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Research Article

Investigation of the complexation activity of pyrimidine-2,4(1H,3H)-dithione with Pd (II) and biological activity of the newly formed complex

Petya Marinova¹, Dimitar Stoitsov², Evelina Varbanova², Yordanka Gaytanska³, Denica Blazheva³✉, Aleksandar Slavchev³, Plamen Penchev²

¹Department of General and Inorganic Chemistry with Methodology of Chemistry Education, Faculty of Chemistry, Paisii Hilendarski University of Plovdiv, Plovdiv, Bulgaria

²Department of Analytical and Computer Chemistry, Faculty of Chemistry, Paisii Hilendarski University of Plovdiv, Plovdiv, Bulgaria

³Department of Microbiology and Biotechnology, Technological Faculty, University of Food Technologies, Plovdiv, Bulgaria

Abstract

This article describes the synthesis of a new Pd (II) complex by using 2,4-dithiouracil (2,4-DTu) as a starting reagent and its structure elucidation. The coordination compound was analyzed by several methods, including melting point determination, UV-Vis, ¹H NMR-solution state, ¹H- and ¹³C-NMR solid state, HSQC, ¹H-1H COSY, ATR and Raman spectroscopy. The metal complex was formed by mixing aqueous solutions of metal salts with the ligand dissolved in DMSO and water, along with NaOH, in a metal-to-ligand-to-base ratio of 1:4:2. Furthermore, the compound's antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as yeasts, was assessed. It should be noted that for the first time a biologically active mixed ligand complex of Pd (II) with 2,4-DTu and 2-Tu was obtained. The ligands 2,4-DTu and 2-thiouracil (2-Tu) are bidentately coordinated to Pd(II) through a deprotonated nitrogen atom and the adjacent heteroatom—sulfur or oxygen, respectively. Tentative structures of the Pd(II) complexes are proposed, in which DMSO-h₆ and H₂O may be located either in the inner or outer coordination sphere. The new Pd(II) complex exhibited a coordination number of 6 or 4, respectively. The newly formed coordination compound of the corresponding pyrimidine-based ligands, i.e. 2,4-dithiouracil and 2-thiouracil, with Pd (II) demonstrated the strongest activity against *Staphylococcus aureus*. It, also, showed a significant improvement in its antifungal activity in comparison with free ligand 2,4-dithiouracil.

Keywords

antimicrobial activity, palladium (II) complexes, 2,4-dithiouracil, 2-thiouracil, ¹H- and ¹³C-NMR solid state

Abbreviations

2,4-DTu – 2,4-dithiouracil; DMSO – Dimethyl sulfoxide; NMR – Nuclear magnetic resonance; HSQC – Heteronuclear Single Quantum Coherence; COSY – Homonuclear correlation spectroscopy; ATR – Attenuated Total Reflection

✉ Corresponding author: Assoc. Prof. Denica Blazheva, PhD, Department of Microbiology and Biotechnology, Technological Faculty, University of Food Technologies, 26 Maritza Blvd., 4002 Plovdiv, Bulgaria, tel.: +359 32 603 607; e-mail: d_blazheva@uft-plovdiv.bg

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Introduction

Since cisplatin was introduced to oncology in 1978, Pt (II) and Pd (II) compounds have been extensively researched with the goal of developing enhanced anticancer agents. For example, the radioisotope palladium-103 is applied in brachytherapy for a treatment of various kinds of cancer (Blasko et al. 2000; Kellar et al. 2005). Moreover, palladium finds application in the alloy production for dental crowns

and bridges because of its ability to resist to corrosion, tarnish and discoloration (Rushforth 2004; Megremis and Carey 2006). Additionally, palladium alloys can be incorporated in the manufacturing of more durable and resistant sterilization surgical instruments (Wataha and Shor 2010; Zhang et al. 2019; Huang et al. 2014). Common medical applications of palladium are presented in Fig. 1.

