ORIGINAL SCIENTIFIC PAPER
DOI: 10.63395/STEDConf14022025931R289
Submitted: 21.08.2025.
Accepted: 14.10.2025.

INTERACTIONS OF HUMAN SERUM ALBUMIN WITH A PALLADIUM(II) COMPLEX CONTAINING A THIOAMIDE MOIETY: DETAILED EXPERIMENTAL AND MOLECULAR DOCKING STUDIES

Marija Ristić, Maja Đukić, Jelena Petronijević, Ivan Jakovljević, Marina Ćendić Serafinović, Nenad Joksimović, Milena Vukić

University of Kragujevac, Faculty of Science, Radoja Domanovića 12, 34000 Kragujevac, Serbia, marija.jeremic@pmf.kg.ac.rs

Coresponding author: Marija Ristić, University of Kragujevac, Faculty of Science, Radoja Domanovića 12, 34000 Kragujevac, Serbia, marija.jeremic@pmf.kg.ac.rs

ABSTRACT

Thioamides and their derivatives are an interesting group of compounds because of their structural variations and also because of the combination of hard and soft donor atoms (S and N) that potentially allow coordination – in a variety of binding modes – to a wide range of metal centers, and also because of their biological significance.

In our previous studies, we have synthesized a palladium(II) complex with a thioamide-type ligand of the formula $[PdL_2Cl_2]$ (L= ethyl 4-[1-amino-2-cyano-3-(methylamino)-3-thioxo-1-propen-1-yl]-1-piperazine-1-carboxylate), whose ability to interact with DNA was investigated fluorometrically. In this work, research was continued in the direction of studying the interactions of the thioamide ligand and its palladium(II) complex with human serum albumin (HSA) in the presence of site-specific markers: warfarin (site I, subdomain IIA), ibuprofen (site II, subdomain IIIA) or methyl orange (site III, subdomain IB), to determine the binding affinity, binding strength and the location of binding site. The results obtained showed that ligand and complex bind moderately to the HSA via site III (subdomain IB), and that the quenching mechanism is static.

Keywords: palladium(II), thioamide, HSA interaction, competitive binding studies, docking simulation.

INTRODUCTION

Thioamides, as sulfur-containing analogs of amides, represent a structurally diverse class of compounds with intriguing chemical and biological properties. Their ability to coordinate with transition metal ions arises from the presence of both soft (S) and hard (N) donor atoms, which enable different binding modes and geometries (Reiss et al., 2012; Desseyn, 1989). These structural features, as well as the pharmacophoric potential of thioamides, have attracted considerable attention in medicinal and coordination chemistry, particularly in the development of metal-based therapeutic agents (Nadeem et al., 2010; Ahmad et al., 2017). Among the transition metal complexes, palladium(II) compounds have attracted considerable interest due to their structural and electronic similarity to platinum(II) analogs, many of which are known chemotherapeutic agents (Matesanz et al., 2013). However, Pd(II) complexes often exhibit greater kinetic lability compared to Pt(II), which can affect their reactivity, biological stability and interaction dynamics with biomolecules. Understanding such interactions is crucial for evaluating the pharmacological potential of these complexes.

Human serum albumin (HSA), the most abundant plasma protein in humans, plays a central role in the transport, metabolism, and distribution of a variety of endogenous and exogenous substances, including drugs, hormones, and metal ions (Mitrović et al., 2024). HSA possesses multiple binding sites, in particular Sudlow's sites I and II (located in subdomains IIA and IIIA, respectively), and a third site in subdomain IB, which can serve as a binding region for small molecules and metal complexes (Mitrović et al., 2024; Zsila, 2013). Investigating the binding

properties of metal-based drugs to HSA is crucial for understanding their in vivo behavior, distribution, and potential side effects (Mitrović et al., 2024; Zsila, 2013).

In our previous work, we reported the synthesis and DNA-binding activity of a Pd(II) complex with a thioamide-type ligand, ethyl 4-[1-amino-2-cyano-3-(methylamino)-3-thioxo-1-propen-1-yl]-1-piperazine-1-carboxylate, forming the complex $[PdL_2Cl_2]$ (Ristić i sar., 2024). Building on this basis, the interactions of this ligand and its Pd(II) complex with HSA are investigated in the present study. In addition, molecular docking simulations were used to obtain an atomistic insight into the binding interactions and to support the spectroscopic results.

