

Communication

Synthesis and Antibacterial Studies of a New Au(III) Complex with 6-Methyl-2-Thioxo-2,3-Dihydropyrimidin-4(1H)-One

Petya Marinova ^{1,*}, Nikola Burdzhiev ², Denica Blazheva ³ and Aleksandar Slavchev ³

¹ Department of General and Inorganic Chemistry with Methodology of Chemistry Education, Faculty of Chemistry, University of Plovdiv, "Tzar Assen" Str. 24, 4000 Plovdiv, Bulgaria

² Department of Organic Chemistry and Pharmacognosy, Faculty of Chemistry and Pharmacy, University of Sofia, 1 J. Bourchier Av., 1164 Sofia, Bulgaria; nburdzhiev@chem.uni-sofia.bg

³ Department of Microbiology, University of Food Technologies, 26 Maritza Blvd., 4002 Plovdiv, Bulgaria; d_blazheva@uft-plovdiv.bg (D.B.); a_slavchev@uft-plovdiv.bg (A.S.)

* Correspondence: marinova@uni-plovdiv.bg

Abstract: This article describes the synthesis of a new metal complex using 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one. The compound was analyzed using several methods, including determining its melting point and employing UV-Vis, IR, ATR, ¹H NMR, HSQC, and Raman spectroscopy for the free ligand. The metal complex was formed by combining 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. The NMR signals of the ligand were assigned using ¹H-¹H COSY, DEPT-135, HMBC, and HMQC spectra. Furthermore, the compound's antimicrobial activity against Gram-positive and Gram-negative bacteria, as well as yeasts, was assessed.

Keywords: antimicrobial activity; gold(III) complexes; 6-methyl-2-thiouracil (6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one)



Citation: Marinova, P.; Burdzhiev, N.; Blazheva, D.; Slavchev, A. Synthesis and Antibacterial Studies of a New Au(III) Complex with 6-Methyl-2-Thioxo-2,3-Dihydropyrimidin-4(1H)-One. *Molbank* **2024**, *2024*, M1827. <https://doi.org/10.3390/M1827>

Academic Editor: Stefano D'Errico

Received: 2 May 2024

Revised: 13 May 2024

Accepted: 15 May 2024

Published: 24 May 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Uracil, a fundamental pyrimidine derivative inherent in nucleic acids [1], assumes pivotal roles owing to its presence as a pyrimidine base with binding sites at the N1, N3, O2, and O4 atoms. Its significance and functionality extend to the enzyme structure and various medicinal applications. Notably, since 1942, 2-thiouracil has found utility in inhibiting thyroid hormone biosynthesis and treating Graves' disease [2], whereas methylthiouracil serves as a recognized antithyroid drug.

In exploring the chemical versatility and practical applications of uracil derivatives, previous studies have provided valuable insights. Oladipo and Isola conducted an extensive review on uracil's coordination possibilities and highlighted practical applications of some of its complexes [3]. Similarly, Masoud et al. delved into the complexing properties and biological activities of nucleic acids [4]. Meanwhile, recent contributions by Marinova and Tamahkyarova shed light on the synthesis and biological activities of metal complexes involving 2-thiouracil and its derivatives [5]. Furthering the exploration of uracil derivatives, Bomfim et al. synthesized Ru(II) complexes with 6-methyl-2-thiouracil as potential antileukemic agents [6]. The biological activities of 2-thiouracil and its metal complexes vary widely, with some being utilized as agents to combat tuberculosis and arthritis, while others exhibit bactericidal and fungicidal properties [3–5,7,8].

Recent advancements have also uncovered novel Cu(II) complexes of 6-methyl-2-thiouracil, highlighting the compound's versatility in metal ion coordination through its oxygen, sulfur, and nitrogen atoms [9]. The possible means of coordination of metal ions with 6-methyl-2-thiouracil are given in Figures 1 and 2. Different variants of chelating or bridge coordination are presented in Figures 3 and 4.

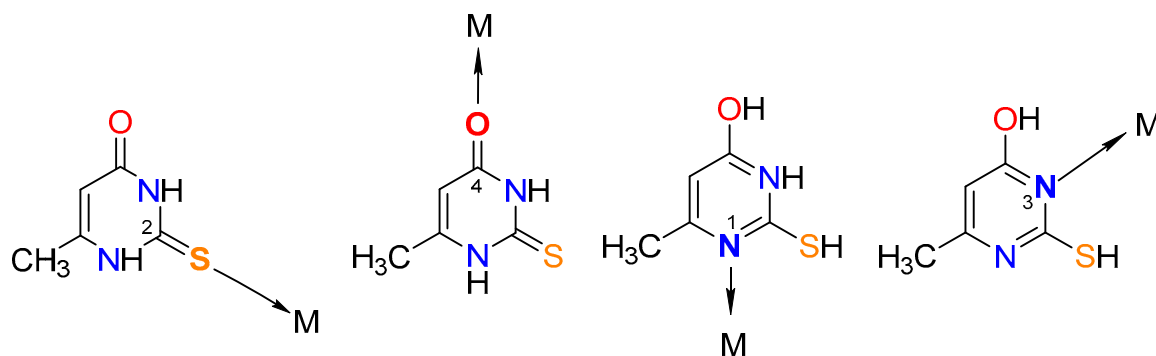


Figure 1. Monodentate coordination of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one with one of the four donor atoms, without any prior deprotonation (the tautomeric form participates in N1- and N3-coordination).

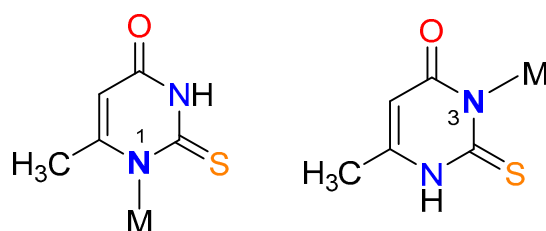


Figure 2. Monodentate coordination with deprotonated ligand who participate as a monoanionic (through a deprotonated nitrogen atom in the first position N1 or a nitrogen atom in the third position N3).

