CZU: 542.913:547.497.1 https://doi.org/10.59295/mabt2026.11

SYNTHESIS OF COORDINATION COMPOUNDS OF SELECTED BIOMETALS WITH PYRIDINE-DERIVED N-METHOXYPHENYLTHIOSEMICARBAZONES EXHIBITING BIOLOGICAL ACTIVITY

Aliona PÎNTEA[1], Roman RUSNAC[1], Viorica RAILEAN[2,3], Olga Garbuz[4], Aurelian GULEA[1]

[1]*Laboratory of "Advanced Materials in Biopharmaceutics and Engineering," Institute of Chemistry, Moldova State University*
[2]*Department of Infectious and Invasive Diseases and Veterinary Administration, Institute of Veterinary Medicine, Nicolaus Copernicus University in Toruń, Szosa Bydgoska 13, 87-100 Toruń, Poland*
[3]*BioColl Team, Centre for Modern Interdisciplinary Technologies, Institute for Advanced Studies, Nicolaus Copernicus University in Toruń, Wileńska 4, 87-100 Toruń, Poland*
[4]*Laboratory of Systematic and Molecular Phylogeny, Institute of Zoology, Moldova State University*

Abstract

The present study reports the synthesis and characterization of coordination compounds of selected biometals Cu(II), Zn(II), Co(III), and Ni(II) with pyridine-derived N-methoxy-phenyl-thiosemicarbazone ligands. The synthesis method is based on the condensation reaction between pyridine carbonyl derivatives and substituted thiosemicarbazides under mild conditions. The biological activity of the obtained compounds was evaluated through antifungal and anticancer assays, highlighting the influence of ligand structure and metal ion nature on biological performance. The outcomes demonstrate an increased activity of the coordination compounds in comparison to the free ligands, particularly for Cu(II) and Ni(II) complexes. The study confirms the potential of these compounds as promising bioactive agents.

Keywords: coordination compounds; biometals; pyridine thiosemicarbazones; structure–activity relationship; antifungal activity; anticancer activity.

SINTEZA COMPUȘILOR COORDINATIVI AI UNOR BIOMETALE CU N METOXIFENILTIOSEMICARBAZONE PIRIDINICE CU PROPRIETĂȚI BIOLOGICE

Rezumat

Lucrarea prezintă sinteza și studiul compușilor coordinativi ai unor biometale Cu(II), Zn(II), Co(III), Ni(II) cu liganzi de tip N-metoxi-fenil-tiosemicarbazonă piridinică. Metoda de obținere se bazează pe reacția de condensare dintre derivați carbonilici piridinici și tiosemicarbazide substituite, în condiții blânde. Activitatea biologică a compușilor a fost evaluată prin teste antimicotice și anticancer, evidențiindu-se influența structurii ligandului și a naturii metalului asupra activității biologice. Rezultatele indică o creștere a activității în cazul combinațiilor coordinative comparativ cu liganzii liberi, în special a combinațiilor coordinative cu Cu(II) și Ni(II). Studiul confirmă potențialul acestor compuși ca agenți bioactivi promițători.

Cuvinte-cheie: compuși coordinativi; biometale; tiosemicarbazone piridinice; relație structură–activitate, activitate antifungică; activitate anticancer.

Introduction

The field of coordination chemistry is currently undergoing a period of intensive development, with a focus on the design and synthesis of compounds with predetermined properties. The aim of this development is to address the pressing challenges of contemporary medicine and pharmacology. The increased interest in coordination compounds of biometals such as Cu, Co, Ni, and Zn with thiosemicarbazone ligands is driven by the multifunctionality

of these systems, which exhibit a broad spectrum of biological activities, including remarkable antitumor, antibacterial, and antifungal properties.

Antonie Ablov and his collaborators have demonstrated that by modifying the peripheral structure of the organic ligand through the introduction of various fragments, such as methoxy-phenyl groups or pyridine nuclei, it is possible to fine-tune the physicochemical and biological properties of the resulting complexes [1, 2]. Pyridine-based thiosemicarbazones, in particular, are of particular interest due to their ability to coordinate metal ions through N,N,S donor atom sets, forming stable structures that can mimic enzymatic processes or selectively interact with cellular targets [3].

