# SYNTHESIS AND BIOLOGICAL ACTIVITY OF *N*-(4-METHOXYPHENYL)-2-OXOPROPANAMIDE 4-PHENYLTHIOSEMICARBAZONE AND ITS COPPER(II) COMPLEXES

## SINTEZA ȘI ACTIVITATEA BIOLOGICĂ A 4-FENILTIOSEMICARBAZONEI *N*-(4-METOXIFENIL)-2-OXOPROPANAMIDEI ȘI A COMPUȘILOR COORDINATIVI AI CUPRULUI(II) CU ACEST LIGAND

**CZU: 547-304.6:546.56:535.243** **https://doi.org/10.59295/spd2024n.81**

**Ianina GRAUR,**
ORCID: 0000-0002-3345-7539
e-mail: ulchina.ianina@usm.md
Moldova State University
**Irina USATAIA,**
ORCID: 0000-0002-6749-0687
e-mail: departamentchimie@mail.ru
Moldova State University
**Vasilii GRAUR,**
ORCID: 0000-0001-8153-2153
e-mail: vasilii.graur@usm.md
Moldova State University
**Victor TSAPKOV,**
ORCID: 0000-0003-1732-3116
e-mail: vtsapkov@gmail.com
Moldova State University
**Aurelian GULEA,**
ORCID: 0000-0003-2010-7959
e-mail: guleaaurelian@gmail.com
Moldova State University
**Olga GARBUZ,**
ORCID: 0000-0001-8783-892X
e-mail: olga.garbuz@sti.usm.md
Institute of Zoology, Moldova State University
**Greta BALAN,**
ORCID: 0000-0003-3704-3584
e-mail: greta.balan@usmf.md
State University of Medicine and Pharmacy „Nicolae Testemitanu”,
Chisinau, Republic of Moldova

***Summary.*** *The new N-(4-methoxyphenyl)-2-oxopropanamide 4-phenylthiosemicarbazone (**HL**) and its 5 copper(II) coordination compounds [Cu(L)X] (X = $Cl^-$ (**I**); $Br^-$ (**II**); $NO_3^-$ (**III**); $CH_3COO^-$ (**IV**); $Cl_2CHCOO^-$ (**V**)) have been synthesized and characterized by elemental analysis, FTIR and NMR spectra. Coordination of 4-phenylthiosemicarbazone **HL** with the copper(II) atom led to increased antibacterial activity against Staphylococcus aureus and Bacillus cereus. Non-coordinated 4-phenylthiosemicarbazone (**HL**) showed the highest activity in relation to $ABTS^{\bullet+}$ cation radicals; its activity is close to that of Trolox, which is used in medicine. The antibacterial and antioxidant activities of the synthesized complexes are influenced by the nature of the acidic residue in their composition.*

***Keywords:*** *antibacterial activity; antioxidant activity; copper(II) complexes; thiosemicarbazone.*

## INTRODUCTION

Thiosemicarbazones and their metal complexes are known for their diverse medicinal applications. Due to their significant interest in pharmacology [1], these compounds have been the subject of extensive research. The biological activity of thiosemicarbazones is linked to their ability to chelate with transition metal ions. Previous studies on the biological properties of thiosemicarbazones and their metal complexes have found that these properties are biologically significant. Previous studies on *N*(4)-substituted thiosemicarbazones have determined that the presence of an additional potential bonding site, along with bulky groups at the *N*(4) position of the thiosemicarbazone moiety, significantly boosts biological activity [2-4].

4-Phenylthiosemicarbazones are notable for their wide range of biological activities, which have been extensively studied and documented in various research fields. These compounds exhibit strong antibacterial and antifungal activities [5]. Their effectiveness against a variety of bacterial and fungal strains makes them potential candidates for developing new antimicrobial agents. The chelation with metal ions also plays a crucial role in their antimicrobial mechanism, as it disrupts the metabolic pathways of the pathogens. These compounds also exhibit antioxidant properties, which are important for protecting cells from oxidative stress and damage caused by free radicals [6]. Their antioxidant activity contributes to their overall biological efficacy, especially in preventing and treating diseases associated with oxidative stress.