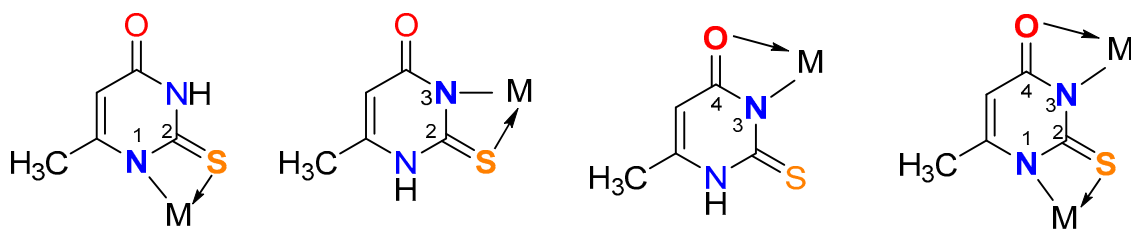


Figure 3. Bidentate coordination with formed chelate.

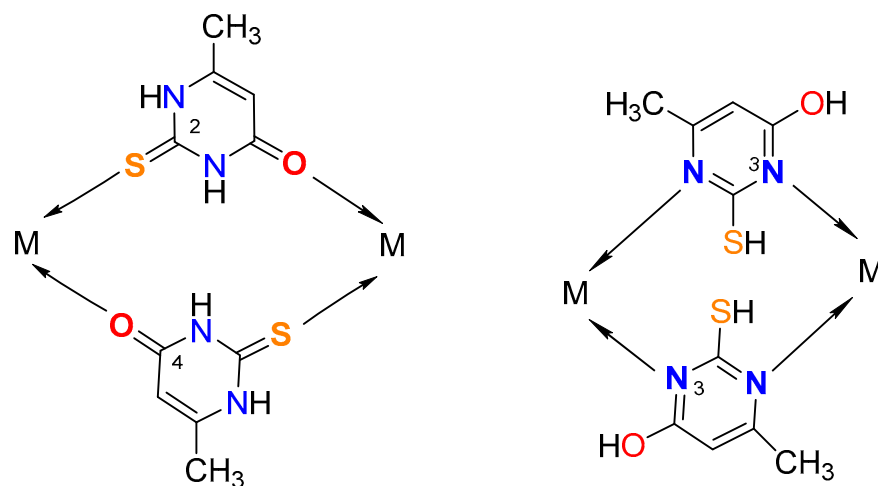


Figure 4. Possible bridge means of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one coordination with metal ions.

Additionally, Fernández-Moreira et al. studied the anticancer properties of gold complexes with various bioligands [10]. Biocompatible citrate-reduced gold nanoparticles

(AuNPs) were modified with a biologically active substance, 2-thiouracil (2-TU), with prospective anticancer properties [11]. The inhibitory effects on cell proliferation of AuNPs, 2-TU, and 2-TU-AuNPs were evaluated using a 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay in the MDA-MB-231 breast cancer cell line. It was demonstrated that AuNPs substantially boosted the inhibitory effect on cell proliferation of 2-TU [11]. Various gold(I), gold(III), and silver(I) metal complexes have been synthesized and their cytotoxicity has been studied [12]. These collective efforts underscore the chemical significance of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one and its derivatives, showcasing their potential coordination modes with metal ions.

The objective of the current study is to synthesize, characterize the structure, and investigate the biological properties of a novel gold complex of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one and to contribute to the understanding of how the coordination of this ligand with gold affects its biological activity, potentially paving the way for the development of new therapeutic agents with targeted antimicrobial or cytotoxic properties.

2. Results and Discussion

The verification of the structure of the metal complex through IR analysis involves comparing the IR spectra of the free ligand with that of its metal complex. Selected experimental data from the IR spectra of the complex with the general formula AuL and of the free ligand 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (L) are presented in Table 1, showing characteristic absorption bands in wavenumbers (cm^{-1}).

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

Assignment	L	AuL
$\nu(\text{OH})$	-	
$\nu(\text{NH})$	3115 sh	3109
$\nu(\text{NH})$	3080	3089
$\nu(=\text{CH})$	3014	
$\nu(\text{C}=\text{O})$	1676 m	1642
	1560 w	1557
$\nu(\text{C}=\text{S})$	1242	1272
	1167 s	1167

In the IR spectrum of the free ligand 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (L), bands at 3115 cm^{-1} and 3080 cm^{-1} are observed, corresponding to the stretching vibrations of N-H groups. These bands in the IR spectrum of the Au(III) complex show no significant change. Additionally, the IR bands of the free ligand at 1676 cm^{-1} and 1242 cm^{-1} are attributed to the stretching vibrations of the $\text{C}^4=\text{O}$ and $\text{C}^2=\text{S}$ groups, respectively. In the gold complex, these stretching vibrations are shifted to lower frequencies by 34 cm^{-1} for $\text{C}^4=\text{O}$ and to higher frequencies by 30 cm^{-1} for $\text{C}^2=\text{S}$ compared to the free ligand 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (L). This shift indicates the participation of the $\text{C}^4=\text{O}$ and $\text{C}^2=\text{S}$ groups of the ligand in coordinating with the Au(III) ion in the complex.

The highest points in the UV spectra of the free ligand were observed at the wavelengths $\lambda_{\text{max}} = 257 \text{ nm}$ and 292 nm . In the electronic spectra of the gold complex, peaks were detected at 257 nm , 272 nm , and 292 nm . We conducted an initial UV analysis of the ligands in dimethyl sulfoxide at room temperature. The spectrum of the ligand is almost identical to the spectrum of the complex. The spectrum of the gold complex exhibited a minor peak around 272 nm , attributed to the electron transition from non-bonding orbitals to π^* orbitals in the ligand. The primary absorption peak in the spectra resulted from the $\pi \rightarrow \pi^*$ electron transition in the ligand. Any d-d transitions, if present, were part of the broad experimental band and overlapped with the ligand's spectrum.

