

FACULTY OF MANAGEMENT HERCEG NOVI

HERCEG NOVI, MONTENEGRO

29 MAY - 01 JUNE 2024

BOOK OF PROCEEDINGS

III INTERNATIONAL CONFERENCE ON ADVANCES IN SCIENCE AND TECHNOLOGY
COAST 2024

Publisher:
Faculty of Management Herceg Novi

Editorial board:
Djordje Jovanović, PhD, Editor-in-Chief, Irena Petrušić, PhD,
Jovana Jovanović, PhD, Ivan Stevović, PhD

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

Print:
"IRTA" d.o.o. Risan

Circulation:
180 copies

CIP - Каталогизација у публикацији
Национална библиотека Црне Горе, Цетиње

INTERNATIONAL conference on advances in science and technology (III ; 2024 ; Herceg Novi)
Book of Abstracts / International conference on advances in science and technology, Herceg Novi, 29 May - 01 June, 2024 : Fakultet za menadžment, 2024 (Herceg Novi). - 181 стр. ; [editorial board Djordje Jovanović, Irena Petrušić, Jovana Jovanović, Ivan Stevović].

ISBN **978-9940-611-08-8**
COBISS.**CG-ID 30617348**

SYNTHESIS, CHARACTERIZATION AND HSA INTERACTIONS OF NEW [PDL$_2$CL$_2$] COMPLEX

Maja B. Đukić[1], Marija S. Ristić[1], Danijela Lj. Stojković[2], Ignjat P. Filipović[1], Marko D. Radovanović[1], Ivan. Ž. Jakovljević[1], Zoran D. Matović[1]
[1] University of Kragujevac, Faculty of Science, Kragujevac 34000, Serbia
[2]University of Kragujevac, Institute for Information Technologies, Department of Science, 34000 Kragujevac, Republic of Serbia

Corresponding author e-mail address: maja.djukic@pmf.kg.ac.rs (M. B. Đukić)

ABSTRACT:
In this report, we have synthesized a new palladium(II) complex, [PdL$_2$Cl$_2$], where L = 5-(methylamino)-3-morpholine-4-ylisothiazole-4-carbonitrile. To an aqueous solution (15 mL) of K$_2$[PdCl$_4$] (0.1632 g, 0.50 mmol) was added 15 mL of a methanolic ligand solution (0.2243 g, 1 mmol). The resulting mixture was stirred for 1 hour at room temperature until the reagents had completely dissolved. The solution was then filtered off under vacuum and washed with diethyl ether, yielding an orange powdery residue. The characterization of the synthesized complex [PdL$_2$Cl$_2$] was carried out by elemental microanalysis, IR spectroscopy and determination of the melting point. The interaction of the new complex with human serum albumin (HSA) was investigated by fluorescence spectroscopy. The high value of the binding constant, K_b, and the Stern-Volmer quenching constant, K_{SV}, are the result of good binding of the complex to HSA.

Keywords: *palladium(II), metal complex, isothiazole ligand, HSA interaction*

---

1. INTRODUCTION

As the leading cause of death, cancer is a global health problem that caused almost 10 million deaths in 2020 [1]. The era of antitumor drugs began with the discovery of cisplatin and its use in medicine [2]. Given the numerous side effects of cisplatin and the emergence of resistance, scientists around the world are working to synthesize new complexes that could be more selective and thus have the greater antitumor potency and fewer side effects [3,4]. An ideal anticancer drug must be able to destroy tumor cells while leaving the adjacent healthy tissue unharmed. Great attention is being paid to platinum group metals, i.e. their complexes, including palladium complexes. Compounds of Pd(II) ions have shown antifungal, antituberculous and antimicrobial effects [5,6]. Due to the presence of sulfur and nitrogen atoms in the structure, thiazole derivatives are good candidates for complexation with Pd(II) ions as soft Lewis acids. In this work, we have described the synthesis and characterization of a new palladium(II) complex, [PdL$_2$Cl$_2$], where L = 5-(methylamino)-3-morpholine-4-ylisothiazole-4-carbonitrile. The characterization of the synthesized complex was carried out by elemental microanalysis, IR spectroscopy and

determination of the melting point. Fluorescence spectroscopy was used to investigate the structural changes in the HSA molecule caused by the addition of the complex and simultaneously to determine the binding constant and the number of binding sites.