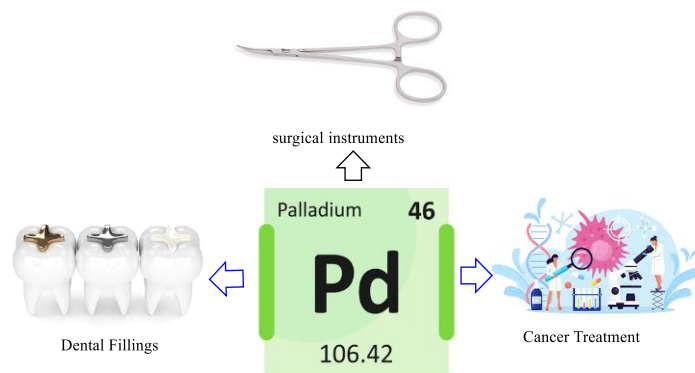


Figure 1. Medical applications of palladium

While palladium is not biologically active the toxicity of its compounds to eukaryotic and prokaryotic cells was already proven (Khan et al. 1991). The reduction of Pd (II) to Pd (0) and its accumulation on cell surfaces were found to be manifested under the influence of *Desulfovibrio desulfuricans* in the presence of format and H₂ as an electron donor (Lloyd et al. 1998). A possible reduction mechanism based on enzyme catalysis was proposed. Furthermore, soluble palladium was found to be removed from solution by *D.*

desulfuricans resting cells and biomass from other organisms acting as biosorbents (Yong et al. 2002; Remoudaki et al. 1999). In addition, unstable methylated palladium species were formed during the interaction of palladium with methylcobalamin where a methylation of the metal has been reported to occur *in vitro* (Scovell 1974). Also, palladium forms complexes with organic ligands such as 2-thiouracil, 2,4-dithiouracil, 6-methyl-2-thiouracil, 6-propyl-2-thiouracil possessing antibacterial and antifungal activities (Fig. 2).

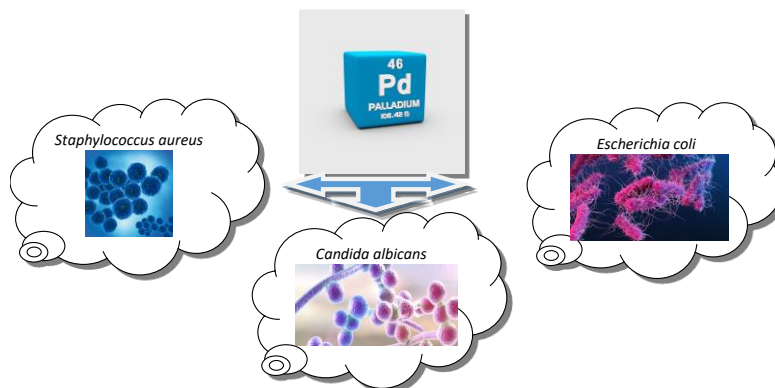


Figure 2. Antibacterial and antifungal activity of palladium complexes with organic ligands such as 2-thiouracil, 2,4-dithiouracil, 6-methyl-2-thiouracil, 6-propyl-2-thiouracil

The abovementioned ligands have well-known biological activities and applications that can be seen on Fig. 3.

Ólmez et al. (2001) obtained new Mn (II), Fe (II), Co (II), Ni (II), Cu (II), Zn (II), Cd (II) and Pd (II) complexes of uracil. Mixed-pyrimidine complexes were evaluated by Bardaj et al. (2007). Weiss et al. (2014) published the *in vitro* bioorthogonal generation of 5-fluorouracil from a biologically

inert precursor during heterogeneous Pd0 catalysis. Palladium metal complexes exhibit various characteristics that make them attractive frameworks for developing anti-cancer drugs (Srivastava 2021). Palladium (II) complexes with 1,2,4-triazolo[1,5-a]pyrimidine, 5,7-dimethyl-1,2,4-triazolo[1,5-a]pyrimidine, 5,7-diphenyl-1,2,4-triazolo[1,5-a]pyrimidine, 5,7-di-tert-butyl-1,2,4-triazolo[1,5-a]pyrimidine were successfully synthesized (Szyk et al. 2000).

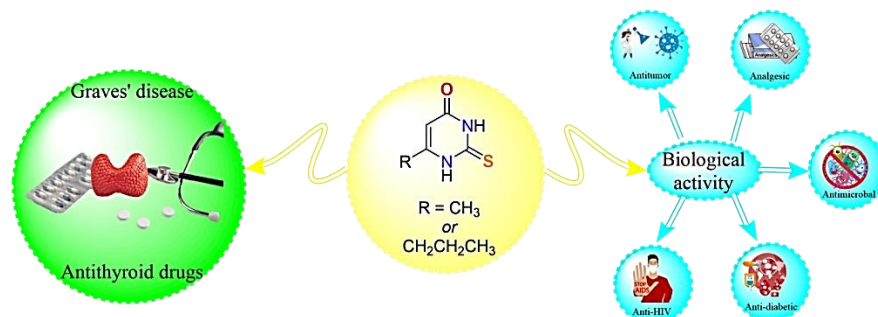


Figure 3. Medical applications of 6-methyl-2-thiouracil, 6-propyl-2-thiouracil, 2,4-dithiouracil and their metal complexes