The gold complex is stable in air and moisture, and it has limited solubility. The reaction of the ligand 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one (L) with the

transition metal ion resulted in a 56% yield of a stable solid compound. The synthesized complex exhibits a yellow-orange color and is sparingly soluble in DMSO and DMF, but insoluble in water, THF, ethanol (C₂H₅OH), ethyl acetate (EtOAc), and cyclohexane (C₆H₁₂). Analytical data, including the yield percentages of the gold complex, are presented in Table 2.

Table 2. Analytical and physical characteristic of metal complexes with 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (L).

Compound	Colour	Yield (%)	Melting Point (°C)	Solubility
L	colorless		330	soluble in DMSO
AuL	yellow-orange	56	>350 °C	limited solubility in DMSO and DMF; insoluble in H ₂ O, EtOH, THF, EtOAc and C ₆ H ₁₂ .

The ¹H NMR spectrum of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (L) displays four signals, including a singlet at 12.29 ppm corresponding to H-1 and H-3 (overlapping resonances) [8]. The olefin proton is observed at 5.68 ppm (H-5), and the signal at 2.06 ppm is assigned to H-1'. These assignments were confirmed through analysis using ¹H-¹H COSY, ¹H-broadband decoupled ¹³C-NMR, DEPT-135, and HMBC spectra, as detailed in Table 3 [8]. You can see the spectra in the Supplementary Materials.

Table 3. ¹H and ¹³C NMR spectral data and ¹H-¹H COSY and HMBC correlations for 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (600.13 MHz (¹H) and 150.903 MHz (¹³C))^a [8].

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)	1'	4 ^b , 6, 1'
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 characterization of the gold complex in solution was obstructed by its low solubility in various solvents without heating, including DMSO. It is known that DMSO can not only be a solvent but also a ligand in the complexation, and upon heating can displace another ligand, leading to the decomposition of the complex. In fact, this is exactly what we observe when trying to dissolve a sample of the complex in DMSO-*d*₆ at a high temperature for NMR. The NMR spectra of this sample showed the presence of free ligands, but no complex. Another sample was prepared via the gentle heating of a small quantity of the complex in DMSO-*d*₆. The ¹H and ¹³C NMR spectra of the AuL complex in DMSO-*d*₆ solution are given in Figures 5 and 6. In the ¹H NMR spectrum of the complex, signals at 2.01, 5.31, 10.8, 10.9 ppm are observed. These signals are characteristic of 6-methyluracil (6-MeU) and not of 2-thiouracil derivatives. It is known that tetrahydropyrimidine-2,4-diones can be obtained through the desulfurization of 2-thioxo-1,2,3,4-tetrahydropyrimidin-4-ones in the presence of a base [13]. Since our complex synthesis is carried out in the presence of NaOH, it is not unexpected that some of the 6-Me-2-Tu is converted to 6-MeU and this is in agreement with the data reported by Novakov et al. [13]. In the ¹³C NMR solution spectrum of the complex, the signals at 174.09 ppm and 160.60 ppm correspond to the C=S and C=O groups, respectively. This indicates a decrease in the chemical shift of 1.78 ppm for the C=S resonance and 0.46 ppm for the C=O signal. These shifts suggest the involvement

of the C=S and C=O groups of the ligand in coordination with the gold. Additionally, the signal at 174.09 ppm looks broader and has lower intensity compared with the same signal in the ligand spectrum. The signal for the C-5 carbon suffers the same broadening, which suggests spatial proximity to the binding site. The ^1H and ^{13}C NMR spectral data in the solution of the complex compared with the free ligand are given in Table 4.

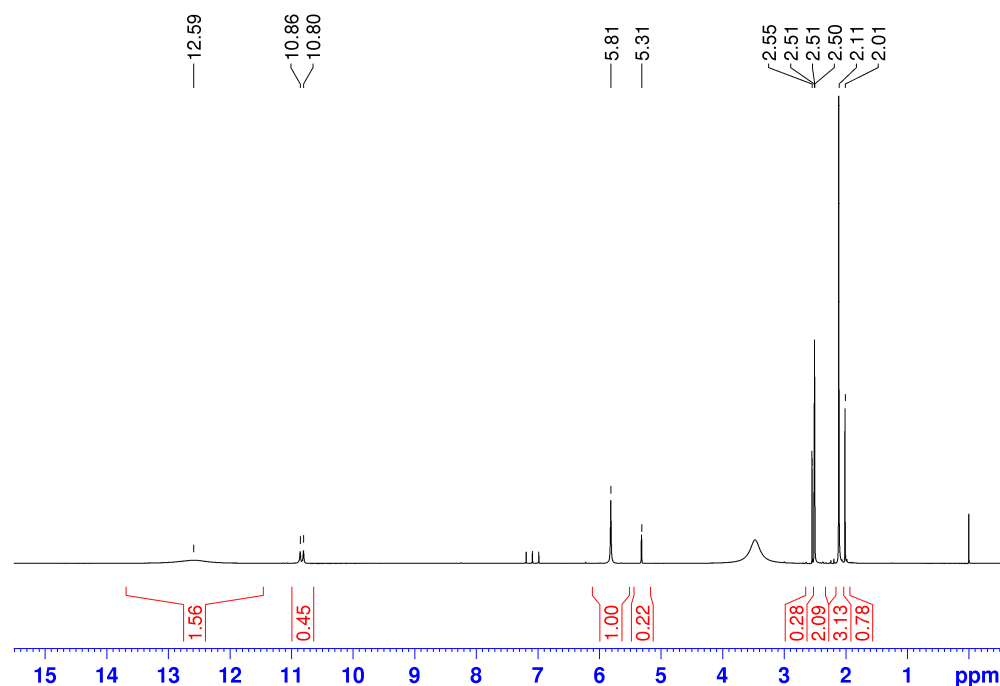


Figure 5. ^1H NMR spectrum of the gold complex in DMSO-d_6 solution.

