Synthesis, Characterization and Antibacterial Studies of New Cu(II) and Pd(II) Complexes with 6-Methyl-2- thiouracil and 6-Propyl-2-thiouracil

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Article

Synthesis, Characterization and Antibacterial Studies of New Cu(II) and Pd(II) Complexes with 6-Methyl-2-thiouracil and 6-Propyl-2-thiouracil

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Abstract: The primary objective of this study is to synthesize, elucidate the structural properties, and explore the biological activity of novel metal complexes derived from 6-methyl-2-thiouracil and 6-propyl-2-thiouracil. The paper outlines a synthesis method and assesses the antimicrobial activity of newly formed Cu(II) and Pd(II) complexes with two substituted 2-thiouracil ligands. The structural attributes of these newly developed compounds are thoroughly discussed based on data derived from melting point analysis, MP-AES for Cu and Pd, UV-Vis, IR, ATR, ^{1}H NMR, ^{13}C NMR and Raman spectroscopy. The interpretation of the complex spectra is assisted by the data for 6-methyl-2-thiouracil and 6-propyl-2-thiouracil obtained from ^{1}H-^{1}H COSY, DEPT-135, HMBC and HMQC spectra. In addition, the antimicrobial activity of these complexes and the free ligands are assessed against both Gram-positive and Gram-negative bacteria, as well as yeasts.

Keywords: 6-methyl-2-thiouracil; 6-propyl-2-thiouracil; complexation; copper(II) complexes; palladium(II) complexes; antimicrobial activity

1. Introduction

Pyrimidine derivatives are documented to possess a wide range of biological activity such as antineoplastic [1], antiviral [2], antimicrobial [3], free radical scavenging [4], anti-inflammatory [5], pain-relieving [6], and anxiety-reducing [7] properties. Thionamides, a class of relatively simple molecules, serve as antithyroid drugs, featuring a sulfhydryl group and a thiourea moiety within a heterocyclic framework. In the United States, the antithyroid drugs in use are Propylthiouracil (also known as 6-propyl-2-thiouracil) and Methimazole (referred to as 1-methyl-2-mercaptoimidazole or Tapazole). Various literature reviews have delved into this subject [8–10].

Notably, a study by Xiao-Ming Mao et al. explored the efficacy of intrathyroid injection of dexamethasone in reducing the relapse rate of hyperthyroidism in patients newly diagnosed with Graves' disease [11]. Furthermore, Propylthiouracil is currently regarded as the preferred treatment for hyperthyroidism during pregnancy [12]. A comprehensive review on the topic of hyperthyroidism and pregnancy was presented by M.G. Fernandez [13]. N.Z. Shaban et al. synthesized metal complexes involving pyrimidine, which encompass Cd and Zn-barbiturate, as well as Cd and Hg-thiouracil compounds [14]. In another study, new complexes of Cu(II), Ni(II), Co(II), and Pd(II) were reported [15]. These complexes featured a Schiff base known as {1-[(5-bromo-2-

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hydroxy-benzylidene)-amino]-4-phenyl-2-thioxo-1,2-dihydro-pyrimidin-5-yl}-phenyl-methanone [15].

The reaction of 5-bromouracil led to the preparation of novel complexes involving Mn(II), Cd(II), Co(II), Ni(II), Cu(II), and Ag(I) [16]. The obtained data demonstrated that these complexes exhibited greater antimicrobial potency compared to the free ligand.

The interest in platinum and palladium complexes stems from their pronounced cytostatic activity. Recently, *cis*-dihalogeno complexes of Pt(II) and Pd(II) were synthesized in conjunction with 6-tert-butyl-2-thiouracil [17]. Oladipo and Isola provided a comprehensive review on the coordination possibilities of uracil and the practical applications of some of its complexes [18]. Furthermore, a series of complexes was synthesized using Rh(III), Ir(III), Pt(II), and Pd(II) in combination with the ligand 6-methyl-2-thiouracil [19].

K. Paizanos et al. successfully obtained new Cu(I) complexes featuring the antithyroid drug 6-propyl-thiouracil [20].

New complexes, including Cd(II) and mixed-ligand peroxo complexes of vanadium, were prepared using 1,3-propanediamine in combination with 2-thiouracil and its 6-methyl derivative [21,22]. L. M. Bomfim et al. conducted a synthesis of Ru(II) complexes involving 6-methyl-2-thiouracil, showing promise as novel antileukemic drug candidates [23].

To date, a multitude of metal complexes have been synthesized using uracil and thiouracil derivatives, involving various metals such as Cu, Fe, Co, Ni, Zn, Mn, Cd, V [24–26], as well as Pd, Pt, and Au, with evaluations of their composition and structure [27]. New thiolate gold(I) complexes, featuring $P(NMe_2)_3$ (HMPT) as a phosphane group, were successfully synthesized [28], with two of these thiolate gold(I) complexes demonstrating effectiveness as potential candidates for chemotherapy.

In vitro screening of the antimicrobial activity of numerous metal complexes derived from thiouracil derivatives was conducted against Gram-positive and Gram-negative bacteria, filamentous fungi, and yeast [15,16,29–32].

Furthermore, the cytotoxic effects of various metal complexes of thiouracil derivatives were investigated against different tumor cell lines [23,28,33–37].

In Scheme 1 (top), the chemical structure of 6-methyl-2-thiouracil and 6-propyl-2-thiouracil was represented. Various metal complexes exhibit a monodentate coordination mode with 2-thiouracil derivatives, binding through different atoms, such as N1 (e.g., Cu(I) complex) [38], N3 (e.g., Pd(II) complex) [39], S (e.g., Ru(II), Cu(I), Sn(IV), Pt(II), Pd(II) complexes) [17,20,34,40], or O (e.g., Co(II), Ni(II), Mn(II), Zn(II) complexes) [41–43].