The findings of local research underscore the pivotal function of copper(II) and zinc(II) in the context of cellular metabolism. The administration of these elements in the form of organometallic chelates has been shown to enhance bioavailability and mitigate toxicity [4,5]. The incorporation of a methoxy-phenyl fragment into the ligand molecule is intended to optimise the lipophilicity of the complex, thereby facilitating its passage across cellular membrane barriers, which is an essential aspect for the efficacy of a pharmaceutical agent [6].

In recent years, interest in coordination compounds of Zn(II) with thiosemicarbazone-type ligands has increased considerably. The reasons for this increase are twofold: firstly, their biological potential and, secondly, the fundamental role that zinc plays in numerous biochemical processes. Due to its redox-inert nature and high biocompatibility, Zn(II) is frequently utilised in the design of compounds with pharmacological potential, with a particular focus on reducing systemic toxicity and increasing biological selectivity [7].

Recent studies demonstrate that methoxy-substituted thiosemicarbazones represent a class of ligands that directly influence their coordination behaviour. The methoxy group ($–OCH_3$), which exhibits an electron-donating character, contributes to an increased electron density on the aromatic ring and on the donor centres involved in the chelation of the metal ion. Consequently, this facilitates the formation of more stable Zn(II) complexes [8].

A significant aspect emphasised in the extant literature is the influence of the methoxy substituent position on the physicochemical and biological properties of coordination compounds. It is evident that ortho isomers are distinguished by pronounced steric effects, which have the potential to restrict ligand access to the metal centre and induce distorted coordination geometries [9]. However, meta isomers demonstrate a balance between steric and electronic effects, favouring the formation of complexes with moderate stability and a relatively ordered structure [10]. Para isomers, due to extended electron delocalization and increased accessibility of donor centres, have been found to generally lead to more stable complexes and often enhanced biological activity [11].

These correlations are supported by numerous structural studies based on X-ray diffraction, which have demonstrated that the position of the substituent directly influences coordination parameters, including metal–ligand bond lengths and the overall geometry of the complex [12].

From a structural point of view, Zn(II) complexes with thiosemicarbazones exhibit tetrahedral or octahedral geometries, depending on the denticity of the ligand and any co-ligands (Smith et al., 2022). As demonstrated in the majority of cases documented in the extant literature, the thiosemicarbazone ligands coordinate to the Zn(II) ion in bidentate (N,S) or tridentate (N,N,S) modes, forming stable chelate rings that significantly contribute to the rigidification of the complex structure. In the case of methoxy-substituted derivatives, it has been observed that the methoxy group does not participate directly in coordination. However, it has been demonstrated that the group exerts influence on the complex stability through inductive and resonance effects exerted on the conjugated system 13]. From a biological activity perspective, Zn(II) thiosemicarbazone complexes have demonstrated a broad spectrum of action. Recent studies highlight significant antimicrobial activity, particularly against Gram-positive bacteria,

as well as antifungal activity dependent on the nature of the substituents and the lipophilicity of the complex [14]. Moreover, a considerable number of studies indicate the antiproliferative potential of these compounds against tumor cell lines, including breast and colon cancer [15].

In particular, it has been found that para-methoxy derivatives of Zn(II) coordination compounds exhibit superior biological activity, which is attributed both to higher structural stability and to an enhanced ability to interact with target biomolecules. This correlation supports the importance of substituent positioning in optimizing the biological properties of these systems [16].

Pyridine-based thiosemicarbazones represent an important class of compounds in coordination chemistry and medicinal chemistry, being extensively studied due to their ability to form stable metal complexes and their diverse biological activity. In this context, derivatives substituted with N-methoxyphenyl groups have attracted particular attention, as the substituent significantly influences the electronic properties of the conjugated system, the coordination ability, and the biological activity of the resulting compounds.

The general synthesis method of these thiosemicarbazones is based on the condensation reaction between pyridine-based carbonyl derivatives (aldehydes or ketones) and substituted thiosemicarbazides, a process well documented in the literature [17]. The reaction mechanism is of the nucleophilic condensation type and proceeds through several successive steps.