2. EXPERIMENTAL

2.1. Materials and physical measurements

$K_2[PdCl_4]$, CH_3OH, human serum albumin (HSA) and phosphate buffered saline (PBS) were purchased from Sigma-Aldrich and used as received. Elemental microanalyses for C, H, N were performed at the Department of Science, Institute for Information Technologies, University of Kragujevac, 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. Fluorescence spectra were performed using an RF-1501 PC spectrofluorometer (Shimadzu, Japan). The melting point was measured on the Stuart melter with an accuracy of ±1 °C.

2.2. Synthesis of the complex

The $[PdL_2Cl_2]$ was synthesized following by the method described elsewhere [7]. To an aqueous solution (15 mL) of $K_2[PdCl_4]$ (0.1632 g, 0.50 mmol) was added 15 mL of a methanolic ligand solution (0.2243 g, 1 mmol). The resulting mixture was stirred for 1 hour at room temperature until the reagents had completely dissolved. The solution was then filtered off under vacuum and washed with diethyl ether, yielding an orange powdery residue. (196 mg, 62.6%). Anal. Calcd. for ($C_{18}H_{24}Cl_2N_8O_2PdS_2$) C: 34.54; H: 3.86; N: 17.90. Found: C: 34.67; H: 3.98; N: 17.68. IR (KBr, ν_{max}/cm^{-1}) 3468.08 ($\nu_{=CH}$), 2964.73, 2918.34, 2860.62 (ν_{CH}), 2210.29 ($\nu_{C\equiv N}$), 1579.86 ($\nu_{C=C}$). Melting point: 229 °C.

2.3. Interactions of the complex with HSA fluorescence spectroscopy

Double distilled water was used to prepare all solutions. The solutions of HSA and complex were prepared by dissolving in a phosphate buffer (PBS, 5×10^{-2} mol dm^{-3}, pH 7.4) 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_b) 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 20.0 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 [8]:

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

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) [8] using the fluorescence intensity data:

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

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

3. RESULTS AND DISCUSSION

In this work, we have synthesised a new palladium(II) complex with the formula [Pd(5-MA-3-MorphCN-ITZ)$_2$Cl$_2$] (Fig. 1). The complex was obtained in good yield as an orange-coloured powder, soluble in DMSO and DMF and is stable both in the solid state and in solution in air. The IR spectrum is consistent with the complex structure.

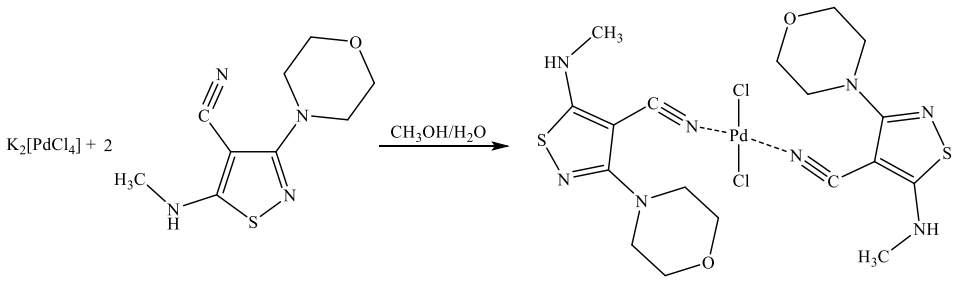

Fig.1. Pathway for the synthesis of [Pd(5-MA-3-MorphCN-ITZ)$_2$Cl$_2$]

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 complex and to determine the quenching constants (k_q), binding constant (K_b), and number of binding sites (n) for the complex formed between the palladium(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 [9]. The fluorescence spectra of HSA with different concentrations of the new palladium(II) complex were recorded and are shown in Fig. 2.