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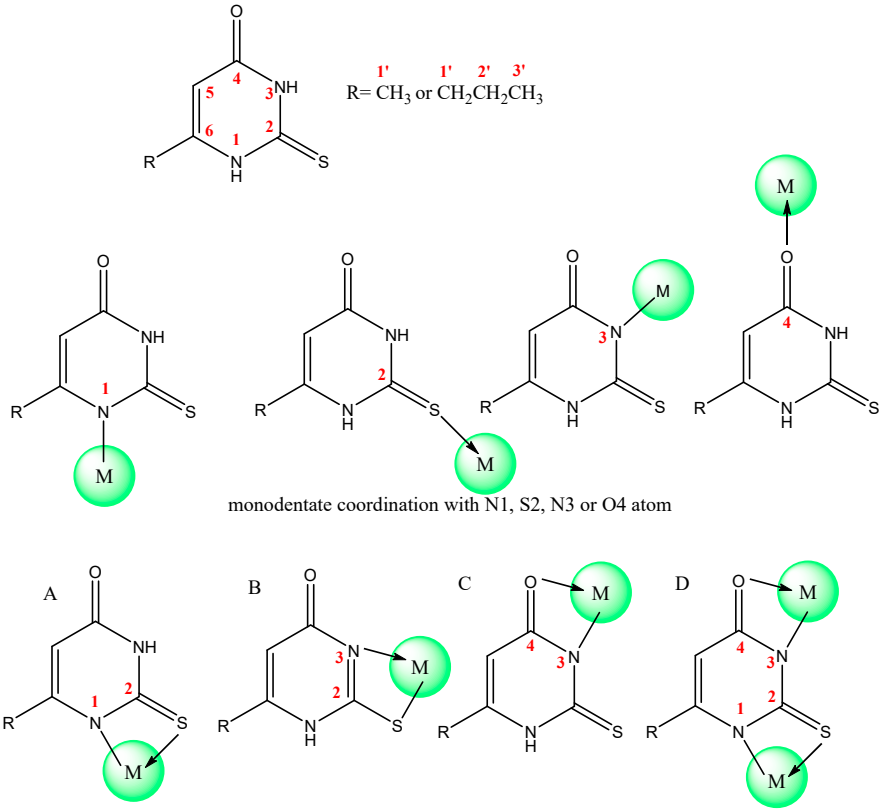

Scheme 1. (Top) Structure of 6-methyl-2-thiouracil and 6-propyl-2-thiouracil, as well as the atoms numbering and monodentate coordination. (Bottom) Representation of coordination binding sites for 6-methyl-2-thiouracil and 6-propyl-2-thiouracil ligands (bidentate chelate).

At the bottom of Scheme 1, the bidentate coordination modes of these ligands are highlighted. There are at least four potential bidentate coordination possibilities (A-D) for both ligands. Some of these modes were examined by Lusty and colleagues [19], who discussed coordination modes represented in (A) and (B) in the presence of platinum and rhodium centers, respectively. These were the most common coordination modes for this ligand class. Complexes with Pt(II), Pd(II), Ru(II) and Zn(II) also display coordination mode (A) [15,19,23,39,44], while coordination mode (B) was observed in Cd(II), Hg(II), Co(II) complexes and peroxo complexes of vanadilum [14,21,22,45]. To date, possibility (C) has not been observed for thiouracil derivatives, except in an osmium/uracil complex [46] and Mn(II), Co(II), Ni(II), Cu(II), Cd(II), Ag(I) with 5-bromo-uracil [16]. Coordination mode (D) was observed in a Ru(II) complex with 2,2′-bipyridine (bipy) [47], tin(IV) complexes [48] and Pt(II) with 5,6-diamino-4-hydroxy-2-mercaptopyrimidine [27]. Tridentate coordination mode with the participation of S2,N3,O4-atoms of the free ligands [14,15,24,31] was reported also.

This paper presents the synthesis of novel metal complexes involving 6-methyl-2-thiouracil and 6-propyl-2-thiouracil. The characterization of these compounds was conducted through various techniques, including melting point determination, UV-Vis, IR, ^{1}H NMR, ^{13}C NMR and Raman spectroscopy. The assignment of NMR signals of the ligands was obtained from ^{1}H-^{1}H COSY, DEPT-135, HMBC, and HMQC spectra. In this study we provide details regarding the synthesis of new Cu(II) and Pd(II) complexes with 6-methyl-2-thiouracil (L1) and 6-propyl-2-thiouracil (L2). Furthermore, the antimicrobial activity against both Gram-positive and Gram-negative bacteria, as well as yeasts, is evaluated.

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2. Materials and Methods

2.1. Spectra measurements

The free ligands 6-methyl-2-thiouracil and 6-propyl-2-thiouracil are purchased from Aldrich Chem. The metal salts $Cu(CH_3COO)_2.H_2O$ and $Pd(NO_3)_2.H_2O$ (Aldrich Chem) and solvents used for synthesis of the complexes were with a p. a. quality. Absorption spectra were registered on a UV-30 SCAN ONDA UV/Vis/NIR Spectrophotometer from 200 to 1000 nm. The IR spectra of L1, L2 and their complexes were registered in KBr pellet on a Bruker FT-IR VERTEX 70 Spectrometer from 4000 cm^{-1} to 400 cm^{-1} at resolution 2 cm^{-1} with 25 scans. The Raman spectra of compounds (the stirred crystals placed in aluminium disc) were measured on a RAM II (Bruker Optics) with a focused laser beam of of Nd:YAG laser (1064 nm) from 4000 to 100 cm^{-1} at resolution 2 cm^{-1} with 25 scans. Additionally, ATR spectra of the complexes were measured (MIRacle Single reflection, PIKE technology) to check if the coordination water was present in them. The NMR spectra of the ligand were registered on a Bruker Avance II NMR spectrometer operating at 600.130 and 150.903 MHz for ^{1}H and ^{13}C, respectively, using the standard Bruker software. The NMR spectra of the metal complexes were measured on a Bruker Avance III HD spectrometer operating at 500.130 and 125.76 MHz for ^{1}H and ^{13}C, respectively, using the standard Bruker software. Measurements were carried out at ambient temperature.

2.2. Microwave Plasma – Atomic emission spectrometry (MP-AES) determination of Cu and Pd in the complexes

0.0200 g of sample were weighted on analytical balance and dissolved with 65% nitric acid, p.a. (Chem-Lab NV) for Cu-complexes and nitric and 37% hydrochloric acid, p.a. (Fluka AG) for Pd-complexes. Blank solutions were prepared as well. After appropriate dilution, concentration of Cu and Pd was determined by MP-AES 4200 (Agilent technologies). Calibration standards were prepared from monoelemental standard solutions – 1000 mg L^{-1} Cu (Merck, Darmstadt, Germany) and 1000 mg L^{-1} Pd (High-purity standards, Charlestone, England). Conventional MP-AES operating conditions were used. Analytes were measured on three emission lines for estimation of potential spectral interferences, i.e. 324.754 nm, 327.395 nm, 510.554 nm for Cu and 340.458 nm, 360.955 nm, 363.470 nm for Pd. Five replicates and 5 seconds measurement were applied for all lines.