MATERIAL AND METHODS OF WORK

Protein binding studies were performed with human serum albumin (HSA) in PBS buffer (10 mM PBS, pH 7.4), using a fixed HSA concentration and varying compound concentrations. The quenching of the emission intensity of HSA tryptophan residues at 361 nm was monitored using the investigated compounds as quenchers with increasing concentrations. Fluorescence spectra were recorded from 300 to 500 nm at an excitation wavelength of 295 nm. We also investigated the competitive interactions between the markers (warfarin as marker for site I of subdomain IIA, ibuprofen as marker for site II of subdomain IIIA and methyl orange as marker for site III of subdomain IB) and the compounds towards HSA using the same method as above. The fluorescence spectra of the investigated compounds were recorded under the same experimental conditions, and no fluorescence emission was detected. The relevant constants were calculated using the Stern–Volmer and Scatchard equations (1) and (2) and plots (Lakowicz , 2006):

$$F_0/F = 1 + k_q \tau_0 [Q] = 1 + K_{sv}[Q] \quad (1)$$

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) (Lakowicz and Weber, 1973) is the lifetime of the fluorophore in the absence of the quencher, and [Q] is the concentration of the quencher (compound). The K_{SV} value is determined as the slope from the plot of F_0/F versus [Q].

$$\log (F_0 - F)/F = \log K_b + n \log [Q] \quad (2)$$

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

COMPUTATIONAL RESEARCH

Dft calculations

In all tested compounds B3LYP functional (Zhang et al., 2005; Lashgari et al., 2005) was used to optimize the geometries together with LANL2DZ basis set. The structures were visualized in a free version of the Discovery Studio Visualizer 3.5.0 Accelrys Software Inc. (https://www.3ds.com/products/biovia). These calculations were performed by Gaussian 09 program package (Frisch et al., 2013).

Molecular docking

The X-ray crystal structure of HSA (PDB ID: 1HK1) was acquired from the Protein Data Bank (PDB) (RCSB PDB: Homepage). Docking processes were carried out using Autodock 4.2 (Morris et al., 2009) software equipped with the graphical user interface (GUI) Auto-DockTools (ADT 1.5.6rc3) (Sanner et al., 2009). Then the polar hydrogen atoms were added, and ADT was used to remove crystal water, Geisteiger charges were added to each atom, and merge non-polar hydrogen atoms to the DNA structure. The structures were then saved in PDBQT file format, for further studies in ADT. For the visualization of the docking results, a free version of the Discovery

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Studio Visualizer 3.5.0 Accelrys Software Inc. (https://www.3ds.com/products/biovia) so as Chimera software have been used (https://www.rbvi.ucsf.edu/chimera).

RESULTS AND DISCUSSION

In order to localize the exact binding site of the investigated compounds to the HSA molecule, competitive experiments were performed with the above-mentioned markers. The binding site markers and HSA were added in equimolar concentrations (2 μM), while the ligand and complex solutions were added in increasing concentrations up to a ratio of 10 (up to 20 μM). The obtained fluorescence data were analyzed using the Stern–Volmer and Scatchard equations and plots (Figs. 1 and 2, equations (1) and (2)). The Stern–Volmer constants (K_{sv}), biomolecular quenching constants (k_q) and binding constants (K_b) for the interactions of investigated compounds with HSA in the presence and absence of the site markers are listed in Table 1. The HSA–ibuprofen, HSA–warfarin and HSA–methyl orange (Fig. 2) adducts show intense emission that decreases with the addition of the studied compounds, and the results show that the studied compounds bind with moderately strong affinity ($K_b = 10^2–10^4$ M^{-1}) to sites IIA, IIIA and IB.

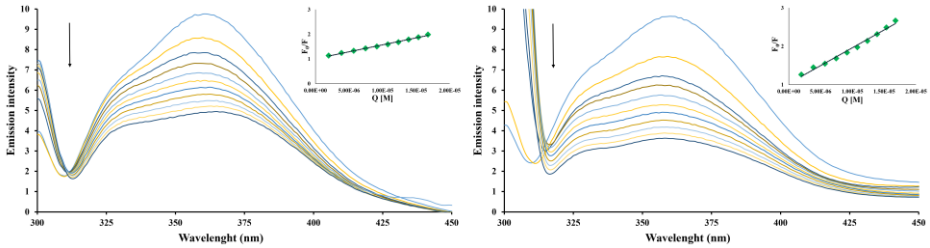

Figure 1. Fluorescence emission spectra of HSA in the presence of different concentrations of the ligand (left) and complex (right) (T = 298 K, pH = 7.4). [HSA] = 2.0 μM. [Compound] = 0-20 μM. The arrow shows the change in intensity as the complex concentration is increased. Inset: plot of F_0/F versus [compound].