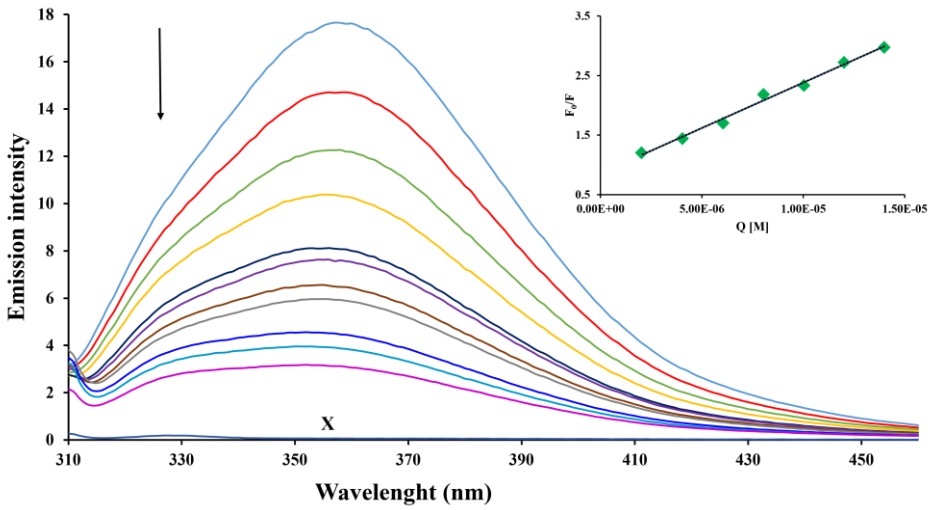

Fig. 2. Fluorescence emission spectra of HSA in the presence of different concentrations of the complex (T = 298 K, pH = 7.4). [HSA] = 2.0 μM. [Complex] = 0-20 μM. Curve x shows the emission spectrum of the complex only. The arrow shows the change in intensity as the complex concentration is increased. 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_b (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_b 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

	K_{SV} (M^{-1})	k_q (M^{-1} s^{-1})	R^{2a}	K_b (M^{-1})	n	R^{2a}
Pd-HSA	1.53 x 10^5	1.53 x 10^{13}	0.9915	3.78 x 10^6	1.28	0.9890
[a] R is the correlation coefficient						

3. CONCLUSION

In this work, we have described the synthesis and characterization of a new palladium(II) complex, [Pd(5-MA-3-MorphCN-ITZ)$_2$Cl$_2$]. The results of elemental microanalysis, melting point, as well as IR spectrum, are in agreement with the proposed structure of the complex. 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.

4. LITERATURE

[1] World Health Organization, International Agency for Reasearch on Cancer, Cancer today, https://gco.iarc.fr/today (2020) (accessed February 2021).
[2] Rosenberg, B., Van Camp, L., Trosko, L. et al. (1969), "Platinum Compounds: a New Class of Potent Antitumour Agents", Nature, Vol. 222, 385-386.
[3] Amo-Ochoa P., González M. V., Pérez M. José, Masaguer R. José, Alonso C., Navarro-Ranninger C. (1996), Cytotoxicity, DNA binding, and reactivity against nucleosides of platinum (II) and (IV) spermine compounds, Journal of Inorganic Biochemistry, 64, 4, 287-299, https://doi.org/10.1016/S0162-0134(96)00082-7.
[4] Bose N. R., Ghosh K. S., Moghaddas S. (1997), Kinetic analysis of the cis-diamminedichloroplatinum(II)-cysteine reaction: Implications to the extent of platinum-DNA binding, Journal of Inorganic Biochemistry, 65, 3, 199-205, https://doi.org/10.1016/S0162-0134(96)00133-X.
[5] Garoufis A., Hadjikakou S.K., Hadjiliadis N. (2009), Palladium coordination compounds as anti-viral, anti-fungal, anti-microbial and anti-tumor agents, Coordination Chemistry Reviews, 253, 9–10, 1384-1397, https://doi.org/10.1016/j.ccr.2008.09.011.
[6] Petrović S. Đ. et al. (2023), Synthesis, characterization, HSA binding, molecular docking, cytotoxicity study, and antimicrobial activity of new palladium(II) complexes with propylenediamine derivatives of phenylalanine, Journal of Inorganic Biochemistry, 246, 112283, https://doi.org/10.1016/j.jinorgbio.2023.112283.
[7] Jovičić Milić S. S. et al. (2022), Synthesis, characterization, DNA interactions and biological activity of new palladium(II) complexes with some derivatives of 2-aminothiazoles, Journal of Inorganic Biochemistry, 233, 111857, https://doi.org/10.1016/j.jinorgbio.2022.111857.
[8] Lakowicz, J. R. (2006), "Principles of Fluorescence Spectroscopy", Springer, New York, USA, 3rd edn.

[9] Lakowicz, J. R. & Weber, G. (1973), "Quenching of fluorescence by oxygen. A probe for structural fluctuations in macromolecules", Biochemistry, Vol. 12, 4161-4170.