2.3. Synthesis of Cu(II) and Pd(II) complexes of 6-methyl-2-thiouracil (L1) and 6-propyl-2-thiouracil (L2)

The added reagents, M, L, OH^-, were in 1:4:2 molar ratio.

2.3.1. Synthesis of Cu(II)L1

0.0008 mol $Cu(CH_3COO)_2.H_2O$ (0.1597 g) in 10 mL H_2O;
0.0032 mol (0.4550 g) of 6-methyl-2-thiouracil (L1) in 10 mL DMSO;
0.0016 mol (0.0640 g) NaOH in 5 mL H_2O.

2.3.2. Synthesis of Pd(II)L1

0.002 mol (0.4609 g) $Pd(NO_3)_2.H_2O$ in 10 mL H_2O
0.008 mol (1.1374 g) of 6-methyl-2-thiouracil (L1) in 10 mL DMSO;
0.004 mol (0.1600 g) of NaOH in 5 mL H_2O.

2.3.3. Synthesis of Cu(II)L2

0.0008 mol $Cu(CH_3COO)_2.H_2O$ (0.1597 g) in 10 mL H_2O;
0.0032 mol (0.5447 g) of of 6-propyl-2-thiouracil (L2) in 10 mL DMSO;
0.0016 mol (0.0640 g) NaOH in 5 mL H_2O.

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2.3.4. Synthesis of Pd(II)L2

0.002 mol (0.4609 g) $Pd(NO_3)_2.H_2O$ in 10 mL H_2O

0.008 mol (1.1318 g) of 6-propyl-2-thiouracil (L2) in 10 mL DMSO;

0.004 mol (0.1600 g) of NaOH in 5 mL H_2O.

5 mL of NaOH was gradually introduced into a ligand solution under continuous stirring maintaining a pH of 8. The corresponding metal salts solution was dropwise from a burette while using an electromagnetic stirrer. Precipitation of the resulting complexes commenced after 2 hours and 24 hours, manifesting as a yellow-green and brown solid for the copper(II) complexes and palladium(II) complexes, respectively. The precipitates were subsequently separated by filtration, rinsed with 10-20 cm^3 of H_2O, and then dried over $CaCl_2$ for a period of 2 weeks.

2.4. Spectral data of the free ligands and their metal complexes

UV-Vis (DMSO) of L1: λ_{max}= 258, 294 nm

IR (cm^{-1}) of L1: 3115 (NH), 3080 (NH), 3014 (=CH), 2932 (CH_3), 2890 (CH_3), 2580, 2407, 1920, 1893, 1863, 1754, 1698, 1676 (C=O), 1637, 1560, 1423, 1384, 1349, 1242 (C=S), 1200, 1194, 1167, 1043, 1032, 993, 962, 933, 874, 838, 808, 729, 656, 598, 580, 553, 548, 513, 457, 416.

IR (cm^{-1}) of Cu(II)L1: 3115 (NH), 3080 (NH), 3003 (=CH), 2931 (CH_3), 2888 (CH_3), 1753, 1637 (C=O), 1578, 1559, 1426, 1385, 1350, 1284, 1241(C=S), 1207, 1199, 1167, 1033, 962, 933, 906, 872, 837, 809, 755, 656, 621, 597, 553, 512, 457.

ATR (cm^{-1}) of Cu(II)L1: 3111 (NH), 3088 (NH), 3001 (=CH), 2934 (CH_3), 2882 (CH_3), 1752, 1699, 1633 (C=O), 1575, 1541, 1424, 1386, 1349, 1283, 1241 (C=S), 1207, 1195, 1164, 1032, 962, 932, 908, 872, 834, 806, 755, 656, 628, 621.

Raman (cm^{-1}) of L1: 3085 (NH), 2921, 1635 (C=O), 1558, 1419, 1382, 1353, 1245 (C=S), 1199, 1177, 1043, 985, 961, 931, 834, 789, 657, 597, 554, 512, 458, 258, 214.

Raman (cm^{-1}) of Cu(II)L1: 3084 (NH), 2916, 1636 (C=O), 1578, 1549, 1418, 1382, 1281, 1244 (C=S), 1207, 1170, 1043, 986, 961, 650, 622, 596, 573, 553, 512, 473, 456, 257, 218.

UV-Vis (DMSO) of Pd(II)L1: λ_{max}= 258, 318 nm

IR (cm^{-1}) of PdL1: 3442 (H_2O), 3111 (NH), 3071 (NH), 3052 (=CH), 2993, 2930 (CH_3), 2892, 2855 (CH_3), 2750, 2697, 1678 (C=O), 1559, 1521, 1466, 1419, 1400, 1366, 1352, 1284, 1244 (C=S), 1193, 1168, 1064, 954, 830, 656, 614, 598, 576, 553, 513, 459.

ATR (cm^{-1}) of Pd(II)L1: 3400 (H_2O), 3105 (NH), 3080 (NH), 3049 (=CH), 2889, 2747, 2694, 1673 (C=O), 1636, 1559, 1515, 1465, 1417, 1399, 1365, 1351, 1285, 1242 (C=S), 1232, 1192, 1166, 1064, 953, 872, 826, 655, 613.

IR (cm^{-1}) of L2: 3112 (NH), 3093 (NH), 3042 (=CH), 2958 (CH_3), 2931 (CH_2), 2873, 2607, 1877, 1777, 1703, 1656 (C=O), 1628, 1557, 1445, 1393, 1336, 1314, 1281, 1243 (C=S), 1193, 1165, 1100, 1039, 1014, 965, 940, 888, 821, 790, 743, 641, 558, 538, 508, 465, 422, 416.

Raman (cm^{-1}) of L2: 3110 (NH), 2929 (CH_2), 2871, 1661 (C=O), 1630, 1548, 1433, 1337, 1243 (C=S), 1184, 1164, 1099, 1039, 1015, 970, 938, 643, 562, 534, 459, 352, 322, 257, 230.