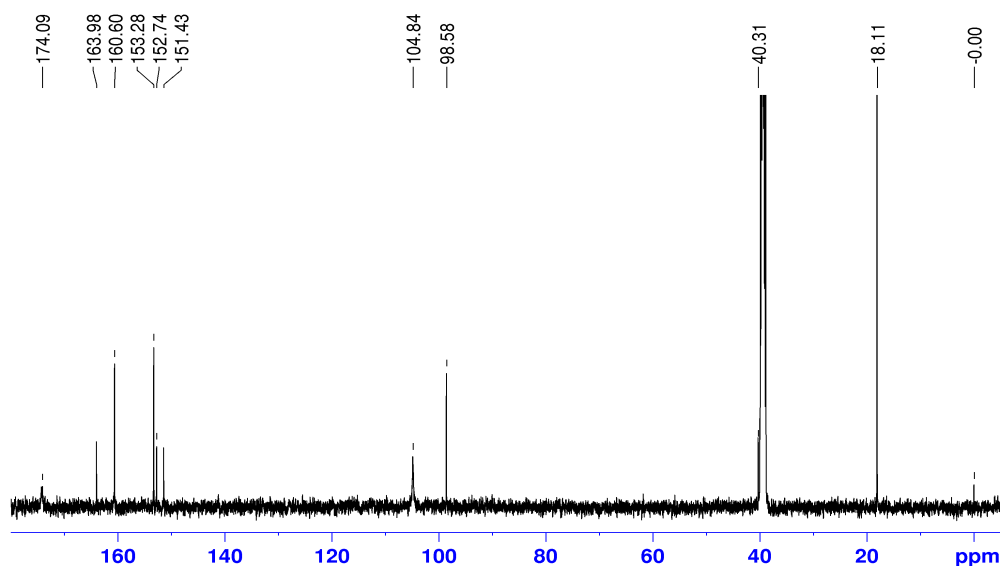


Figure 6. ^{13}C NMR spectrum of the gold complex in DMSO-d_6 solution.

However, solid-state NMR spectroscopy provides better distinction between the complex and the free ligand, as illustrated in Figure 7. The ^{13}C NMR spectrum of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one obtained using the cross-polarization (CP) experiment reveals five distinct signals. Two of these signals are observed at chemical shifts of 174.6 ppm and 163.0 ppm, corresponding to the carbon atoms in the C=S (thione) and C=O (ketone) functional groups, respectively. Detailed chemical shift data are summarized in Table 5.

Table 4. ¹³C NMR spectral data in solution of complex and ¹H of 6-methyl-uracil.

Atom	δ (¹³ C) ppm 6-Methyl-2-Thioxo-2,3-Dihydropyrimidin-4(1H)-One	δ (¹³ C) ppm AuL	δ (¹³ C) ppm 6-MeU	δ (¹ H) ppm 6-MeU
1 (NH)	-	-	-	10.86
2 (C=S)/C=O	175.87	174.09	151.43	
3 (NH)	-	-	-	10.80
4 (C=O)	161.06	160.60	163.98	
5	103.72	104.84	98.58	5.31
6	153.20	153.28	152.74	
1'	18.11	18.11		2.01
DMSO		40.31		

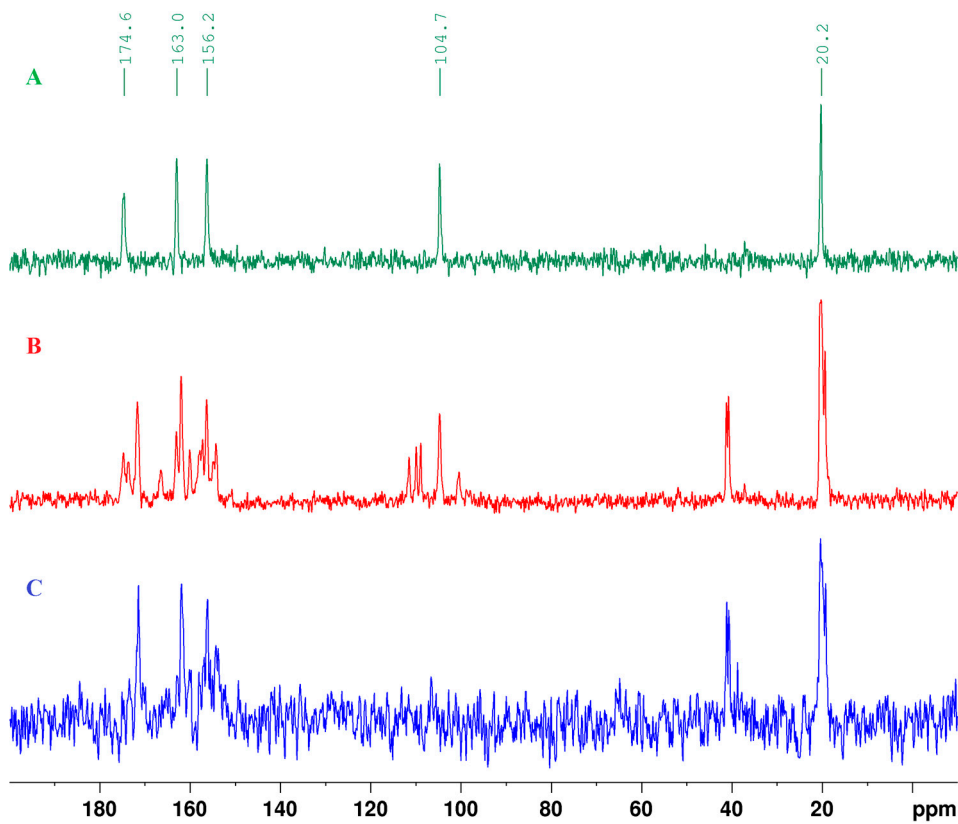


Figure 7. ¹³C NMR acquired with MAS at 15 kHz. (A)—CP spectrum of the ligand; (B)—CP spectrum of the complex; (C)—CPPI spectrum of the complex.