In the first step, activation of the carbonyl group occurs, where the carbon atom of the C=O group may be protonated in a mildly acidic medium, leading to an increase in the electrophilicity of the carbonyl carbon and facilitating subsequent nucleophilic attack. In the second step, the terminal nitrogen atom of the $–NH_2$ group in thiosemicarbazide acts as a nucleophile, attacking the carbonyl carbon and forming a carbinolamine-type intermediate. This step is essential for initiating the condensation process and determines the pathway of product formation.

Subsequently, the carbinolamine intermediate undergoes a dehydration step, with the elimination of a water molecule, leading to the formation of the azomethine double bond (–C=N–), characteristic of the final thiosemicarbazone structure.

In the final stage, the obtained product is stabilized through extended π-conjugation within the aromatic system and the azomethine fragment, through possible intramolecular hydrogen bonding, as well as through thioamide tautomeric equilibria, which contribute to structural rigidification and the thermodynamic stability of the compound. Thus, the synthesis mechanism explains both the efficient formation of pyridine thiosemicarbazones and their structural and functional properties, relevant to their applications in coordination and bioorganic chemistry.

Overview of ligand synthesis

The synthesis of thiosemicarbazones is carried out under relatively mild conditions, making the method efficient and widely used in organic and coordination chemistry. The reaction is typically performed in polar protic solvents such as ethanol, methanol, or hydroalcoholic mixtures, which facilitate both the dissolution of the reactants and the progression of the condensation steps. These conditions are well documented in the literature regarding the synthesis of thiosemicarbazone derivatives and the optimization of carbonyl–hydrazinic condensation reactions [18–19].

The process is catalyzed by small amounts of glacial acetic acid, which acts as an activating agent for the carbonyl group without generating significant side reactions. The reaction is conducted under moderate reflux conditions at temperatures ranging between 60–80°C, ensuring suitable reaction kinetics without degradation of sensitive compounds. The reaction time varies depending on the structure of the reactants, typically between 1 and 4 hours, and the optimal molar ratio is generally 1:1 between the carbonyl compound and thiosemicarbazide [20].

Once the reaction has been completed, the reaction mixture is gradually cooled to room temperature. This process induces precipitation of the condensation product due to its low

solubility in the reaction medium. The obtained solid product is then isolated by filtration, followed by purification steps through recrystallization from suitable solvents, such as ethanol, in order to obtain a compound of high purity required for subsequent stages of structural characterization and property evaluation [21,22].

Materials and methods

The scientific research was carried out within the Laboratory of "Advanced Materials in Biopharmaceutical and Technical Applications" of the Institute of Chemistry of the State University of Moldova. The syntheses were performed using reagents purchased from "Sigma-Aldrich", "Acros Organics", or "Alfa Aesar", which were used without prior purification.

FTIR spectra were recorded on powder samples using a Bruker ALPHA instrument, in the wavenumber range of 4000–400 cm^{-1}. Nuclear Magnetic Resonance (NMR) spectra were recorded at room temperature using a Bruker DRX400 spectrometer. Acetone-d_6 was used as the solvent.

The antifungal activity was evaluated according to the serial dilution method described in the literature. The tests were carried out in the Microbiology Laboratory of the National Agency for Public Health in Chisinau.

The synthesis procedure of ligands HL1–HL3 was performed according to the method described in reference [23], as shown in Fig. 1.