Marques (2013) draws attention specifically to polynuclear polyamine complexes which have garnered significant interest, as they can form DNA adducts that conventional drugs cannot achieve (through long-distance intra- and interstrand cross-links) while the complexes are also able to overcome often the acquired resistance to cisplatin. Ravindra et al. (2014) reported of platinum group elements in the environment, as well as their health risk. Gebel et al. (1997) determined that the divalent palladium salts PdCl₂, K₂PdCl₄, Pd(NH₃)₂I₂, Pd(NH₃)₄Cl₂, and transpalladium (II) were neither genotoxic in the cytokinesis-block micronucleus test with human lymphocytes nor in the bacterial SOS chromotest. Bünger et al. (1996) investigated the cyto- and genotoxic effects of coordination complexes of platinum, palladium and rhodium *in vitro*. Zalevska (2023) presented an overview and structured analysis of the recent literature on the antibacterial and antifungal properties of palladium complexes with organic ligands, covering studies published within the last three years. Kielhorn et al. (2002) published an overview of the palladium exposure and health effects in humans. Shaheen et al. (2007) synthesized of homobimetallic palladium(II) complexes with 2-thiouracil (2-Tu) ligands. *In vitro* cytotoxicity assessment of new

metal complexes was done. The structure of [Pd₂(TU)(PPh₃)₃Cl₂] complex was determined by X-ray diffraction (Shaheen et al. 2024). Kuzovlev et al. (2015) obtained new palladium and copper complexes with substituted pyrimidine-2-thiones and 2-thiouracils. The structure of [Pd(L₄)₂Cl₂] complex was investigated by X-ray analysis. Jin et al. (2012) synthesized new Pd(II) complexes with heterocyclic-N/NS ligands. Fernández-Galán et al. (1999) obtained a new allyl palladium complexes with thionate ligands. An X-ray crystal structure analysis reveals a square-planar arrangement of two thionate ligands around the Pd atom (Fernández-Galán et al. 1999). Recently, Marinova et al. synthesized Cu(II), Pd(II) and Au(III) complexes with 2-thiouracil (Marinova et al. 2022), new Cu(II) and Pd(II) Complexes with 6-Methyl-2-Thiouracil and 6-Propyl-2-Thiouracil (Marinova et al. 2023), Au(III) and Cu(II) complexes with 2,4-Dithiouracil (2,4-DTu) (Marinova et al. 2024b), Au(III) complex with 6-Methyl-2-Thioxo-2,3-Dihydropyrimidin-4(1H)-One (Marinova et al. 2024a) and presented the synthesis and biological activities of some metal complexes of 2-thiouracil and its derivatives (Marinova et al. 2024c).

This work presents the synthesis and structure elucidation of new biologically active palladium

complex with 2,4-DTu and 2-Tu. The structure of the compound was elucidated mainly by ^1H NMR-solution state, ^1H - and ^{13}C -NMR solid state, HSQC and ^1H - ^1H COSY spectroscopy. Additionally, the structure elucidation was supported by the data obtained from melting point determination, UV-Vis, ATR, Raman and elemental analyses based on microwave plasma atomic emission spectrometry (MP-AES) and inductively coupled plasma optical emission spectrometry (ICP-OES). This is the first time when a mixed ligand palladium complex containing 2,4-DTu and 2-Tu as ligands and possessing antimicrobial activity has been reported.

Materials and Methods

Reagents and apparatus. Only high-purity A.C.S. grade reagents were purchased from Aldrich Chem for the synthesis of the Pd (II) complex. The free ligand 2,4-dithiouracil is purchased from Aldrich Chem. The metal salts $\text{NH}_4[\text{PdCl}_4]$ (Aldrich Chem) and solvents used for synthesis of the complexes were with a high purity generally equal to A.C.S. grade and suitable for use in many laboratory and analytical applications. The melting point of the 2,4-DTu ligand is in the range (279 - 281°C) whereas the melting point for Pd (II) complex is above 350°C. Additional study showed that the melting point for 2-Tu is 300°C.

Synthesis of Pd (II) complex of 2,4-dithiouracil—general procedure. The coordination compound was prepared by mixing aqueous solutions of the metal salt with the ligand dissolved in a blend of DMSO and water, accompanied by NaOH, in a molar ratio of metal to ligand to base of 1:4:2. This procedure yielded a neutral complex, which appeared as an orange-brown solid precipitate. The precipitate was subsequently separated through filtration, rinsed thoroughly with water, and dried over calcium chloride (CaCl_2) for two weeks. The aqueous solution containing 56.9 mg (0.2 mmol) of $\text{NH}_4[\text{PdCl}_4]$ metal salt in 5 mL of water was introduced gradually into a solution of 2,4-dithiouracil in 5 mL of DMSO, incorporating 115.4 mg (0.8 mmol). The ligand solution had been pretreated with a sodium hydroxide solution, consisting of 16.0 mg (0.4 mmol) of NaOH dissolved in 5 mL of water.