Table 5. ¹³C spectral data (in ppm) for 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one (L) and its complexes with Au(III) acquired with solid-state CP MAS.

Atom	L	AuL
1 (NH)	-	-
2 (C=S)	174.6	171.7; 173.6; 174.7
3 (NH)	-	-
4 (C=O)	163.0	160.1; 162.0; 163.0; 166.5
5	104.7	100.4; 104.7; 108.9; 109.9; 111.5
6	156.2	154.3; 154.7; 156.3; 157.2; 157.8
1'	20.2	19.3; 20.2; 20.4
DMSO- _{H6}	-	40.7; 41.2

In the ^{13}C NMR solid-state spectrum of AuL, there are a lot of signals, as can be seen from Table 5. There is a set of signals corresponding to the starting ligand (Figure 7). As can be seen from the signals corresponding to C-5, there are at least four more signals arising either from different coordination of the ligand, the presence of more than one ligand in the complex with different coordination, or because of different crystallization forms of the complex. The signals at ca. 41 ppm unambiguously show the presence of DMSO in the outer coordination sphere of the Au. There is a shift and a split in the signals for C=S and C=O, which shows that the C=S and C=O groups of the ligand participate in the coordination with the gold.

The ATR spectrum of a sample of the recently prepared AuL was measured to check if the coordination water was present in the complex. The ATR spectrum showed no trace of water in the complex. After recording the ATR spectrum again after the sample was allowed to stand for a while, we found the presence of water, which is in agreement with the results obtained by NMR.

Elemental analysis results for the metal ion were obtained using microwave plasma-atomic emission spectrometry. This result can be utilized to determine the tentative average composition of the complex. Considering that the ratio of ligand to 6-MeU is approximately 4:1 (^1H NMR) and the presence of water and DMSO in the sample, we can propose the following tentative formulas for the complexes, as presented in Table 6.

Table 6. Elemental analysis data for the metal ion of the complex.

Metal Complex	Composition *	Formula	Molecular Weight	W(M)% calc./exp.
Au(III)L	[4LAu].6-MeU.DMSO.16H ₂ O	C ₂₇ H ₆₈ N ₁₀ O ₂₃ S ₅ Au	M = 1258.17 g/mol	15.7/16.2 ± 1.2
	[4LAu]. 6-MeU.2DMSO.6H ₂ O	C ₂₉ H ₅₄ N ₁₀ O ₁₄ S ₆ Au	M = 1156.15 g/mol	17.0/16.2 ± 1.2
	[4LAu]. 6-MeU.2DMSO.5H ₂ O	C ₂₉ H ₅₂ N ₁₀ O ₁₃ S ₆ Au	M = 1138.14 g/mol	17.3/16.2 ± 1.2

* Tentative average composition of different complexes.

Various metal complexes demonstrate a monodentate coordination mode with 2-thiouracil derivatives, binding primarily through the sulfur (S) atom. Examples include the Au(III), Pt(II), and Pd(II) complexes [11,12,14] (refer to Figure 1). The Pt(II) and Pd(II) complexes with 6-tert-butyl-2-thiouracil reported by Golubyatnikova et al. highlight sulfur as the primary coordinating atom in these complexes [14]. In the case of the Au(III)L complex, based on the experimental data acquired, we suggest a monodentate coordination via the S atom and/or monodentate coordination via the O atom. Specifically, we propose that two ligands participate in the coordination via the S2 atoms, while another two ligands coordinate via the O4 atoms (Figure 8).

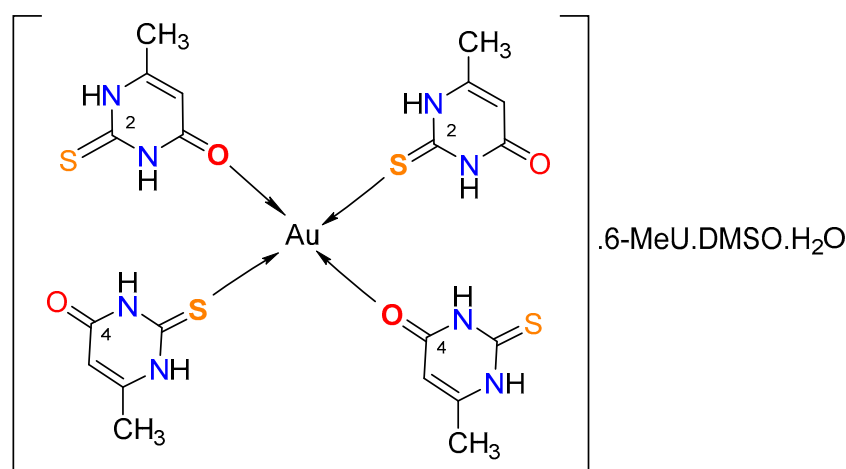


Figure 8. The representation of suggested coordination binding sites for 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one.

6-Methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one exhibited the strongest activity against *Enterococcus faecalis* ATCC 19433 and *Candida albicans* ATCC 10231. No activity was detected against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 8739 and *Saccharomyces cerevisiae*. In the inhibition zones of the rest of the test microorganisms, except for *Pseudomonas aeruginosa* ATCC 9027, single-cell colonies were observed, which is a sign that some of the cells of the microorganisms were not sensitive to the tested concentration of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one. The complex of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one with Au(III) showed a different antimicrobial spectrum. Unlike the ligand, it was active against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 8739 and *Saccharomyces cerevisiae*, and had no effect on *Proteus vulgaris* G and *Listeria monocytogenes* ATCC 8787. Only in the inhibition zone of *Klebsiella pneumoniae* ATCC 13883 were single-cell colonies detected. The highest activity was expressed against *S. aureus*, and the lowest was expressed against *E. coli* ATCC 8739, *B. subtilis*, and *S. cerevisiae*. It is established that Au(III) has antimicrobial activity [15,16] and its addition to the ligand 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one broadened its antimicrobial spectrum and increased its activity against *S. aureus* and *K. pneumoniae*.