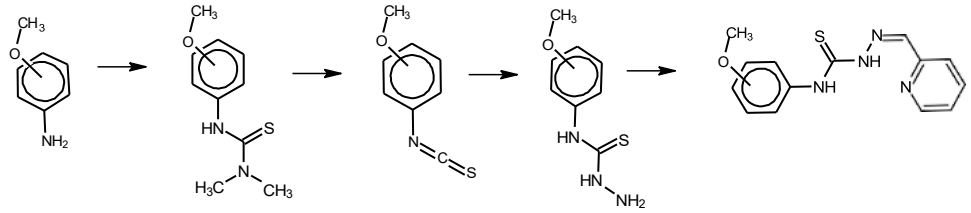


Figure 1. Synthetic scheme of thiosemicarbazones HL^1–HL^3

A mixture of equimolar amounts of a pyridine-based carbonyl compound and N-methoxyphenyl-thiosemicarbazide is dissolved in ethanol. To the resulting solution, 3 drops (~100 µL) of glacial acetic acid are added, and the mixture is heated under reflux for 3 hours with continuous stirring. The progress of the reaction is monitored by thin-layer chromatography (TLC). Once the reaction mixture has been cooled to room temperature, the resulting solid product is isolated by filtration, washed with cold ethanol, and dried under vacuum. The purification of the substance is achieved through recrystallization from ethanol. The condensation method between pyridine-based carbonyl derivatives and N-methoxyphenyl-thiosemicarbazide represents an efficient and versatile approach for obtaining functionalized thiosemicarbazones. This synthetic strategy allows the modulation of the physicochemical and biological properties of the compounds, being particularly useful in the development of ligands for coordination chemistry and bio-pharmaceutical applications, whose structures are presented in Fig. 2.

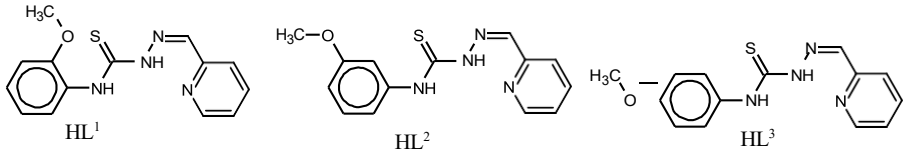


Figure 2. Structural formulas of the synthesized ligands

The synthesis of coordination compounds [Cu(L)Cl], [Cu(L)(H_2O)NO_3], [Co($L)_2$]Cl, [Zn(HL)Cl_2], and [Ni($Ly)_2$], was carried out according to the general synthetic procedure described in reference [24]. In a conical flask, 1 mmol of ligand and 1 mmol of $MX_2 \cdot nH_2O$ are introduced in 20 mL of ethanol. The reaction mixture is stirred for 2 hours at 70–75 °C. After completion, the mixture is cooled to room temperature; the resulting precipitate is filtered, washed with cold ethanol, and dried in a desiccator to constant mass.

Biological evaluation methods of antifungal activity

The antimycotic properties of the synthesized compounds were investigated *in vitro* against the laboratory strain *Candida albicans*. The activity was determined in Sabouraud liquid nutrient medium (pH = 6.8). Inocula were prepared from fungal cultures aged 3–7 days. Their concentration in suspension was adjusted to (2–4) × 10^6 colony-forming units per millilitre [23–24].

The evaluation of the antifungal activity of organic compounds and synthesized coordination complexes represents a fundamental stage in the study of their biological properties, being essential for identifying potential antifungal agents. In particular, pyridine-based thiosemicarbazone derivatives and their metal coordination complexes are extensively studied due to their ability to interact with fungal biological systems and to exhibit significant biological effects. Numerous studies have shown that the antifungal activity of these compounds is influenced both by the structure of the ligand and by the nature of the metal center, with complexes often exhibiting higher activity than the free ligand due to increased lipophilicity and improved transport across cellular membranes.

The antifungal testing procedure begins with the preparation of biological material, which involves the selection of standardized fungal strains such as *Candida albicans*, cultivated on appropriate nutrient media such as Sabouraud or RPMI 1640. The fungal suspension is adjusted to a standardized concentration expressed in colony-forming units per millilitre to ensure experimental reproducibility. Control of incubation parameters, including temperature and exposure time, is essential for obtaining comparable and reproducible results.

Subsequently, the tested compounds are dissolved in suitable solvents such as dimethyl sulfoxide or ethanol, yielding homogeneous solutions that are subjected to biological evaluation. One of the most commonly used methods is the disc diffusion assay (Fig. 3), which allows a preliminary assessment of antifungal activity. In this method, a solid medium uniformly inoculated with the fungal suspension serves as a support for discs impregnated with the tested compound. During incubation, the substance diffuses into the medium, and in the presence of antifungal activity, a clear inhibition zone forms around the disc, corresponding to suppression of fungal growth.