ATR (cm^{-1}) of L2: 3088 (NH), 3038 (=CH), 2957 (CH_3), 2928 (CH_2), 2872, 1702, 1653 (C=O), 1627, 1554, 1445, 1392, 1336, 1313, 1281, 1242 (C=S), 1191, 1164, 1100, 1039, 1014, 965, 940, 887, 816, 787, 743, 640.

IR (cm^{-1}) of Cu(II)L2: 3451 (OH), 3093 (NH), 3042 (=CH), 2962 (CH_3), 2914 (CH_2), 2873, 1694, 1651 (C=O), 1553, 1497, 1453,1403, 1381, 1346, 1313, 1275, 1232 (C=S), 1210, 1191, 1166, 1008, 1021, 954, 876, 832, 754, 741, 703, 669, 658, 589, 571, 559, 549, 528, 468, 414, 403.

Raman (cm^{-1}) of Cu(II)L2: 3000, 2967, 2914, 2876, 1685, 1660 (C=O), 1631, 1595, 1500, 1438, 1416, 1381, 1276, 1230 (sh., C=S) 1212, 1165, 1089, 1021, 975, 878, 704, 670, 593, 581, 563, 531, 473, 438, 337, 307, 247, 219.

ATR (cm^{-1}) of Cu(II)L2: 3416 (OH), 3093 (NH), 3037 (=CH), 2959 (CH_3), 2913 (CH_2), 2871, 1692, 1642 (C=O), 1551, 1494, 1452, 1401, 1381, 1345, 1310, 1275, 1231 (C=S), 1210, 1190, 1165, 1105, 1015, 966, 953, 875, 831, 787, 754, 738, 703, 657, 644.

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IR (cm^{-1}) of PdL2: 3437 (H_2O), 3200, 3158, 3117 (NH), 3080 (NH), 2962 (CH_3), 2872, 1657 (C=O), 1595, 1545, 1467, 1428, 1379, 1338, 1320, 1261 (C=S), 1202, 1175, 1091, 1023, 972, 916, 882, 832, 789, 748, 697, 643, 606, 548, 468.

ATR (cm^{-1}) of PdL2: 3402 (H_2O), 3081 (NH), 2944 (CH_3), 2917 (CH_2), 2867, 2829, 1703, 1643 (C=O), 1616, 1564, 1516, 1445, 1425, 1386, 1329, 1286, 1223 (C=S), 1184, 1169, 1100, 997, 965, 908, 887, 860, 819, 787, 763, 745, 680, 643, 607.

Raman spectra of PdL1 and PdL2 cannot be measured; the samples burned at 1 mW.

2.5. Antimicrobial assay

Antimicrobial activity of 6-methyl-2-thiouracil, 6-propyl-2-thiouracil and their complexes against Gram-positive bacteria—*Enterococcus faecalis* ATCC 19433, *Staphylococcus aureus* ATCC 25923, *Listeria monocytogenes* ATCC 8787, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, and Gram-negative bacteria—*Escherichia coli* ATCC 8739, *Salmonella enterica* subsp. *enterica* ser. Enetritidis ATCC 13076, *Pseudomonas aeruginosa* ATCC 9027, *Proteus vulgaris* G, *Klebsiella pneumoniae* ATCC 13883, and the yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae* was tested using the agar diffusion method. A suspension of each test microorganism (10^6 cfu/cm^3) was spread on the surface of PCA (Scharlau) nutrient medium for *C. albicans* and the bacteria and Wort agar (Sharlau) for *S. cerevisiae*. Wells of 7 mm diameter were made in the inoculated agar medium. 50 µL of the tested substance solution (10 mg/cm^3 in DMSO) was pipetted into the wells. The Petri dishes were incubated at 37°C (for the bacteria and *C. albicans*) and 30°C (for *S. cerevisiae*) for 24–48 h. The inhibition zones were measured. Zones with diameter more than 7 mm were considered as zones of inhibition. Each test was carried out in triplicates, and the accumulated data are presented as mean values.

3. Results and discussion

3.1. Synthesis of the metal complexes

The interaction of metal ions with L1 and L2 in molar ratio of metal:ligand:base (1:4:2) resulted in the formation of the complexes with suggested formulas given in **Table 1**. The results of elemental analysis for the metal ions were determined by Microwave Plasma – Atomic emission spectrometry. They allow to suppose the tentative average composition of different complexes.

Table 1. Elemental analyses data for the metal ions of the complexes.

metal complex	composition*	formula	Molecular weight	W(M)% calc./exp.
Cu(II)L1	[3LCu.(DMSO)]	$C_{17}H_{24}N_6O_4S_4Cu$	M=568.22 g/mol	11.2 / 11.6±0.6
Pd(II)L1	[5LPd.(DMSO)].H_2O	$C_{27}H_{38}N_{10}O_7S_6Pd$	M=913.46 g/mol	11.6 / 11.1±0.6
Cu(II)L2	[LCu.H_2O.$(OH^-)_2$.$(DMSO)_2$]	$C_{11}H_{26}N_2O_6S_3Cu$	M=442.07 g/mol	14.4 / 14.3±0.7
Pd(II)L2	[4LPd.$(DMSO)_2$].H_2O	$C_{32}H_{54}N_8O_7S_6Pd$	M=961.63 g/mol	11.1 / 11.5±0.5

*tentative average composition of different complexes.

All of the complexes are stable in air and moisture and their solubility is limited. We found that the reaction of L1 and L2 with the transition metal ions afforded a 40-72 % yield of a stable solid compound. The obtained complexes have a yellow-green or brown colour and are limited solubility in DMSO and DMF, except Cu(II)L2 (soluble in DMSO only) and insoluble in water, THF, C_2H_5OH, EtOAc, and cyclohexane. The analytical data including the yield percentage of the complexes are presented in **Table 2**.

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Table 2. Analytical and physical characteristic of metal complexes with 6-methyl-2-thiouracil.

complexes	Colour	Yield (%)	Melting point (°C)	Solubility
L1	colorless		330	soluble in DMSO
Cu(II)L1	yellow-green	61	>350 °C	soluble in DMSO, DMF, C_2H_5OH, H_2O and insoluble in THF, EtOAc and C_6H_{12}.
Pd(II)L1	brown	72	>350 °C	soluble in DMSO, DMF and insoluble in H_2O, THF, C_2H_5OH, EtOAc and C_6H_{12}.
L2	colorless		218-220	soluble in DMSO
Cu(II)L2	yellow-green	43	260-263 °C	soluble in DMSO and insoluble in H_2O, THF, C_2H_5OH, EtOAc and C_6H_{12}.
Pd(II)L2	brown	70	255-257 °C	soluble in DMSO, DMF and insoluble in H_2O, THF, C_2H_5OH, EtOAc and C_6H_{12}.

