SYNTHESIS AND BIOLOGICAL ACTIVITY OF 1-(AZEPAN-1-YL)PROPANE-1,2-DIONE 4-PHENYLTHIOSEMICARBAZONE AND ITS COPPER(II) COMPLEXES

SINTEZA ȘI ACTIVITATEA BIOLOGICĂ A 4-FENILTIOSEMICARBAZONEI 1-(AZEPAN-1-IL)PROPAN-1,2-DIONEI ȘI A COMPUȘILOR COORDINATIVI AI CUPRULUI(II) CU ACEST LIGAND

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Sumar. *A fost sintetizată o nouă tiosemicarbazonă 4-feniltiosemicarbazonă 1-(azepan-1-il) propan-1,2-dionă (HL) şi cinci compuşi coordinativi ai cuprului(II) [Cu(L)X] (X = Cl - (I); Br - (II); NO 3 - (III); CH 3 COO - (IV); Cl 2 CHCOO - (V)).Compuşii au fost studiaţi prin analiză elementară, spectroscopie FTIR, precum şi spectre RMN ^{1}H şi ^{13}C. Coordinarea 4-feniltiosemicarbazonei HL la atomul de cupru(II) a dus la creşterea activităţii antibacteriene şi antifungice. Activitatea compuşilor sintetizaţi este influenţată de natura restului acid prezent în compoziţia acestora. Cel mai activ compus este complexul IV.*

Cuvinte-cheie: *activitatea antibacteriană; activitatea antifungică; compuşi coordinativi ai cuprului(II);4-feniltiosemicarbazonă.*

INTRODUCTION

Thiosemicarbazones have attracted significant interest due to their diverse coordination behavior, notable biological potential, structural versatility, and ability to function as ion sensors. They are now widely recognized as a key group of sulfur-containing ligands, especially for transition metal ions. This growing interest is largely attributed to their impressive biological activities, which are closely linked to their capacity to form metal complexes.

Various 4-phenylthiosemicarbazones and their coordination compounds have been described in the literature as biologically active compounds. These compounds exhibit antimicrobial [1, 2], antifungal [3], antioxidant [4] and cytotoxic [5, 6] properties. Although 4-phenylthiosemicarbazones are frequently encountered in the literature, pyruvic acid amides 4-phenylthiosemicarbazones are practically not described. Although pyruvic acid amides themselves are often biologically active [7], the presence of their fragment in thiosemicarbazone may affect their properties. Because bearing two pronucleophilic and two proelectrophilic sites, this structural scaffold exhibits distinct chemical properties and unparalleled biological activity [8].

In this study, a new 1-(azepan-1-yl)propane-1,2-dione 4-phenylthiosemicarbazone (**HL**) and its five copper(II) coordination compounds were synthesized, and their antibacterial and antifungal activities were studied.

MATERIAL AND METHOD

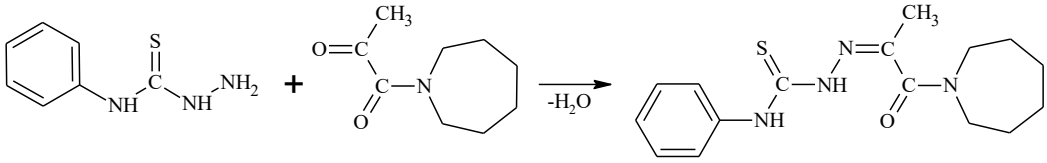
Pyruvic acid, 4-phenylthiosemicarbazide, oxalyl chloride, sodium carbonate, dichloromethane, dimethylformamide, azepane, copper(II) chloride dihydrate, copper(II) bromide, copper(II) nitrate trihydrate, copper(II) dichloroacetate monohydrate, copper(II) acetate monohydrate were obtained from Sigma-Aldrich and used without further purification. The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-400, using $CDCl_3$ as a solvent. FTIR spectra were obtained on a Bruker ALPHA FTIR spectrophotometer at room temperature in the range of 4000-400 cm^{-1}. The determination of copper content in the synthesized coordination compounds, using titration methods, performed similarly to the literature procedures [9]. The resistance of solutions of complexes in methanol (20°C, 0.001 M) was measured using an R-38 rheochord bridge.

1-(Azepan-1-yl)propane-1,2-dione

1-(Azepan-1-yl)propane-1,2-dione was synthesized according to the procedure described in [10] with some modifications. Pyruvic acid (8.80 g, 0.100 mol) was dissolved in 10 mL of CH_2Cl_2 in a flat-bottomed flask. To catalyze the reaction, three drops of dimethylformamide were introduced into the mixture. A separate solution of oxalyl chloride (15.24 g, 0.120 mol) in 10 mL of CH_2Cl_2 was then gradually added to the reaction mixture under stirring at 0°C. The flask, equipped with a reflux condenser and sealed with a calcium chloride drying tube, was stirred for 1.5 hours at room temperature. The result of reaction was yellow oily liquid. A mixture of sodium carbonate (8.3 g, 0.100 mol), azepane (9.9 g, 0.100 mol) and 10 mL of CH_2Cl_2 was stirred at 0°C. 2-Oxopropanoyl chloride was added dropwise to the cooled suspension and the mixture was stirred at room temperature for an hour. The resulted mixture was filtered and washed three times with water. The compound was then purified by column chromatography using a 1:1 mixture of ethyl acetate and hexane. Yield 60%. FT-IR (ν_{max}, cm^{-1}): 1710 ($C{=}O_{ketone}$); 1628 ($C{=}O_{amide}$).