3. Materials and Methods

The free ligand 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one was purchased from Aldrich Chem. The metal salts $\text{NH}_4[\text{AuCl}_4] \cdot \text{H}_2\text{O}$ (Aldrich Chem) and solvents used for synthesis of the complexes were of high purity, generally equal to A.C.S. grade and were suitable for use in many laboratory and analytical applications.

3.1. Spectral Measurements

Absorption spectra were registered on a UV-30 SCAN ONDA UV/Vis/NIR spectrophotometer from 200 to 1000 nm. The IR spectra of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one and its complex were registered in KBr pellets on a Bruker FT-IR VERTEX 70 spectrometer from 4000 cm^{-1} to 400 cm^{-1} at a resolution of 2 cm^{-1} with 25 scans. The Raman spectra of the compound (the stirred crystals placed on an aluminum disc) were measured on a RAM II (Bruker Optics, Ettlingen, Germany) with a focused laser beam of d:YAG laser (1064 nm) from 4000 to 100 cm^{-1} at a resolution of 2 cm^{-1} with 25 scans. Additionally, the ATR (Madison, WI, USA) spectrum of the complex was measured (MIRacle Single reflection, PIKE technology) to check if the coordination water was present. The NMR spectra of the ligand were registered on a Bruker Avance II NMR spectrometer operating at 600.130 and 150.903 MHz for ^1H and ^{13}C , respectively, using the standard Bruker software. The NMR spectra of the metal complex were measured on a Bruker Avance III HD spectrometer operating at 500.130 and 125.76 MHz for ^1H and ^{13}C , respectively, equipped with a tunable multinuclear BBO probe-head for the measurements in solution state and with a 2.5 mm Cross-Polarization Magic Angle Spinning (CPMAS) probe-head for the solid-state experiments. CP MAS and Cross-Polarization with Polarization Inversion (CPPI) MAS spectra were recorded at an MAS speed of 15 kHz and α -glycine was used as an external reference (α -glycine carbonyl C—176.03 ppm). Measurements were carried out at ambient temperature.

3.2. Antimicrobial Assay

The antimicrobial activity of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one and its complex with Au(III) 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, and *Klebsiella pneumoniae* ATCC 13883 and the yeasts *Candida albicans* ATCC 10231 and *Saccharomyces cerevisiae*. The procedure involved spreading a suspension of each test microorganism (10^6 cfu/cm^3) onto specific nutrient agar media. Wells with a 7 mm diameter were created

in the agar, and 50 μL of the tested substance solution (10 mg/cm^3 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 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one and its Au(III) complex against a range of pathogenic microorganisms.

4. Experimental Part

4.1. Synthesis and Characterization

4.1.1. Synthesis of Au(III) Complex of 6-Methyl-2-Thioxo-2,3-Dihydropyrimidin-4(1H)-One—General Procedure

The metal complex was synthesized by combining aqueous solutions of the metal salt with the ligand dissolved in a mixture of DMSO and water, along with NaOH, in a metal-to-ligand-to-base ratio of 1:4:2. This process resulted in the formation of a non-charged complex, observed as a yellow-orange precipitate. The precipitate was then isolated by means of filtration, washed multiple times with water, and subsequently dried over CaCl_2 for a period of 2 weeks.

The aqueous solution containing 71.4 mg (0.2 mmol) of $\text{NH}_4[\text{AuCl}_4]\cdot\text{H}_2\text{O}$ metal salt in 10 mL of water was added dropwise to a solution of 113.7 mg (0.8 mmol) of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one in 5 mL of DMSO. The ligand solution had been previously alkalized with an aqueous solution of sodium hydroxide, consisting of 16.0 mg (0.4 mmol) of NaOH in 5 mL of water.

The metal complex was obtained following a previously described procedure [7,8], with variations in the reaction duration (specifically 24 h for the gold(III) complex) and/or utilizing various solvents [6,14,17–19].

4.1.2. Spectral Data of the Free Ligand and Its Metal Complex

UV-Vis (DMSO) of L: $\lambda_{\text{max}} = 257, 292 \text{ nm}$

IR (cm^{-1}) of L: 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.

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

Raman spectra of gold complex could not be measured; the samples burned at 1 mW.

UV-Vis (DMSO) of Au(III) complex: $\lambda_{\text{max}} = 257, 272, 292 \text{ nm}$

IR (cm^{-1}) of Au(III) complex: 3447, 3188, 3109, 3089, 2926, 2870, 1698, 1641, 1595, 1556, 1422, 1383, 1349, 1271, 1241, 1199, 1194, 1166, 1030, 838, 809, 655, 626, 597, 552, 513, 458.

ATR (cm^{-1}) of Au(III) complex: 3086, 2882, 1636, 1583, 1549, 1418, 1383, 1350, 1265, 1241, 1194, 1165, 1029, 951, 932, 907, 871, 833, 805, 744, 655, 624.

^1H -NMR of Au(III) complex: 12.59 (br. s, 2H, NH), 5.81 (s, 1H, H-5), 2.55 (H-DMSO), 2.11 (s, 3H, H-1').