IR Verification of the structures of the metal complexes can be easily achieved by comparing the IR spectra of the free ligands with that of their metal complexes. The selected experimental data from the IR spectra of the complexes Cu(II)L1 and Pd(II)L1 and of the free ligand, cm^{-1} is given in **Table 3**.

Table 3. Selected experimental IR data (in KBr, wavenumber in cm^{-1}) for 6-methyl-2-thiouracil and its complexes.

assignment	L1	Cu(II)L1	Pd(II)L1
ν(OH)	-	-	3442
ν(NH)	3115 sh	3115	3111
ν(NH)	3080	3080	3071
ν(=CH)	3014	3003	3052
ν(C=O)	1676 m	1637	1678
	1560 w	1559	1559
ν(C=S)	1242	1242	1244
	1167 s	1167	1168

In the IR spectra of the free ligand L1 the bands at 3115 cm^{-1} and 3080 cm^{-1} are observed which may refer to the stretching vibrations of N-H groups. In the spectrum of the Cu(II) complex the same bands are observed at the same frequencies. In the IR spectrum of the Pd(II)L1 these bands are shifted to the lower frequencies as compared to the free ligand bands with 4 cm^{-1} and 9 cm^{-1}, respectively. This fact shows that the two NH groups of the ligand participate in the coordination for palladium complex. The L1 IR bands at 1676 and 1242 cm^{-1} can be attributed to the stretching vibration of C^4=O and C^2=S groups, respectively. The stretching vibration of C^4=O in CuL1 complex is shifted to the lower frequency with 39 cm^{-1} as compared to this stretching in the free ligand. The same bands in IR spectrum of the PdL1 complex are not changed. In the ATR spectrum of PdL1 complex the band at 3400 cm^{-1} may refer to the stretching vibrations of molecular H_2O. This band missed for CuL1 complex.

In the IR spectrum of the free ligand L2 the bands at 3112 cm^{-1} and 3093 cm^{-1} are observed which may refer to the stretching vibrations of N-H groups. In the spectrum of the copper complex the same bands are observed at the same frequencies. In the IR spectrum of the PdL2 complex these bands are shifted; the first with +5 cm^{-1} and the second with -13 cm^{-1} as compared to those in the free ligand spectrum. This fact shows that the two NH groups of the ligand participate in the coordination for PdL2. In the IR spectrum of L2 the bands at 1656 and 1243 cm^{-1} can be attributed to the stretching vibrations of C^4=O and C^2=S groups, respectively. The band for C^2=S group is shifted to the lower frequencies as compared to the free ligand spectrum with 11 cm^{-1} and to the higher frequencies with 18 cm^{-1} for the CuL2 and PdL2 complexes, respectively. The band at 3451 and 3437 cm^{-1} in the IR spectrum of the two complexes may refer to the stretching vibrations of OH^- (CuL2) and molecular H_2O (PdL2), **Table 4**. The solid state ATR spectra confirm these findings.

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Table 4. Selected experimental IR data (in KBr, wavenumber in cm^{-1}) for 6-propyl-2-thiouracil and its complexes.

assignment	L2	Cu(II)L2	Pd(II)L2
ν(OH)	-	3451	3437
ν(NH)	3112	-	3117
ν(NH)	3093	3093	3080
ν(=CH)	3042	3042	
ν(C=O)	1656	1651	1657
	1557	1553	1545
ν(C=S)	1243	1232	1261
	1165	1166	1175

The stretching vibrations of C^4=O and C^2=S appear at 1635 and 1245 cm^{-1} in the Raman spectrum of 6-metyl-2-thiouracil and 1661 and 1243 cm^{-1} in that for 6-propyl-2-thiouracil, respectively. In the Raman spectrum of the CuL2 the band for C=S group is shifted with 13 cm^{-1} to the lower frequency.

The ^{1}H NMR spectrum of 6-metyl-2-thiouracil (L1) shows four signals; a singlets at 12.29 ppm is for H-1 and H-3 (overlap resonances). The olefin proton appears at 5.68 ppm (H-5), and 2.06 ppm is for H-1′. These assignments are confirmed by ^{1}H-^{1}H COSY, ^{1}H-broadband decoupled ^{13}C-NMR, DEPT-135 and HMBC spectra and are given in **Table 5**.

Table 5. ^{1}H and ^{13}C NMR spectral data and ^{1}H-^{1}H COSY and HMBC correlations for **6-methyl-2-thiouracil** [600.13 MHz (^{1}H) and 150.903 MHz (^{13}C)][a].

Atom	δ (^{13}C) ppm	DEPT-135	δ (^{1}H) ppm	Multiplicity (*J*, Hz)	^{1}H-^{1}H COSY	HMBC
1 (NH)			12.29	s		
2 (C=S)	175.87	C				
3 (NH)			12.29	s		
4 (C=O)	161.06	C				
5	103.72	CH	5.68	d (0.9)	7	4[b], 6, 7
6	153.20	C				
1′	18.11	CH_3	2.06	d (0.7)	5	5, 6

a) In DMSO-*d*6 solution. All these assignments were in agreement with COSY, HMQC and HMBC spectra. b) These correlations are weak.

The ^{1}H NMR and ^{13}C NMR spectral data of the Cu(II) and Pd(II) complexes are presented in **Tables 6** and **7**, respectively.

Table 6. ^{1}H NMR spectral data (in ppm) for complexes of 6-methyl-2-thiouracil with Cu(II) and Pd(II).

Atom	L1 (6-methyl-2-thiouracil)	Cu(II)L1 Multiplicity (*J*, Hz)	Pd(II)L1 Multiplicity (*J*, Hz)
1 (NH)	12.29 s	12.24 s	12.24 s and 10.80
2 (C=S)	–	–	–
3 (NH)	12.29 s	12.29 s	12.30 s and 10.86
4 (C=O)	–	–	–
5	5.68	5.68 s	5.68 s and 5.31
6	-	-	-
1′	2.06	2.07	2.07 and 2.01

DMSO-$_{H6}$ - 2.54 s 2.54 s.

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Table 7. ^{13}C NMR spectral data (in ppm) for complexes of 6-methyl-2-thiouracil with Cu(II) and Pd(II).