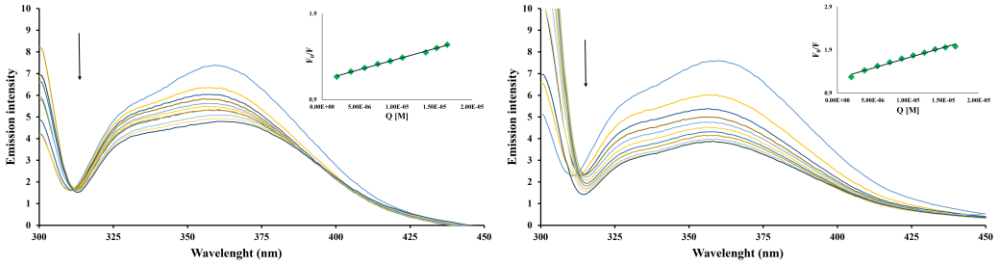

Figure 2. Fluorescence emission spectra of HSA in the presence of different concentrations of the ligand (left) and complex (right) with specific marker MO (T = 298 K, pH = 7.4). [HSA] = [MO] 2.0 μM. [compound] = 0-20 μM. The arrow shows the change in intensity as the complex concentration is increased. Inset: plot of F_0/F versus [compound].

The binding constants were observed to change and decrease in the presence of the site markers, with the largest decrease observed in the presence of methyl orange, suggesting that the complexes may compete with this site marker and bind to site IB. However, slight changes in the constants in the presence of the other two site markers (warfarin and ibuprofen) cannot be completely ignored. For this reason, docking experiments are also performed in this study.

Table 1. HSA constants (K_{sv}, k_q, K_b) and number of binding sites (n) for the interactions of L and PdL in the absence and the presence of site markers.

System	K_{SV} (M^{-1})	k_q (M^{-1} s^{-1})	K_b (M^{-1})	n
L-HSA	5.63×10^4	5.63×10^{12}	2.29×10^4	0.92
L-HSA-WF	3.38×10^4	3.38×10^{12}	8.12×10^2	0.64
L-HSA-IP	4.84×10^4	4.84×10^{12}	4.46×10^4	1.0
L-HSA-MO	2.43×10^4	2.43×10^{12}	$\mathbf{2.05 \times 10^2}$	0.55
PdL-HSA	9.31×10^4	9.31×10^{12}	1.80×10^4	0.85
PdL-HSA-WF	7.16×10^4	7.16×10^{12}	3.86×10^3	0.72
PdL-HSA-IP	4.83×10^4	4.83×10^{12}	2.07×10^3	0.71
PdL-HSA-MO	4.77×10^4	4.77×10^{12}	$\mathbf{7.58 \times 10^2}$	0.60

Docking results

Calculated data of the molecular docking are represented in Table 2. In case of ligand L (with BSA) values of ΔG for three binding sites are: IIA = -7.98 kcal·mol^{-1}, IIIA = -5.99 kcal·mol^{-1} and IB = -6.75 kcal·mol^{-1}. Further, PdL compound gave lower negative energy values (IIA = -7.00 kcal·mol^{-1}, IIIA = -5.89 kcal·mol^{-1} and IB = -7.15 kcal·mol^{-1}).

Table 2. Overview of molecular docking calculations for L and PdL with HSA.

Compound	Δ*G* (kcal mol^{-1})		
	IIA	**IIIA**	**IB**
L	- 6.71	- 5.99	**- 6.75**
PdL	- 7.00	- 5.89	**- 7.15**

Thus, the represented data (Table 2) predicts stronger binding to HSA in the case of the PdL complex system. The best docking poses are presented in Figure 3, left (between L and HSA) and Figure 3, right (between PdL and HSA). Results obtained from docking experiments show that the preferred binding site would be in domain IB, which agrees with experimental binding studies.

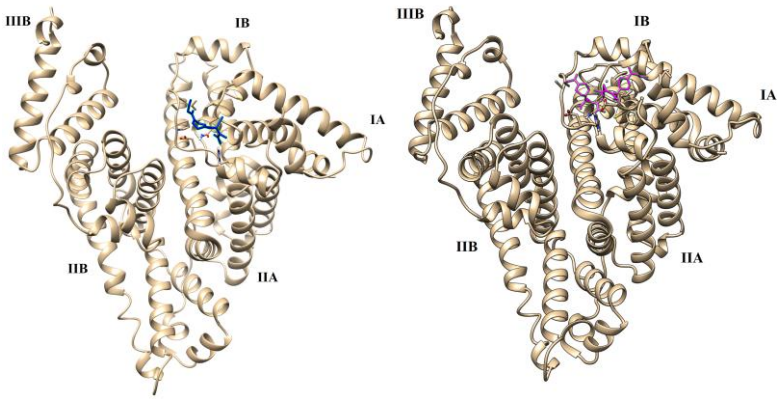

Figure 3. Interactions between **L** and HSA (left) and **PdL** and HSA (right).