Spectral measurements. Absorption spectra were registered on a UV-30 SCAN ONDA UV/Vis/NIR

Spectrophotometer from 200 to 1000 nm. Three maxima at $\lambda_{\text{max}}=257$ nm, 286 nm, 356 nm were found for 2,4-DTu and only one at $\lambda_{\text{max}}=295$ nm was found for 2-Tu. For Pd (II) complex two maxima were found at $\lambda_{\text{max}} = 257$ nm and 292 nm. Solid-state NMR spectra were measured on a Bruker Avance III HD 500 MHz NMR spectrometer by using 2.5 mm Cross-Polarization Magic Angle Spinning (CP MAS) probe head. α -glycine was utilized as external reference (α -glycine carbonyl C-176.03 ppm) with a MAS speed of 15 kHz applied for measuring the CP MAS and Cross-Polarization with Polarization Inversion (CPPI) MAS spectra. NMR measurements were done at room temperature. The Raman spectra of both 2,4-DTu and Pd (II) complex were measured in the range from 4000 cm^{-1} to 100 cm^{-1} on a RAM II (Bruker Optics) spectrometer with a resolution of 2 cm^{-1} and 25 scans by placing stirred crystals within aluminum disc. The ATR spectral measurements in the range from 4000 cm^{-1} to 600 cm^{-1} for both 2,4-DTu and Pd (II) complex were performed with PIKE MIRacle Single Reflection technology. The working frequencies of the Bruker Avance II NMR liquid state spectrometer were 600.130 MHz and 150.903 MHz for the made ^1H and ^{13}C NMR spectra measurements for 2,4-dithiouracil (2,4-DTu). The ^1H and ^{13}C NMR solution state spectra measured for the metal complex were obtained on a Bruker Avance III HD spectrometer operating correspondingly at 125.76 MHz and 500.130 MHz. Both NMR instruments were working with a standard Bruker software.

A portion of the dried complex was weighted on analytical balance and 4 mL aqua regia (37% HCl, pa, Merck, Darmstadt, Germany, 63% HNO_3 , trace metal analysis, Fisher chemical, Pittsburgh, Pennsylvania, USA) were added for dissolution. Sample was diluted to 10 mL with deionized water (18 $\text{M}\Omega\cdot\text{cm}$ PURELAB Chorus 2+ ELGA Veolia). Mass concentrations of sulfur and palladium were determined by Inductively Coupled Plasma – Optical Emission Spectrometry (ICP-OES iCap 6000, Thermo Scientific, Waltham, MA, USA) after applying appropriate dilution factor for the sample. Procedural blanks were prepared following the same steps. Pd (50 mg. L^{-1} , Romautoplast Bulgaria Inc) and S (1000 mg. L^{-1} , CPACChem, 6000 Bogomilovo, Bulgaria) were used for preparation of calibration solutions. Two wavelengths per analyte

were monitored in axial mode as it follows: S – 180.731 nm and 182.034 nm, Pd – 340.458 nm and 324.270 nm.

Microwave Plasma – Atomic Emission Spectrometry (Agilent technologies, Santa Clara, CA, USA) was used as an alternative method for Pd (wavelengths: 340.458 nm and 363.470 nm). Five

second measurement and 3 replicates were performed for all emission lines. The analytical data including yield percentage of the complex were recorded in Table 1. The results from the elemental analyses are presented in Table 2 by mean \pm confidence interval estimated with confidence level (P=95%) and number of replicates (n=3).

Table 1. Analytical and physical characteristics of the metal complex with 2,4-dithiouracil

Complex	Color	Yield, %	mp, °C	Solubility
Pd(II)L	orange-brown	41	>350	Soluble in DMSO and insoluble in H ₂ O, THF, C ₂ H ₅ OH, EtAc, and C ₆ H ₁₂ .

Table 2. Elemental analyses of complex

Metal complex	Composition*	Formula	Molecular weight	W(M)% calc./exp. ^a	W(M)% calc./exp. ^b	W(S)% calc./exp. ^a
Pd(II)L	2,4-DTu.2-Tu.Pd. DMSO.H ₂ O	C ₁₀ H ₁₄ N ₄ O ₃ S ₄ Pd	472.92 g.mol ⁻¹	22.5/23.5 \pm 6.1	22.5/23.2 \pm 6.0	27.1/26.7 \pm 7.6

a Measurement performed with inductively-coupled plasma optical emission spectrometry (ICP-OES).

b Measurement performed with microwave plasma atomic emission spectrometry (MP-AES).