Atom	δ (^{13}C) ppm, L1	Cu(II)L1	Pd(II)L1
1 (NH)	–	–	–
2 (C=S)	175.87	175.86	175.86 / ?
3 (NH)	–	–	–
4 (C=O)	161.06	161.01	161.01 / ?
5	103.72	103.69	103.69 and 98.71
6	153.20	153.12	153.12 / ?
1′	18.11	18.06	18.06 and 18.20

The ^{1}H NMR and ^{13}C NMR spectra in DMSO-d6 solution of CuL1 show the same values of chemical shift to those of L1 but the solid-state NMR differentiates between them, see **Tables 6, 7, 11** and **Figure 1**. The ^{13}C NMR spectrum of the L1 registered with crosspolarisation (CP) experiment shows five signals. Two of them, at 174.6 and 163.0 ppm, are for the C=S and C=O, respectively.

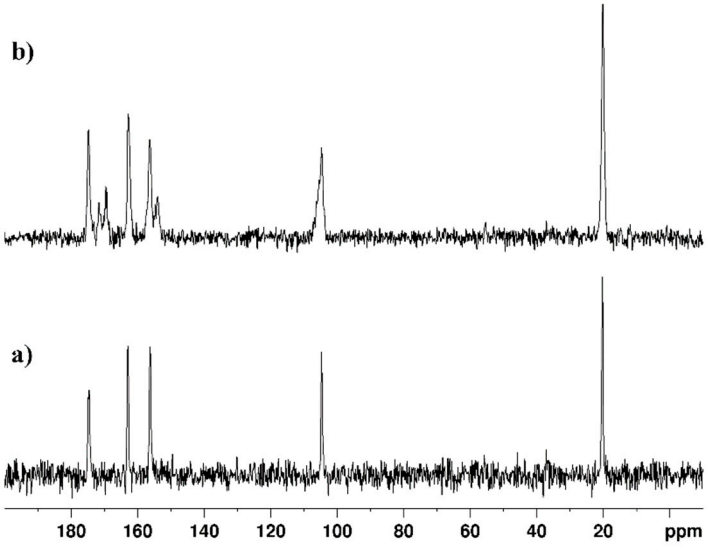

Figure 1. Solid-state NMR of the ligand L1 (a) and its complex with copper (b).

In the ^{13}C NMR solid state spectrum of CuL1 the two couples at 171.7 / 174.8 ppm and 162.9 / 169.5 ppm are for C=S and C=O groups, respectively. This means that the resonance for C=S is upfield shifted with 2.9 ppm and the signal for C=O is downfield shifted with 6.5 ppm, respectively. This fact shows that the C=S and C=O groups of the ligand participate in the coordination with the copper. The two couples at 104.7 / 105.4 ppm and 154.0 / 156.4 ppm, Table 11, are for the carbon atom in 5 and 6 position, respectively.

The ^{1}H NMR solution spectrum of PdL1 shows that the signals for the two amine protons (H-1 and H-3) are upfield shifted with 1.49 and 1.43 ppm. Also, all resonances of the free ligand are present in excess; this could be from the remaining reagent, L1, or from the decomposition of PdL1 in the solution. In the ^{13}C NMR solution spectrum of the PdL1 only methyl and olefin resonances of the coordinated ligand can be seen; the others are indistinguishable from the noise.

The ^{1}H NMR spectrum of 6-propyl-2-thiouracil (L2) shows six signals: two broad singlets at 12.20 ppm (H-1) and 12.31 ppm (H-3). The olefin proton appears at 5.67 ppm (H-5), and 2.32 ppm is for H-1′, 1.54 ppm for H-2′, 0.87 ppm for H-3′. These assignments are confirmed by ^{1}H-^{1}H COSY, ^{1}H-broadband decoupled ^{13}C-NMR and DEPT-135 spectra and are given in **Table 8**.

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Table 8. ^{1}H and ^{13}C NMR spectral data and ^{1}H-^{1}H COSY and HMBC correlations for **6-propyl-2-thiouracil** [500.13 MHz (^{1}H) and 150.903 MHz (^{13}C)][a].

Atom	δ (^{13}C) ppm	DEPT -135	δ (^{1}H) ppm	Multiplicity (*J*, Hz)	^{1}H-^{1}H COSY
1 (NH)	-	-	12.20	s	
2 (C=S)	176.08	C	-		
3 (NH)	-	-	12.31	s	
4 (C=O)	161.22	C	-		
5	103.06	CH	5.67	s	
6	156.74	C			
1′	33.21	CH_2	2.32	t(7.5)	2′
2′	20.58	CH_2	1.54	sx(7.4)	1′, 3′
3′	13.26	CH_3	0.87	t(7.4)	2′

a) In DMSO-*d*6 solution. All these assignments were in agreement with COSY spectra.

In the ^{1}H NMR and ^{13}C NMR solution spectra of CuL2 only the free ligand resonances are presents. In the solid state ^{13}C NMR spectrum there are resonances for both free and coordinated ligand. In the proton spectrum of CuL2 in DMSO-d6 appears a singlet at 2.54 ppm for DMSO-h6 that was coordinated to Cu(II) in the process of synthesis. The data about L2 and CuL2 are summarized and shown in **Tables 9–11** and **Figure 2. a,b,c**.

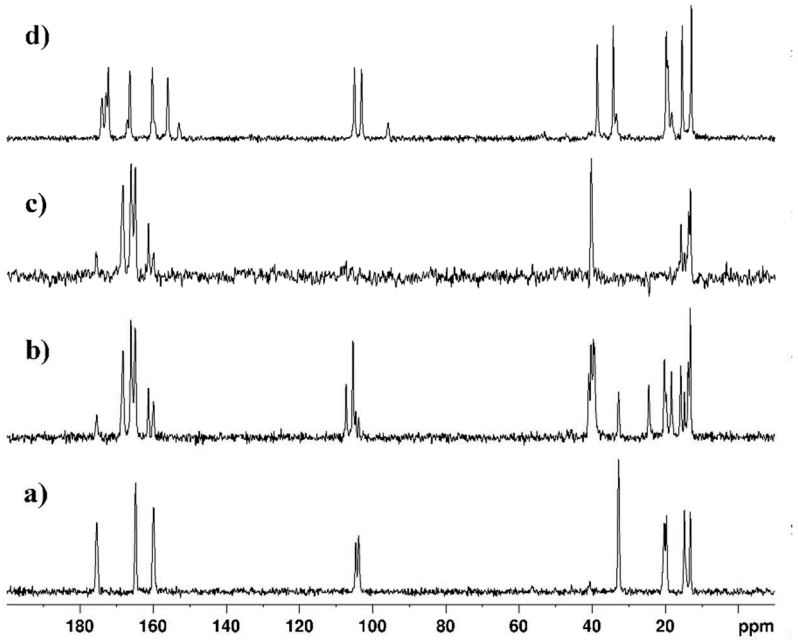

Figure 2. Solid-state NMR of the ligand L2 and its complexes: a) ^{13}C CP spectrum of L2; b) ^{13}C CP spectrum of Cu(II)L2; c) ^{13}C CPPI spectrum of Cu(II)L2; d) ^{13}C CP spectrum of Pd(II)L2.