The biological activity of 4-phenylthiosemicarbazones is largely attributed to their ability to form stable complexes with transition metal ions. This chelating ability enhances their interaction with biological molecules, leading to various therapeutic effects. The presence of the phenyl group at the *N*(4) position further enhances their activity by providing additional binding sites and increasing their lipophilicity, which improves their cellular uptake and bioavailability. Due to these diverse biological activities, 4-phenylthiosemicarbazones are of great interest in medicinal chemistry and pharmaceutical research.

In this work, a new *N*-(4-methoxyphenyl)-2-oxopropanamide 4-phenylthiosemicarbazone (**HL**) and its five copper(II) coordination compounds were synthesized, and their biological activities: antibacterial and antioxidant was studied.

## MATERIAL AND METHOD

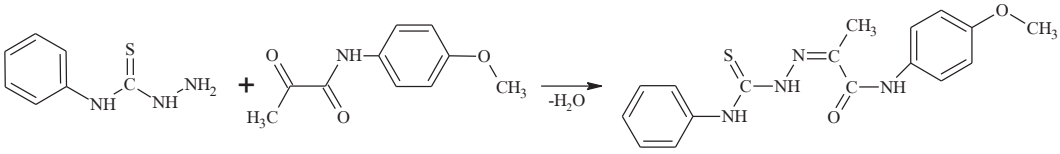
Pyruvic acid, 4-phenylthiosemicarbazide, oxalyl chloride, tributylamine, dichloromethane, dimethylformamide, 4-methoxyaniline, copper(II) chloride dihydrate, copper(II) bromide, copper(II) nitrate trihydrate, copper(II) dichloroacetate, copper(II) acetate monohydrate were obtained from Sigma-Aldrich 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 [7]. The resistance of solutions of complexes in methanol (20°C, 0.001 M) was measured using an R-38 rheochord bridge.

### *N*-(4-methoxyphenyl)-2-oxopropanamide

*N*-(4-methoxyphenyl)-2-oxopropanamide was synthesized according to the procedure described in [8] with some modifications. Pyruvic acid (8.80 g, 0.100 mol) was dissolved in 10 mL $CH_2Cl_2$ in a flat-bottomed flask. Three drops of dimethylformamide were added to the reaction mixture as a catalyst. Oxalyl chloride (15.24 g, 0.120 mol) was dissolved in 10 mL $CH_2Cl_2$ and added dropwise to the reaction mixture with stirring at 0°C. The flask with a reflux condenser was closed with a calcium chloride tube and the reaction mixture was stirred for 1.5 hours at room temperature. As a result, a yellow oily liquid was formed. A mixture of tributylamine (18.5 g, 0.100 mol), 4-methoxyaniline (12.3 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 mixture was filtered, and the resulting precipitate was purified by recrystallization from acetone to obtain a white precipitate. Yield 40%, mp. 129-130°C. FT-IR ($\nu_{max}$, $cm^{-1}$): 3333 (NH); 1714 ($C{=}O_{ketone}$); 1669 ($C{=}O_{amide}$).

### *N*-(4-methoxyphenyl)-2-oxopropanamide 4-phenylthiosemicarbazone (HL)

*N*-(4-methoxyphenyl)-2-oxopropanamide (3.86 g, 20.0 mmol) was dissolved in 25 mL of ethanol. 4-Phenylthiosemicarbazide (3.34 g, 20.0 mmol) was added to this solution while stirring and heating (Scheme 1). The resulting pale-yellow precipitate was filtered, washed with a small amount of ethanol, and air-dried. Yield 80%, mp. 207-210°C.




Scheme 1. *N*-(4-methoxyphenyl)-2-oxopropanamide 4-phenylthiosemicarbazone (**HL**)

### General method for synthesis of coordination compounds I-V

The ethanolic solution of 4-phenylthiosemicarbazone **HL** was mixed with ethanolic solution of copper(II) salt in equal molar amounts. The green precipitates have formed during stirring. The resulting precipitates were filtered off, washed with a small amount of ethanol and dried.