As can be seen from Table 2, there is no statistically significant difference between the calculated and experimentally determined mass percentages of Pd and S.

Antimicrobial assay. The antimicrobial activity of 2,4-dithiouracil and its Pd (II) complex against various microorganisms was evaluated using the agar diffusion method. The tested microorganisms included Gram-positive bacteria such as *Enterococcus faecalis* ATCC 19433, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 8787, *Bacillus subtilis* ATCC 6633, and *Bacillus cereus* ATCC 11778, as well as Gram-negative bacteria including *Escherichia coli* ATCC 8739, *Salmonella enterica* subsp. *enterica* ser. *Enteritidis* ATCC 13076, *Pseudomonas aeruginosa* ATCC 9027, *Proteus vulgaris* G, *Klebsiella pneumoniae* ATCC 13883, and the yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae*. The procedure involved spreading a suspension of each test microorganism (10⁶ cfu.cm⁻³) onto specific nutrient agar media. Wells of 7 mm diameter were created in the agar, and 50 μ L of the tested substance solution (10 mg/cm³ in DMSO) was added to each well. The

Petri dishes were then incubated at appropriate temperatures (37°C for bacteria and *C. albicans*, and 30°C for *S. cerevisiae*) for 24-48 h. After incubation, the inhibition zones around each well were measured, with zones larger than 7 mm considered as zones of inhibition. Each test was performed in triplicate, and the results were reported as mean values of the inhibition zone diameters. This methodology allowed for the assessment of the antimicrobial effectiveness of both 2,4-dithiouracil and its Pd (II) complex against a range of pathogenic microorganisms.

Results and Discussion

Pyrimidine-2,4(1*H*,3*H*)-dithione will be referred in the article as 2,4-dithiouracil (2,4-DTu). The structure of 2,4-DTu has already been completely verified by ATR, Raman, 1D and 2D NMR in a previous work (Marinova et al. 2024b), where the complexation activity of 2,4-DTu was investigated for the synthesis of new Au (III) and Cu (II) complexes. The ¹H NMR spectrum of the Pd (II) complex showed more signals compared to the ¹H NMR spectrum of 2,4-DTu.

Table 3. ^1H NMR spectral data for the Pd(II) complex [500.13 MHz (^1H)]^a

Atom	2,4-DTu ^b	2-Tu ^b	2,4-DTu.2-TuPd ^c
1	12.88, s	12.26, s	13.46, m, (2-Tu)
2			
3	13.62, s	12.43, s	14.20, s, (2,4-DTu)
4			
5	6.50,dd (7.1 Hz, 1.5Hz)	5.81,d (8.24 Hz)	7.09, s, (2,4-DTu) 7.09, s, (2-Tu)
6	7.26,dd (6.9 Hz, 5.6 Hz)	7.40,dd (7.6Hz, 5.6 Hz)	7.55, s, (2,4-DTu) 7.84, s, (2-Tu)

a In DMSO- d_6 solution. All these assignments were in agreement with the HSQC and ^1H - ^1H COSY spectrum;

b The corresponding assignments concern the free uncoordinated ligands—2,4-dithiouracil (2,4-DTu) and 2-thiouracil (2-Tu).

c Spectral data suggest that 2,4-DTu and 2-Tu are ligands coordinated to Pd(II) in our complex.

There are six signals that can be found at 12.26 ppm, 12.43 ppm, 12.88 ppm, 13.46 ppm, 13.62 ppm and 14.20 ppm indicating the presence of NH groups (Table 3). Also, there are 7 signals at 5.81 ppm, 6.50 ppm, 7.09 ppm, 7.26 ppm, 7.40 ppm, 7.55 ppm and 7.84 ppm. A possible reason for observing a higher number of signals in the ^1H NMR spectrum of Pd (II) complex could be the desulfurization of 2,4-DTu under the alkaline conditions of the synthesis due to the use of NaOH (Marinova et al. 2024b; Novakov et al. 2005). As a result, the sulfur atom at $\text{C}^4=\text{S}$ was replaced with an oxygen atom producing 2-Tu in the reaction mixture. This phenomenon was previously described for the synthesis of Au (III) and Cu (II) complexes with 2,4-DTU used as a starting reagent (Marinova et al. 2024b). Based on our previous studies (Marinova et al. 2022; Marinova et al. 2024b), the ^1H signals at 5.81 ppm and 7.40 ppm were assigned correspondingly to the H-5 and H-6 protons of 2-Tu, while those at 6.50 ppm and 7.26 ppm were for the H-5 and H-6 protons of 2,4-DTu, respectively. Furthermore, the singlets at 12.26 ppm and 12.43 ppm were for NH-1 and NH-3 groups of 2-Tu and those at 12.88 ppm and at 13.62 ppm corresponded to NH-1 and NH-3 groups of 2,4-DTu. In addition, the HSQC spectrum showed the following weak CH correlations – (6.51 ppm - 116.89 ppm) and (7.27 ppm - 136.55 ppm) for 2,4-DTu, as well as (5.81 ppm - 105.04 ppm) and (7.41 ppm - 141.69 ppm) for 2-Tu. As it becomes clear all of the abovementioned assignments were for the free uncoordinated ligands.