Table 9. ^{1}H NMR spectral data (in ppm) for complexes of 6-propyl-2-thiouracil with Cu(II) and Pd(II).

Atom	L2 (6-propyl-2-thiouracil)	Cu(II)L2 Multiplicity (*J*, Hz)	Pd(II)L2 Multiplicity (*J*, Hz)
1 (NH)	12.20 s	12.20 s	12.20 and 10.77 s
2 (C=S)	-	-	-
3 (NH)	12.31 s	12.31 s	12.32 and 10.87 s
4 (C=O)	-	-	-
5	5.67 s	5.67 s	5.68 and 5.31 s and t(1.8)

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6	-	-	-
1′	2.32 t(7.5)	2.32 t(7.4)	2.32 and 2.25 t(7.3) and t(7.3)
2′	1.54 sx(7.4)	1.54 sx(7.5)	1.54 and 1.48 sx(7.6) and m
3′	0.87 t(7.4)	0.88 t(7.3)	0.87 and 0.82 t(7.3) and m
DMSO	-	2.54 s	2.54 s

In DMSO-*d*6 solution.

Table 10. ^{13}C NMR spectral data (in ppm) for complexes of 6-propyl-2-thiouracil with Cu(II) and Pd(II).

Atom	δ (^{13}C) ppm, L2	Cu(II)L2	Pd(II)L2
1 (NH)	-	-	-
2 (C=S)	176.08		176.02
3 (NH)	-	-	-
4 (C=O)	161.22		164.19 and 161.09
5	103.06		98.03
6	156.74		156.61 and 156.33 and 151.71
1′	33.21	33.24	33.56 and 33.14
2′	20.58	20.55	20.50 and 20.24
3′	13.26	13.25	13.20

In DMSO-*d*6 solution.

Table 11. ^{13}C NMR spectral data (in ppm) for L1 and L2 and some of the complexes aquired with crosspolarisation experiments, external reference α-glycine carbonyl C (176.03 ppm).

Atom	L1	L2	Cu(II)L1	Cu(II)L2	Pd(II)L2
1 (NH)					
2 (C=S)	174.6	175.5	171.7/174.8	168.3/175.4	172.3/172.9/174.0
3 (NH)					
4 (C=O)	163.0	164.8	162.9/169.5	164.9/166.1	160.3/166.4/167.0
5 (CH)	104.7	103.8/104.6	104.7/105.4	103.8/104.6/105.4/107.2	95.8/103.1/105.0
6 (C)	156.2	159.9	154.0/156.4	159.9/161.3	153.0/156.0/159.7
1′	20.2	32.7	20.1	32.7/39.3/39.6/40.9	33.4/34.2/38.6
2′		19.6/20.2		18.2/19.7/20.2/24.4	18.2/19.3/19.7
3′		13.1/14.7		13.1/13.6/14.7/15.7	12.9/15.4
DMSO				40.3	

The ^{13}C NMR spectrum of the L2 registered with crosspolarisation experiment shows seven signals. The two signals at 175.5 and 164.8 ppm are for the C=S and C=O, respectively. In the ^{13}C NMR spectrum with crosspolarisation experiment of CuL2 the signal for C=S is upfield shift of 7.2 ppm. This fact showed that the C=S group of the ligand participate in the coordination with the copper. Also, there appears a signal at 40.3 ppm that confirms the coordination of DMSO-h6 to Cu(II).

There are six couples of signals in ^{1}H NMR spectrum of Pd(II) complex with 6-propyl-2-thiouracil. In each couple one of the resonances is for the free ligand (the L2) other for the coordinated L2. The couples at 12.20 / 10.77 ppm and 12.32 / 10.87 ppm are for NH at first and third position. The couple at 5.68 / 5.31 ppm is for olefin proton 5. The three couples at 2.32 ppm (t,7.3) / 2.25 (t,7.3) ppm, 1.54 ppm (sx, 7.6) / 1.48 ppm (m) and 0.87 ppm (t, 7.3) / 0.82 ppm (m) are for propyl protons (H-1′), (H-2′) and (H -3′), respectively. There appears a singlet at 2.54 ppm that is for DMSO-h6. It is interesting to note that all resonances are shifted for coordinated L2 as compared to free L2 but the shifts are higher for NHs. In PdL2 the singlets for NH-1 and NH-3 are upfield shifted with 1.43 and 1.44 ppm, respectively.

The ^{13}C NMR solution spectrum of PdL2 shows seven groups of signals. The two signals with the highest chemical shift, at 176.02 ppm and 164.19 ppm, are for the C=S and C=O in PdL2,

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respectively. There is also a resonance at 161.09 ppm (free ligand), a.e. there is downfield shift of 2.97 ppm. This fact shows that the C=O group of the ligand participate in the coordination with Pd(II).

In the CP NMR spectrum of PdL2 the signal for C=S is upfield shifted with 3.2 and 2.6 ppm. In the same spectrum there are three signals for C=O, Table 11. One is upfield shifted with 4.5 ppm and the other downfield shifted with 2.2 ppm. This fact showed that the C=S and C=O groups of the ligand participate in the coordination with the palladium in two different ways.

The ATR spectra of CuL2 and PdL2 show the presence of H_2O and/or OH^-.

According to the solid-state NMR, clear shifts in the signals of the carbon atoms were observed in the complex of Cu(II) with L1, indicating the presence of the complex. There are also signals corresponding to the starting ligand L1, which may or may not be included in the second coordination sphere.