### Antioxidant activity

The antioxidant activity of the synthesized compounds was investigated using the $ABTS^{\bullet+}$ method described in [9]. The $ABTS^{\bullet+}$ radical was produced by mixing a 7 mM solution of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) from Sigma with a 2.45 mM solution of potassium persulfate ($K_2S_2O_8$), also from Sigma, at 25°C in the dark for 12-20 hours at room temperature. The resulting solution was diluted by combining it with acetate-buffered saline (0.02 M, pH 6.5) until an absorbance of 0.70 ± 0.01 units at 734 nm was attained. The studied compounds were diluted in DMSO, with concentrations ranging from 1 to 100 μM. Next, 20 μL of each compound's dilution was added to a 96-well microtiter plate, followed by the addition of 180 μL of the $ABTS^{\bullet+}$ working solution was dispensed using the dispense module of the hybrid reader (BioTek). The reduction in absorbance at 734 nm was accurately measured after a 30-minute incubation at 25°C. All measurements were conducted in triplicate, with DMSO serving as the negative control, and blank samples were run using solvent without $ABTS^{\bullet+}$. The hybrid reader used for the measurement was the Synergy H1 from BioTek. Each experiment was repeated three times, and the resulting data were averaged.

### Antibacterial activity

Standard strains of *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 11778), were used to study antibacterial activity of the **HL** and coordination compounds **I**–**V**. The minimum inhibitory concentrations (MICs, μg $mL^{-1}$) and minimum bactericidal concentrations (MBCs, 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 *N*-(4-methoxyphenyl)-2-oxopropanamide 4-phenylthiosemicarbazone (**HL**) was synthesized through a two-step process. In the first step, *N*-(4-methoxyphenyl)-2-oxopropanamide was generated by reacting pyruvic acid, oxalyl chloride, and 4-methoxyaniline in $CH_2Cl_2$. In the second step, the resulting *N*-(4-methoxyphenyl)-2-oxopropanamide was combined with 4-phenylthiosemicarbazide in ethanol, with both compounds used in equal molar amounts. Its structure was confirmed via NMR spectroscopy.

Five novel coordination compounds were synthesized using 4-phenylthiosemicarbazone (**HL**) as a base. Copper(II) complexes **I-V** were produced by reacting **HL** with

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 52-70 $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$ suggesting that these complexes are 1:1 electrolytes.

The spectrum of **HL** contains the absorption bands that correspond to groups: two NH, C=O, C=N and C=S. One of the ν(N-H) absorption bands disappears from the FTIR spectra of the complexes **I-V**, which points to deprotonation of the **HL**. But the position of the absorption band of the second NH group shifts to the low–frequency region. Beside this the spectra show shifts to the low–frequency region of the ν(C=O) and ν(C=N) absorption bands. Also was observed the disappearance of the ν(C=S) and appearance of the ν(C–S) absorption bands.

The antibacterial activity of the **HL** and complexes **I-V** were studied on a series of Gram-positive bacteria *Staphylococcus aureus* and *Bacillus cereus*. The obtained results in the form of minimum inhibitory/bactericidal 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 activity. Regarding *Staphylococcus aureus*, the nature of the acidic residue in the composition of copper complexes does not affect the activity of these compounds, except for complex **I**, which showed the greatest activity. Complex **I** also exhibits the greatest activity towards *Bacillus cereus*. The activity of other complexes is influenced by the nature of the acidic residue in the composition of the copper(II) complexes. The activity of the complexes decreases in the following order: $Cl^- > NO_3^- \approx Cl_2CHCOO^- > CH_3COO^- > Br^-$.

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

| Compound | *Staphylococcus aureus* (ATCC 25923) | | *Bacillus cereus* (ATCC 11778) | |
|---|---|---|---|---|
| | MIC | MBC | MIC | MBC |
| **HL** | >1000 | >1000 | >1000 | >1000 |
| **I** | 0.4883 | 0.9766 | 0.9766 | 1.953 |
| **II** | 3.906 | 7.813 | 15.63 | 15.63 |
| **III** | 3.906 | 7.813 | 1.953 | 3.906 |
| **IV** | 3.906 | 7.813 | 3.906 | 7.813 |
| **V** | 3.906 | 7.813 | 1.953 | 3.906 |

The antioxidant activity of the compounds was determined by the $ABTS^{\bullet+}$ method (Table 2). 4-Phenylthiosemicarbazone **HL** is the most active one. Its coordination to the

copper(II) atom did not lead to an increase in the activity of the resulting complexes **I-V**. Complex **IV** showed the greatest activity among all the complexes. The activity of other complexes is influenced by the nature of the acidic residue in the composition of the copper(II) complexes. The activity of the complexes varies according to the following order: $CH_3COO^- > Cl^- > Br^- > Cl_2CHCOO^- > NO_3^-$.