The ^1H singlet at 7.09 ppm with an area of 2.33, as well as the most intensive HSQC correlation (7.09 ppm - 115.61 ppm) corresponded to the H-5 protons and C-5 carbons in 2,4-DTu and 2-Tu which are coordinated to Pd (II). The singlets at 7.55 ppm and 7.84 ppm were for the H-6 protons in the coordinated 2,4-DTu and 2-Tu, respectively, supported by the HSQC correlations (7.55 ppm - 137.42 ppm) and (7.84 ppm - 152.29 ppm). ^1H - ^1H COSY spectrum showed weak correlations between the H-5 and H-6 protons in the coordinated 2-Tu and 2,4-DTu – (7.09 ppm - 7.84 ppm) and (7.09 ppm - 7.55 ppm). The singlet at 14.20 ppm probably corresponded to the NH-3 proton in the coordinated 2,4-DTu while the multiplet at 13.46 ppm can be assigned to the NH-1 proton in the coordinated 2-Tu. The N1 atom in 2,4-DTu and N3-atom in 2-Tu are deprotonated and coordinated with Pd (II). Most probably under the alkaline conditions of the synthesis of the Pd (II) complex, the nitrogen atoms of 2,4-DTu and 2-Tu become deprotonated in the reaction mixture, as in this way the conversion of these ligands into some of their tautomeric forms during their complexation with Pd (II) was stimulated as described in a previous study (Marinova et al. 2024b). The ^1H singlet at 2.54 ppm and the HSQC correlation (2.54 ppm - 40.05 ppm) showed that DMSO- h_6 participates in the Pd (II) complex. The area of the signal at 2.54 ppm is 5.66 suggesting that there is only one molecule DMSO- h_6 in the coordination compound. Moreover, the ratio of the summed areas of the ^1H signals registered respectively for the protons in the

complex and DMSO- h_6 is 1.21 which indicates additionally that there cannot be more than one molecule DMSO- h_6 in the structure. There is also a signal at 41.26 ppm in the ^{13}C NMR CP-MAS solid state spectrum indicating the possible presence of DMSO- h_6 in the complex. The broad peak at 3.05 ppm in the ^1H NMR solid state spectrum possibly indicated the presence of DMSO- h_6 and H_2O in the Pd (II) complex. ^{13}C NMR CP-MAS solid state spectrum of the Pd (II) complex showed signals at 188.53 ppm and 173.89 ppm confirming the presence of $\text{C}^4=\text{S}$ and $\text{C}^2=\text{S}$ groups in the coordinated ligands. Also, there is a signal at 149.96 ppm for the C-6 carbon in the coordinated 2-Tu whereas the signal at 137.68 ppm correspond to C-

6 in the coordinated 2,4-DTu. The signal at 117.26 ppm was for the C-5 carbons in 2,4-DTu and 2-Tu.

Raman and ATR spectra of the Pd(II) complex showed bands at 1254 cm^{-1} and 1263 cm^{-1} , respectively, which correspond to $\nu(\text{C}=\text{S})$. Additionally, there is an ATR band at 3178 cm^{-1} for $\nu(\text{N-H})$ indicating the presence of NH groups in the complex. Furthermore, the ATR band at 3071 cm^{-1} indicates $\nu(\text{C-H})$ whereas the Raman and ATR bands at 1547 cm^{-1} and 1558 cm^{-1} were for $\nu(\text{C}=\text{C})$. ATR confirms the presence of water in the Pd(II) complex. The vibrational data for 2,4-DTu (Marinova et al. 2024b) and Pd (II) complex is presented in Table 4.