CONCLUSIONS

This study investigated the interactions of a thioamide-type ligand L and its Pd(II) complex PdL with human serum albumin (HSA) using fluorescence spectroscopy and molecular docking. Experimental results demonstrated that both compounds bind to HSA with moderate affinity (K_b in the range of 10^2–10^4 M^{-1}), with subdomain IB identified as the preferred binding site, as confirmed by competitive binding experiments with specific site markers. Molecular docking further supported that the PdL complex exhibits a somewhat stronger affinity for HSA compared to the free ligand, with binding energy values indicating a more stable interaction at subdomain IB. These findings contribute to a better understanding of the interaction mechanisms between metal-organic compounds and plasma proteins and may be valuable for the further development of metal-based therapeutics with improved pharmacokinetic properties.

DECLARATIONS OF INTEREST STATEMENT

The authors affirm that there are no conflicts of interest to declare in relation to the research presented in this paper.

LITERATURE

Ahmad, S., Nadeem, S., Anwar, A., Hameed, A., Tirmizi, S. A., Zierkiewicz W., Abbas, A., Isab A. A., & Alotaibi, M. A. (2017). Synthesis, characterization, DFT calculations and antibacterial activity of palladium(II) cyanide complexes with thioamides. *Journal of Molecular Structure*, *1141*, 204–212.

Chimera software https://www.rbvi.ucsf.edu/chimera.

Dassault Systèmes BIOVIA. (2016). *Discovery Studio (Version 17.2.0)* [Computer software]. Dassault Systèmes.

Desseyn, H. O. (1989). Complexes of chelates of amides and thioamides. *Pure and Applied Chemistry*, *61*(5), 867–872.

Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Scalmani, G., Barone, V., Mennucci, B., Petersson, G. A., Nakatsuji, H., Caricato, M., Li, X., Hratchian, H. P., Izmaylov, A. F., Bloino, J., Zheng, G., Sonnenberg, J. L., Hada, M., Ehara, M., ... Fox, D. J. (2013). *Gaussian 09, Revision D.01*. Gaussian, Inc.

Lashgari, M., Arshadi, M. R., & Parsafar, G. A. (2005). A simple and fast method for comparison of corrosion inhibition powers between pairs of pyridine derivative molecules. *Corrosion Science, 61*, 778–783.

Matesanz, A. I., Leitao, I., & Souza, P. (2013). Palladium(II) and platinum(II) bis(thiosemicarbazone) complexes of the 2,6-diacetylpyridine series with high cytotoxic activity in cisplatin resistant A2780cisR tumor cells and reduced toxicity. *Journal of Inorganic Biochemistry, 125*, 26–31.

Mitrović, M., Djukić, M. B., Vukić, M., Nikolić, I., Radovanović, M. D., Luković, J., Filipović, I. P., Matić, S., Marković, T., Klisurić, O. R., Popović, S., Zoran D. Matović, Z. D. & Ristić, M. S. (2024). Search for new biologically active compounds: in vitro studies of antitumor and antimicrobial activity of dirhodium(II,II) paddlewheel complexes. *Dalton Transactions*, *53*, 9330–9349.

Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of Computational Chemistry*, *30*(16), 2785–2791.

Nadeem, S., Bolte, M., Ahmad, S., Fazeelat, T., Tirmizi, S. A., Rauf, M. K., Sattar S. A., Siddiq S., Hameed, A. & Haider, S. Z. (2010). Synthesis, crystal structures and, antibacterial and antiproliferative activities in vitro of palladium(II) complexes of triphenylphosphine and thioamides. *Inorganica Chimica Acta*, *363*, 3261–3269.

Reiss, A. & Mureşeanu, M. (2012). Transition metal complexes with ligand containing thioamide moiety: Synthesis, characterization and antibacterial activities. *Journal of the Chilean Chemical Society*, *57*(4), 1409–1414.

Ristić, M. et al. (2025). Interactions of human serum albumin with a palladium(II) complex containing a thioamide moiety: detailed experimental and molecular docking studies. *STED Conference 14*(2), 298-294.

Ristić, M. S., Djukić, M. B. & Kosović Perutović M. (2024). DNA Interactions of Palladium (II) Complex Containing a Thioamide-Type Ligand. 27th Congress of the Society of Chemists and Technologists of Macedonia (SCTM), Ohrid, N. Macedonia 2024, p. 157. ISBN: 978-9989-760-20-4.

Sanner, M. F. (1999). Python: a programming language for software integration and development. *Journal of Molecular Graphics and Modelling, 17*, 57-61.

Zhang, S. G., Lei, W., Xia, M.Z. & F.Y. Wang (2005). QSAR study on *N*-containing corrosion inhibitors: Quantum chemical approach assisted by topological index. *Journal of Molecular Structure: THEOCHEM*, *732*, 175–182.

Zsila, F. (2013). Subdomain IB Is the Third Major Drug Binding Region of Human Serum Albumin: Toward the Three-Sites Model. *Molecular Pharmaceutics, 10*, 1668–1682.