In the case of ligand L2 the situation is more complicated. The ligand itself shows doubled signals for the carbon atoms C-5, C-2′ and C-3′ possibly due to a different spacial arrangement of these atoms in the crystalline lattice of the ligand (Figure 2, a). In the case of the Cu(II)L2 complex, the ligand signals can still be observed, accompanied by the complex signals. What is different in this case is the presence of DMSO included in the complex as a ligand. Despite the presence of many C-1′ signals around it in the CP spectrum (Figure 2, b), the DMSO signal can be easily identified in the CPPI spectrum (Figure 2, c).

The ^{13}C spectrum of Pd(II)L2 obtained by cross-polarisation (CP) (Figure 2, d) presents other complications. There are multiple signals for each type of carbon atom in the ligand, indicating the presence of more than one palladium complex or a palladium complex with two ligands in the coordination sphere simultaneously.

3.2. Antimicrobial activity

Table 12 depicts the results from the antimicrobial assay of 6-methyl-2-thiouracil and its complexes.

Table 12. Antimicrobial activity of 6-methyl-2-thiouracil and its complexes.

Test microorganisms	Complexes		
	6-methyl-2-thiouracil	Cu(II)L1	Pd(II)L1
	Inhibition zone, mm		
Staphylococcus aureus ATCC 25923	-	8	-
Escherichia coli ATCC 8739	-	11*	10*
Eterococcus faecalis ATCC 19433	11	13	-
Salmonella enterica ssp. *enterica* ser. Enetritidis ATCC 13076	-	13	8
Pseudomonas aeruginosa ATCC 9027	9	12	9
Proteus vulgaris G	9*	11*	9*
Bacillus subtilis ATCC 6633	9*	9	10*
Bacillus cereus ATCC 11778	9*	8	9*
Listeria monocytogenes ATCC 8787	9*	11	8
Klebsiella pneumoniae ATCC 13883	9*	13*	11*
Candida albicans ATCC 10231	11	11	9/10*
Saccharomyces cerevisiae	-	9	-

well diameter – 7 mm. * Inhibition zone with single cell colonies.

The highest antimicrobial activity was exhibited by the Cu(II)L1 complex. It was active against all of the test-microorganisms. The Pd(II)L1 complex did not inhibit the growth of *S. aureus, E. faecalis* and *S. cerevisiae*. 6-methyl-2-thiouracil showed the smallest antimicrobial specter – it was not active against *S. aureus, E. coli, S. enterica* and *S. cerevisiae.* The addition of Cu(II) improved the antimicrobial activity of 6-methyl-2-thiouracil against all of the test-microorganisms, except *B. cereus*. Still, there

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were single cell colonies in the inhibition zones against *E. coli, P. vulgaris and K. pneumoniae.* This is indicative of different resistance in the microbial population. The addition of Pd(II) on the other hand, led to loss of activity against *E. faecalis,* and lower activity against *L. monocytogenes* and *C. albicans.*

The inhibition zones diameters for 6-propyl-2-thiouracil are presented in Table 13.

Table 13. Antimicrobial activity of 6propyl-2-thiouracil and its complexes.

Test microorganisms	Complexes		
	6-propyl-2-thiouracil	Cu(II)L2	Pd(II)L2
	Inhibition zone, mm		
Staphylococcus aureus ATCC 25923	-	8	11/16*
Escherichia coli ATCC 8739	-	10*	-
Eterococcus faecalis ATCC 19433	-	12	15
Salmonella enterica ssp. *enterica* ser. Enetritidis ATCC 13076	8	12	15
Pseudomonas aeruginosa ATCC 9027	8	12	14
Proteus vulgaris G	10	9*	-
Bacillus subtilis ATCC 6633	8	12*	12
Bacillus cereus ATCC 11778	10*	8	11/15*
Listeria monocytogenes ATCC 8787	8	9	14
Klebsiella pneumoniae ATCC 13883	11*	12*	12*
Candida albicans ATCC 10231	12	11	11
Saccharomyces cerevisiae	11*	9	8

well diameter – 7 mm. * Inhibition zone with single cell colonies.

It showed higher antimicrobial activity against *P. vulgaris* and both yeasts strains in comparison with the complexes with Cu(II) and Pd(II). The Cu(II) complex inhibited the growth of all test-microorganisms to a different degree – the best results were obtained against *E. faecalis, S. enterica* and *P. aueruginosa. P. vulgaris* was resistant to the tested concentration of the Pd(II) complex (unlike against 6-propyl-2-thiouracil), but all other test-microorganisms were more sensitive towards its action in comparison with 6-propyl-2-thiouracil. This was also true in comparison with the Cu(II) complex except for *E. coli, P. vulgaris,* and *S. cerevisiae.* Similarly to 6-methyl-2-thiouracil and its complexes there were single cells in some inhibition zones (e.g. 6-propyl-2-thiouracil against *B. cereus, K. pneumonia, and S. cerevisiae*). There were two distinct inhibition zones of the Pd(II) complex against *S. aureus* and *B. cereus* – a smaller clear zone and an additional larger zone with single cell colonies within. A more concentrated sample would probably inhibit completely the growth of these microorganisms and will significantly increase the antimicrobial activity of the complex.

5. Conclusions

The structure of new complexes was discussed by melting point, MP-AES for Cu and Pd, UV-Vis, IR, ATR, ^{1}H NMR, ^{13}C NMR and Raman spectroscopy. All measured spectra showed that a complexation occurred of Cu(II) / Pd(II) and 6-methyl-2-thiouracil and 6-propyl-2-thiouracil. Water and OH^-, as well as solvent (DMSO-h6) were present in some of the complexes. The suggested average composition of the obtained complexes was calculated from the results of determination of metal ions by Microwave Plasma-Atomic emission spectrometry together with analysis of ^{1}H-NMR spectra. The antimicrobial activity of the ligand and its complexes against Gram-positive and Gram-negative bacteria and yeasts was investigated. In general, addition of metal ions improved the antimicrobial activity of both 6-methyl-2-thiouracil and 6-propyl-2-thiouracil. The complex of Cu(II) with 6-methyl-2-thiouracil and Pd(II) with 6-propyl-2-thiouracil demonstrated the highest activity against the test microorganisms.

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N. B, P.P.; writing—review and editing, P.M.; P.P.; N.B; supervision, P.M.; project administration, S.T.; funding acquisition, P.P.

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