Table 4. Raman and ATR bands for 2,4-DTu and Pd (II) complex

2,4-DTu		Pd(II) complex	
Raman, cm^{-1}	ATR, cm^{-1}	Raman, cm^{-1}	ATR, cm^{-1}
3097($\nu(\text{C-H})$)	3168 ($\nu(\text{NH})$)	2904	3178($\nu(\text{NH})$)
3080 ($\nu(\text{C-H})$)	3096($\nu(\text{C-H})$)	1814	3071 ($\nu(\text{C-H})$)
3058	3080 ($\nu(\text{C-H})$)	1605	1605
1605	2994	1547 ($\nu(\text{C}=\text{C})$)	1558 ($\nu(\text{C}=\text{C})$)
1547 ($\nu(\text{C}=\text{C})$)	2923	1494	1496
1491	2894	1357	1473
1425	2720	1278	1438
1359	1921	1254 ($\nu(\text{C}=\text{S})$)	1357
1357	1695	1241	1282
1254 ($\nu(\text{C}=\text{S})$)	1673	1189	1263 ($\nu(\text{C}=\text{S})$)
1230	1610	1130	1221
1189	1565 ($\nu(\text{C}=\text{C})$)	1089	1184
1118	1486	984	1136
1076	1411	820	1083
984	1368	694	1015
965	1358	684	984
858	1319	615	949
683	1252 ($\nu(\text{C}=\text{S})$)	444	835
611	1230	353	803
461	1211	254	737
444	1123	198	715
399	1099		691
387	1076		604
229	984		
	965		
	858		
	820		
	792		
	695		
	680		
	614		

Similar structures of Pd(II) complex with two 2,4-DTu ligands which are bidentatly coordinated to the metal center with their deprotonated nitrogen and one of its adjacent sulfur atoms was previously proposed (Lusty et al. 1983). In our case, there are 2,4-DTu and 2-Tu bidentatly coordinated to Pd(II) by a deprotonated nitrogen and its adjacent heteroatom – S or O, respectively. The tentative structures of the Pd(II) complex are proposed on Fig. 4, where DMSO-h₆ and H₂O are present in the

inner or outer coordination sphere of the compound. Pd(II) complex with coordination number 6 was proposed by Marinova et.al., where the ligands bonded to Pd(II) were DMSO-h₆, H₂O, 6-methyl-2-thiouracil or 6-propyl-2-thiouracil (Marinova et al. 2023). 6-Methyl-2-thiouracil or 6-propyl-2-thiouracil were coordinated to Pd(II) through the sulfur and oxygen atoms and one of their adjacent nitrogen atoms from the NH groups.

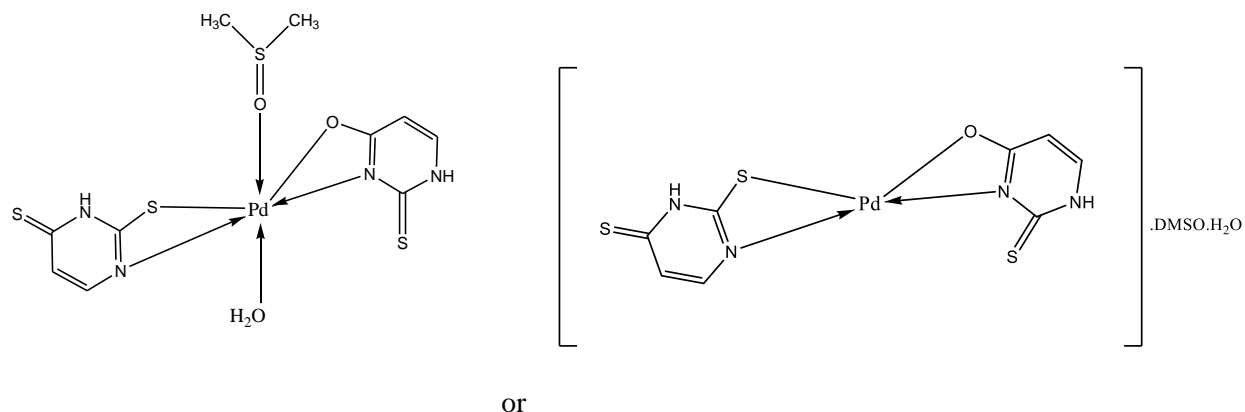


Figure 4. Tentative structures for Pd (II) complex

UV-Vis spectra showed a maximum at $\lambda_{max}=257$ nm for 2,4-DTu and at $\lambda_{max}=295$ nm for 2-Tu. Correspondingly, two maxima at $\lambda_{max}=257$ nm and $\lambda_{max}=292$ nm were found for the Pd(II) complex which indicate the presence of 2,4-DTu and 2-Tu in the compound. The results from the conducted antimicrobial assays during the investigation of the

biological activity of 2,4-dithiouracil and its Pd (II) complex are presented in Table 5. The complex of 2,4-dithiouracil with Pd(II) demonstrated the strongest activity against *Staphylococcus aureus*. In this case the increase in activity in comparison with the ligand was not significant.