**Table 2.** $IC_{50}$ values of the synthesized substances towards $ABTS^{\bullet+}$ radical cation

| Compound | $IC_{50}$, μM |
|---|---|
| **HL** | 33.9 |
| **I** | 87.8 |
| **II** | 90.4 |
| **III** | ≥100 |
| **IV** | 66.3 |
| **V** | 94.9 |
| Trolox | 33.0 |

## CONCLUSIONS

The novel *N*-(4-methoxyphenyl)-2-oxopropanamide 4-phenylthiosemicarbazone (**HL**) and its five coordination compounds were synthesized and characterized. The studied complexes showed antibacterial and antioxidant activity. In the case of antibacterial activity, the coordination of 4-phenylthiosemicarbazone (**HL**) with the copper(II) atom led to an increase in the activity of the resulting complexes. Conversely, for antioxidant activity, the most active substance is **HL**, with an activity close to that of Trolox, a compound used in medicine. In both cases, the activity of copper complexes is influenced by the nature of the acid residue.

## REFERENCES

1. WEST, D.X., PADHYE, S.B., SONAWANE, P.S. Structural and physical correlations in the biological properties of transition metal heterocyclic thiosemicarbazone and *S*-alkyldithiocarbazate complexes. In: *Complex Chemistry*. 2005. nr. 76, p. 1-50. ISSN 978-3-540-46631-4.
2. JAIN, S. K., GARG, B. S., BHOON, Y. K. (1986). Characterization of copper (II) complexes of N4, N4-disubstituted thiosemicarbazones of 2-acetylpyridine by combined evaluation of electronic and ESR parameters. In: *Spectrochimica Acta Part A: Molecular Spectroscopy*. 1986. nr. 42, p. 959-968. ISSN 0584-8539.
3. HOSSAIN, M. E., ALAM, M. N., BEGUM, J., ALI, M. A., NAZIMUDDIN, M., SMITH, F. E., HYNES, R. C. The preparation, characterization, crystal structure and biological activities of some copper(II) complexes of the 2-ben-

zoylpyridine Schiff bases of S-methyl-and S-benzyldithiocarbazate. In: *Inorganica Chimica Acta*. 1996. nr. 249, p. 207-213. ISSN 1873-3255.

4. BERALO, H., GAMBINO, D. Abreaf survey of semicarbazones and their transition metal co mplexes. In: *Journal of Inorganic Chemistry*. 2004. nr. 4, p. 159. ISSN 2090-2034.
5. JOSEPH, M., KURIAKOSE, M., KURUP, M. P., SURESH, E., KISHORE, A., BHAT, S. G. Structural, antimicrobial and spectral studies of copper (II) complexes of 2-benzoylpyridine N (4)-phenyl thiosemicarbazone. In: *Polyhedron*. 2006. nr. 25, p. 61-70. ISSN 1873-3719.
6. KUMAR, L. V., SUNITHA, S., NATH, G. R. Antioxidant, antidiabetic and anticancer studies of nickel complex of Vanillin-4-Methyl-4-Phenyl-3-Thiosemicarbazone. In: *Materials Today: Proceedings*. 2021. nr. 41, p. 669-675. ISSN 2214-7853.
7. LEOVAC, V. M., VOJINOVIĆ, L. S., MESAROŠ-SEČENJI, K. F., ČEŠLJEVIĆ, V. I. Transition metal complexes with thiosemicarbazide-based ligands, part 46: Synthesis and physico-chemical characterization of mixed ligand cobalt (III)-complexes with salicylaldehyde semi-, thiosemi-an. In: *J. Serb. Chem. Soc*. 2003. nr. 68, p. 919-927. ISSN 0352-5139.
8. KOZLOVIČ, K. *Sinteza izbranih amidov piruvične kisline*. Summary of the PhD thesis in chemical sciences, Ljubljana, 2019, 46 p.
9. GARBUZ, O., GUDUMAC, V., TODERAS, I., GULEA, A. Antioxidant Properties of Synthetic Compounds and Natural Products. Action Mechanisms. Monograph. Chișinău: CEP USM, 2023, ISBN 978-9975-62-516-6.

*N.B. The work was carried out within the subprograms "Synthesis and study of new materials based on complexes with multifunctional ligands and with useful properties in medicine, biology and technology" (code: 010602) and "Evaluation of the structure and functioning of the animal world and aquatic ecosystems under the influence of biotic and abiotic factors in the context of ensuring ecological security and the well-being of the population" (code: 010701) within the institutional project.*