^{13}C -NMR of Au(III) complex: 174.09 (C=S), 160.60 (C=O), 153.28 (C-6), 104.84 (C-5), 40.31 (C-DMSO), 18.11 (C-1')

4.1.3. Microwave Plasma—Atomic Emission Spectrometry (MP-AES) Determination of Au in the Complex

Samples with a mass of 20.0 mg were weighed out on an analytical balance and dissolved with 5 mL of aqua regia, comprising 65% nitric acid, p.a. (Chem-Lab, NV, Zedelgem, Belgium) and nitric and 37% hydrochloric acid, p.a. (Fluka AG, Buchs, Switzerland) for the Au complex. A blank solution was prepared as well. After appropriate dilution, the concentration of Au was determined by MP-AES 4200 (Agilent technologies, Santa Clara,

CA, USA). Calibration standards were prepared from an Au monoelemental standard solution in 2% HCl. Conventional MP-AES operating conditions were used. The analyte was measured on three emission lines for the estimation of potential spectral interferences, i.e., 242.795 nm, 267.595 nm, and 312.278 nm. Five replicates and 5 s measurement were applied for all lines.

4.2. Antimicrobial Activity of Au(III) Complex of 6-Methyl-2-Thioxo-2,3-Dihydropyrimidin-4(1H)-One

The antimicrobial activity of the Au(III) complex of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one was evaluated and compared with the activity of the ligand and 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one itself. The experimental results are summarized in Table 7.

Table 7. Antimicrobial activity of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one and its Au(III) complex.

Test Microorganisms	DMSO	Compounds	
		6-Methyl-2-Thioxo-2,3-Dihydropyrimidin-4(1H)-One	Au(III)L
		Inhibition Zone, mm	
<i>Staphylococcus aureus</i> ATCC 25923	-	-	16
<i>Escherichia coli</i> ATCC 8739	-	-	8
<i>Enterococcus faecalis</i> ATCC 19433	-	11	11
<i>Salmonella enterica</i> ssp. <i>enterica</i> ser. <i>Enteritidis</i> ATCC 13076	-	-	11
<i>Pseudomonas aeruginosa</i> ATCC 9027	-	9	9
<i>Proteus vulgaris</i> G	-	9 *	-
<i>Bacillus subtilis</i> ATCC 6633	-	9 *	8
<i>Bacillus cereus</i> ATCC 11778	-	9 *	9
<i>Listeria monocytogenes</i> ATCC 8787	-	9 *	-
<i>Klebsiella pneumoniae</i> ATCC 13883	-	9 *	12 *
<i>Candida albicans</i> ATCC 10231	-	11	10
<i>Saccharomyces cerevisiae</i>	-	-	8

Well diameter—7 mm. * Inhibition zone with single-cell colonies.

5. Conclusions

This paper describes the synthesis of a novel complex of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one with gold(III). The structure of the new complex is elucidated through various analytical techniques, including melting point analysis, UV-Vis and IR spectroscopy, ATR, as well as solution- and solid-state NMR and Raman spectroscopy for the free ligand. Based on the spectral data obtained, we propose the coordination binding sites of the ligands within the complex. Two molecules of the ligand acted via oxygen atom and two through sulfur atom in the coordination of gold ions. In addition, we observed a solvent molecule in the composition of the complex, as indicated by the signals at 2.54 ppm and 40.31 ppm in the ^1H and ^{13}C NMR spectra of the complex solution. The ATR spectrum shows the presence of water, which is in agreement with the results obtained by NMR. This observation underscores the potential structural complexity and unique properties of the synthesized gold(III) complex.

The antimicrobial activity of 6-methyl-2-thioxo-2,3-dihydropyrimidin-4(1H)-one and its complex with Au(III) was investigated against Gram-negative, Gram-positive bacteria, and yeasts. The addition of Au(III) broadened the antimicrobial spectrum of the ligand and it exhibited its highest activity against *S. aureus*.

Supplementary Materials: The following supporting information can be downloaded online: Figure S1. Structure of the ligand 6-methyl-2-thiouracil; Figure S2. The ^1H NMR spectrum of the ligand, 6-methyl-2-thiouracil; Figure S3. ^1H -broadband decoupled ^{13}C -NMR spectrum of the ligand, 6-methyl-2-thiouracil;

Figure S4. The DEPT-135 spectrum of 6-methyl-2-thiouracil; Figure S5. The ^1H - ^1H COSY NMR spectrum of the ligand, 6-methyl-2-thiouracil; Figure S6. The HMQC NMR spectrum of the ligand, 6-methyl-2-thiouracil; Figure S7. The HMBC NMR spectrum of the ligand, 6-methyl-2-thiouracil; Figure S8. The Raman spectrum of 6-methyl-2-thiouracil; Figure S9. The IR spectrum of 6-methyl-2-thiouracil; Figure S10. The UV-Vis spectra of free ligand (orange) and its complex with gold (blue); Figure S11. The IR spectrum of the gold complex; Figure S12. The ATR spectrum of Au(III) complex; Figure S13. The IR spectrum of the complex, Au(III) with 6-methyl-2-thiouracil (red) and ligand (blue); Figure S14. ^{13}C CP MAS of 6-methyl-2-thiouracil acquired at 15 kHz; Figure S15. ^{13}C CP MAS of Au(III) complex acquired at 15 kHz; Figure S16. ^{13}C CPPI MAS of Au(III) complex acquired at 15 kHz.

Author Contributions: Conceptualization, P.M. and D.B.; methodology, P.M.; formal analysis, N.B., D.B. and A.S.; investigation, D.B., A.S. and N.B.; resources, N.B.; data curation, P.M.; writing—original draft preparation, P.M. and N.B.; writing—review and editing, P.M. and N.B.; supervision, P.M.; funding acquisition, P.M. All authors have read and agreed to the published version of the manuscript.

Funding: We acknowledge the financial support from the Fund for Scientific Research of the Plovdiv University, project CII 23-Xφ-006.

Data Availability Statement: The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Acknowledgments: We acknowledge the financial support from the Fund for Scientific Research of the Plovdiv University, project CII 23-Xφ-006 with project administration, Slava Tsoneva, S.T.

Conflicts of Interest: The authors declare no conflicts of interest.