Table 5. Antimicrobial activity of 2,4-dithiouracil and its Pd (II) complex

Test microorganisms	DMSO	Complexes	
		2,4-dithiouracil	Pd (II)L
Inhibition zone, mm			
<i>Staphylococcus aureus</i> ATCC 25923	-	14±1.00	15±0.58
<i>Eterococcus faecalis</i> ATCC 19433	-	12±1.00	12±0.58
<i>Listeria monocytogenes</i> ATCC 8787	-	12±1.00	11±1.15
<i>Bacillus subtilis</i> ATCC 6633	-	11±0.58	11±0.58
<i>Bacillus cereus</i> ATCC 11778	-	11±0.58	13±1.00
<i>Escherichia coli</i> ATCC 8739	-	14±0.58	12±0.58
<i>Salmonella enterica ssp. enterica ser. Enteritidis</i> ATCC 13076	-	14±0.00	10±1.53
<i>Pseudomonas aeruginosa</i> ATCC 9027	-	13±1.00	9±0.58
<i>Proteus vulgaris</i> G	-	12*±0.58	11±0.58
<i>Klebsiella pneumoniae</i> ATCC 13883	-	12*±0.58	-
<i>Candida albicans</i> ATCC 10231	-	10±0.58	14±1.00
<i>Saccharomyces cerevisiae</i>	-	11±1.00	13±0.58

* presence of single-cell colonies in the inhibition zone.

There was an increase in the antimicrobial effect of the complex against *Bacillus cereus*. The addition of Pd(II) caused a slight decrease in activity against *Listeria monocytogenes* and no change in the inhibition effect against the rest of the Gram-positive bacteria included in the test. The Pd(II) complex showed lower activity against the Gram-negative test-microorganisms in comparison with the ligand and even total loss of activity against *Klebsiella pneumoniae*. Similar results were reported for the Pd (II) complex with 6-methyl-2-thiouracil in our previous study (Marinova et al. 2023). On the other hand, the newly synthesized complex exhibited a significant improvement in its antifungal activity against *Saccharomyces cerevisiae* and *Candida albicans*. Given the scarcity of antifungal agents (Wu et al. 2023; Rapp 2004) and the increase in antifungal resistance (Fisher et al. 2022; Hossain et al. 2022), this result is a promising basis of future research. Improvement in the solubility of the compound would probably further increase its antimicrobial activity. The data from the antimicrobial assay is comparable with the results presented for the antimicrobial activity of the Au (III) and Cu (II) complexes described in our previous study (Marinova et al. 2024a).

Conclusions

Further investigation on the complexation activity of 2,4-DTu with Pd (II) was described. The coordination complex was synthesized following a previously reported methodology (Marinova et al. 2022, 2023, 2024a,b,c), with modifications to the reaction time (notably 24 h for the palladium(II) derivative) and/or employing alternative solvents (Lusty et al. 1981,1983). We used different solvents, pH, starting salt and reaction time for obtaining the new complex compared to Lusty et al. (1983). Once again the combination of solution-state 1D and 2D NMR was proven to be the key approach for the determination of the type and quantity of the ligands coordinated to Pd (II). Additional studies including solid state NMR, elemental analyses, ATR and Raman spectroscopies have supported the structure elucidation of the newly formed Pd (II) complex. The complex of 2,4-dithiouracil with Pd (II) demonstrated the strongest activity against *Staphylococcus aureus*. It, also, demonstrated a significant improvement in its

antifungal activity in comparison with 2,4-dithiouracil.

Author Contributions

Conceptualization - P.M. and P.P.; methodology - P.M., and D.B.; formal analysis - P.M., D.S., E.V., D.B., A.S., and Y.G.; investigation - P.M., E.V. and D.B.; resources - P.M.; data curation - P.M. and D.B.; writing - original draft preparation, P.M., D.S., D.B. and A.S.; review and editing, P.M., and D.B.; visualization, P.M. and D.B.; supervision - P.M.; project administration - P.P.; funding acquisition - P.P.

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Institutional Review Board Statement

The protocol carried out in this research did not involve the use of animals.

Informed Consent Statement

Not applicable.

Data Availability Statement

The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding authors.

Conflicts of Interest

The authors declare no conflicts of interest.

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