1-(Azepan-1-yl)propane-1,2-dione 4-phenylthiosemicarbazone (HL)

1-(Azepan-1-yl)propane-1,2-dione (3.38 g, 20.0 mmol) was dissolved in 25 mL of ethanol with heating. 4-Phenylthiosemicarbazide (3.34 g, 20.0 mmol) was added to this solution and the mixture continued to stir while heating for an hour (Scheme 1). The resulting pale-yellow precipitate was filtered, washed with a small amount of ethanol, and air-dried. Yield 83%, mp. 105-107°C.



Scheme 1. 1-(Azepan-1-yl)propane-1,2-dione 4-phenylthiosemicarbazone (HL)

General method for synthesis of copper(II) coordination compounds I-V

4-Phenylthiosemicarbazone (**HL**) was dissolved in ethanol, after that the corresponding copper(II) salt was added to the solution in a molar ratio of 1:1. As a result, precipitation of various shades of green formed. The resulting precipitates were filtered off, washed with a small amount of ethanol and dried.

Antibacterial and antifungal activity

Standard strains of *Staphylococcus aureus* (ATCC 25923) were used to study antibacterial activity and fungi *Candida albicans* (ATCC 10231), *Cryptococcus neoformans* (CECT 1043) were used to study antifungal activity of the HL and coordination compounds **I–V**. The minimum inhibitory concentrations (MICs, μg mL^{-1}), minimum

bactericidal concentrations (MBCs, μg mL^{-1}) and minimum fungicidal concentration (MFC, μg mL^{-1}) were determined using the method of serial dilutions in liquid broth. The tested substances were dissolved in DMSO at a concentration of 10 mg mL^{-1}. The next dilutions were made using 2% of peptone bullion.

RESULTS AND DISCUSSIONS

The novel 1-(azepan-1-yl)propane-1,2-dione 4-phenylthiosemicarbazone (**HL**) was synthesized through a two-step process. In the first step, 1-(azepan-1-yl)propane-1-,2-dione was obtained by reaction between pyruvic acid, oxalyl chloride, and azepane in CH_2Cl_2. In the second step, the resulting 1-(azepan-1-yl)propane-1,2-dione reacted with 4-phenylthiosemicarbazide in ethanol in equal molar amounts. Its structure was confirmed via ^{1}H,^{13}C NMR spectroscopy.

Five novel coordination compounds with 4-phenylthiosemicarbazone (HL) were synthesized. Copper(II) complexes I-V were obtained by reaction between HL and copper(II) salts in a 1:1 molar ratio in hot ethanolic solutions. Elemental analyses of the complexes on copper suggest the general formulas [Cu(L)X] (X = Cl^- (I); Br^- (II); NO_3^- (III); CH_3COO^- (IV); Cl_2CHCOO^- (V)). The molar conductivity values of the synthesized complexes I-V are in the range 60-70 $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$ suggesting that these complexes are 1:1 electrolytes.

The FTIR spectrum of HL contains the absorption bands that correspond to groups: NH, C=O, C=N and C=S. The coordination of 4-phenylthiosemicarbazone (HL) to the copper(II) atom is confirmed by the following changes in the spectra. The ν(N-H) absorption bands disappears from the FTIR spectra of the complexes I-V, which points to deprotonation of the HL. The ν(C=O) and ν(C=N) absorption bands shifted to the low frequency region. The ν(C=S) absorption band disappeared from the spectra of copper(II) complexes, but ν(C–S) absorption band appeared.

The antibacterial activity of the HL and complexes I-V were studied on a series of Gram-positive bacteria *Staphylococcus aureus* and antifungal activities were studied towards fungi *Candida albicans* and *Cryptococcus neoformans*. The obtained results in the form of minimum inhibitory/bactericidal/fungicidal concentrations are shown in Table 1. The synthesized 4-phenylthiosemicarbazone HL does not show activity against the studied microorganisms. Its coordination to copper(II) led to increased antibacterial and antifungal activity. The activity of copper(II) complexes is influenced by the nature of the acid anion. The activity of the complexes towards *Staphylococcus aureus* decreases in the following order: $CH_3COO^- > Cl^- \approx Cl_2CHCOO^- > Br^- \approx NO_3^-$. The activity of the complexes towards *Candida albicans* decreases in the following order: $Cl^- \approx CH_3COO^- > Cl_2CHCOO^- > Br^- \approx NO_3^-$. And the order for the activity towards *Cryptococcus neoformans* is: $CH_3COO^- > Cl^- \approx Cl_2CHCOO^- \approx Br^- > NO_3^-$. The most active complex is complex IV.

Table 1. Antibacterial and antifungal activity of HL and complexes I-V as MIC and MBC values in μg mL^{-1}

Compound	*Staphylococcus aureus* (ATCC 25923)		*Candida albicans* (ATCC 10231)		*Cryptococcus neoformans* (CECT 1043)	
	MIC	MBC	MIC	MFC	MIC	MFC
HL	-	-	500.0	-	250.0	-
I	7.813	7.813	62.50	-	31.25	125.0
II	15.63	31.25	250.0	-	62.50	250.0
III	15.63	62.50	250.0	-	125.0	125.0
IV	3.906	3.906	62.50	-	15.63	125.0
V	7.813	7.813	125.0	-	62.50	250.0

CONCLUSIONS

The novel 1-(azepan-1-yl)propane-1,2-dione 4-phenylthiosemicarbazone (**HL**) and its five copper(II) coordination compounds were synthesized and characterized. The studied complexes manifest antibacterial and antifungal activity. The coordination of the 4-phenylthiosemicarbazone (**HL**) to the copper(II) atom leads to the increasing of activity. The activity of copper(**II**) complexes is influenced by the nature of the acid anion. The complex **IV** is the most active one.

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