References

- Garrett, R.H.; Grisham, C.M. *Principles of Biochemistry with a Human Focus*; Brooks/Cole Thomson Learning: Pacific Grove, CA, USA, 2001; p. 939. ISBN 0-03-097369-4.
- Astwood, E.B. The chemical nature of compounds which inhibit the function of the thyroid gland. *J. Pharmacol. Exp. Ther.* **1943**, *78*, 79–89.
- Oladipo, M.A.; Isola, K.T. Coordination Possibility of Uracil and Applications of Some of Its Complexes: A Review. *Res. J. Pharm. Biol. Chem. Sci.* **2013**, *4*, 386–394. [CrossRef]
- Masoud, M.S.; Ramadana, M.S.; Ramadana, A.M.; Al-Saify, M.H. Complexing Properties and Applications of Some Biologically Active Nucleic Acid Constituents. *Int. J. Innov. Res. Technol. Sci. Eng.* **2020**, *6*, 23–39. Available online: <https://ijisset.org/storage/Volume6/Issue6/IJISSET-060524.pdf> (accessed on 14 May 2024).
- Marinova, P.E.; Tamahkyarova, K.D. Synthesis and Biological Activities of Some Metal Complexes of 2-Thiouracil and Its Derivatives: A Review. *Compounds* **2024**, *4*, 186–213. [CrossRef]
- Bomfim, L.M.; de Araujo, F.A.; Dias, R.B.; Sales, C.B.S.; Gurgel Rocha, C.A.; Correa, R.S.; Soares, M.B.P.; Batista, A.A.; Bezerra, D.P. Ruthenium(II) complexes with 6-methyl-2-thiouracil selectively reduce cell proliferation, cause DNA double-strand break and trigger caspase-mediated apoptosis through JNK/p38 pathways in human acute promyelocytic leukemia cells. *Sci. Rep.* **2019**, *9*, 11483–11500. [CrossRef] [PubMed]
- Marinova, P.; Tsoneva, S.; Frenkeva, M.; Blazheva, D.; Slavchev, A.; Penchev, P. New Cu(II), Pd(II) and Au(III) complexes with 2-thiouracil: Synthesis, Characteration and Antibacterial Studies. *Russ. J. Gen. Chem.* **2022**, *92*, 1578–1584. [CrossRef]
- Marinova, P.; Hristov, M.; Tsoneva, S.; Burdzhiev, N.; Blazheva, D.; Slavchev, A.; Varbanova, E.; Penchev, P. Synthesis, Characterization, and Antibacterial Studies of New Cu(II) and Pd(II) Complexes with 6-Methyl-2-Thiouracil and 6-Propyl-2-Thiouracil. *Appl. Sci.* **2023**, *13*, 13150–13168. [CrossRef]
- Skrobanska, M.; Zabiszak, M.; Taras-Goslinska, K.; Nowak, M.; Kaczmarek, M.T.; Frymark, J.; Michalska, D.; Jastrzab, R. Potentiometric and spectroscopic studies of the complex formation in the 6-methyl-2-thiouracil and copper(II) ion system. *Polyhedron* **2022**, *223*, 115964–115970. [CrossRef]
- Fernández-Moreira, V.; Herrera, R.P.; Concepción Gimeno, M. Anticancer properties of gold complexes with biologically relevant ligands. *Pure Appl. Chem.* **2018**, *91*, 247–269. [CrossRef]
- Lorenzana-Vázquez, G.; Pavel, I.; Meléndez, E. Gold Nanoparticles Functionalized with 2-Thiouracil for Antiproliferative and Photothermal Therapies in Breast Cancer Cells. *Molecules* **2023**, *28*, 4453–4466. [CrossRef] [PubMed]
- Goitia, H.; Villacampa, M.D.; Laguna, A.; Gimeno, M.C. Cytotoxic Gold(I) Complexes with Amidophosphine Ligands Containing Thiophene Moieties. *Inorganics* **2019**, *7*, 13–26. [CrossRef]
- Novakov, I.A.; Orlinson, B.S.; Navrotskii, M.B. Desulfurization of 2-Thioxo-1,2,3,4-tetrahydropyrimidin-4-ones with Oxiranes and 2-Haloacetonitriles. *Russ. J. Org. Chem.* **2005**, *41*, 607–609. [CrossRef]
- Golubyatnikova, L.G.; Khisamutdinov, R.A.; Grabovskii, S.A.; Kabal'nova, N.N.; Murinov, Y.I. Complexes of Palladium(II) and Platinum(II) with 6-tert-Butyl-2-thiouracil. *Russ. J. Gen. Chem.* **2017**, *87*, 117–121. [CrossRef]

15. Shareena Dasari, T.P.; Zhang, Y.; Yu, H. Antibacterial Activity and Cytotoxicity of Gold(I) and (III) Ions and Gold Nanoparticles. *Biochem. Pharmacol.* **2015**, *4*, 199–203. [[CrossRef](#)] [[PubMed](#)]
16. Zhang, Y.; Dasari, T.; Deng, H.; Yu, H. Antimicrobial Activity of Gold Nanoparticles and Ionic Gold. *J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev.* **2015**, *3*, 286–327. [[CrossRef](#)] [[PubMed](#)]
17. Shaban, N.Z.; Masoud, M.S.; Awad, D.; Mawlawia, M.A.; Sadek, O.M. Effect of Cd, Zn and Hg complexes of barbituric acid and thiouracil on rat brain monoamine oxidase-B (in vitro). *Chem.-Biol. Interact.* **2014**, *208*, 37–46. [[CrossRef](#)] [[PubMed](#)]
18. Lusty, J.R.; Peeling, J.; Abdel-Aal, M.A. Complexes of 6-Methyl-2-thiouracil with Rhodium, Iridium, Platinum and Palladium. *Inorg. Chim. Acta* **1981**, *56*, 21–26. [[CrossRef](#)]
19. Teleb, S.M.; Askar, M.E.; El-Kalyoubi, S.A.; Gaballa, A.S. Synthesis, characterization and antimicrobial activities of some 5-bromouracil–metal ion complexes. *Bull. Chem. Soc. Ethiop.* **2019**, *33*, 255–268. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.