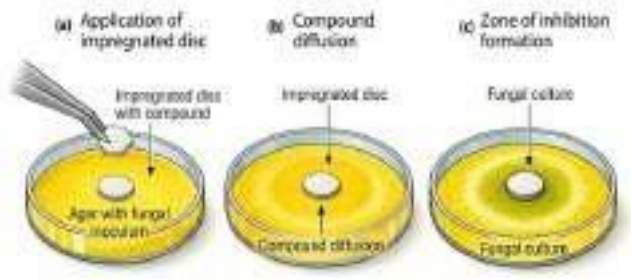


Figure 3. Schematic representation of the disc diffusion method for the evaluation of antifungal activity: (a) application of the compound-impregnated disc onto the inoculated medium, (b) diffusion of the compound into the agar, (c) formation of the inhibition zone.

The image illustrates the uniform distribution of the fungal inoculum on the surface of the agar medium and the appearance of circular inhibition zones around the discs impregnated with the active substance. Clear zones indicate inhibition of microbial growth and are directly correlated with the sensitivity of the fungus to the tested compound. The diameter of these zones is used to evaluate antifungal activity and can be correlated with minimum inhibitory concentration values, according to standardized protocols.

Despite the efficacy of this approach for rapid screening, a more precise quantitative assessment of antifungal activity is achieved through the utilisation of the broth dilution method, particularly the microdilution variant. This involves preparing a series of dilutions of the compound in a liquid nutrient medium, followed by inoculation of each sample with the standardized microorganism (Fig. 4). After incubation, fungal growth is assessed by observing turbidity or through spectrophotometric measurements, and the lowest concentration at which no visible growth is detected is defined as the minimum inhibitory concentration (MIC).

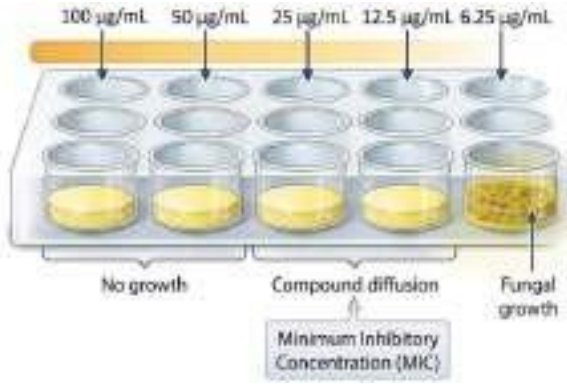


Figure 4. Determination of the minimum inhibitory concentration (MIC) by the microdilution method: variation of compound concentration in wells and assessment of fungal growth inhibition

The figure shows a 96-well microtiter plate used for the determination of the minimum inhibitory concentration (MIC), in which the concentration of the compound decreases progressively along the dilution series. Wells exhibiting turbidity indicate fungal growth, whereas clear wells indicate complete inhibition of growth.

The MIC is defined as the lowest concentration at which no visible growth is observed, typically determined either by optical density measurements or by visual inspection. In order to distinguish between fungistatic and fungicidal effects, the minimum fungicidal concentration (MFC) is also determined. This involves transferring samples from wells without visible growth onto solid media and assessing the ability of the microorganisms to regrow.

The absence of growth confirms the lethal effect on the microorganisms, whereas regrowth indicates only temporary inhibition of fungal development (Fig. 5).

The image illustrates the transfer of samples from a liquid medium (with no visible growth) onto a solid medium for the evaluation of fungal viability. The presence of colonies indicates that the microorganisms were not completely destroyed, whereas their absence confirms the fungicidal effect of the compound.

This method enables a clear distinction between fungistatic and fungicidal activity and is used as a complementary approach to MIC determination. In the case of coordination complexes, antifungal activity is influenced by both the nature of the metal and the structure of the ligand.

Studies indicate that transition metals such as copper or nickel can enhance biological activity through mechanisms including the generation of reactive oxygen species and interaction with fungal enzymes.

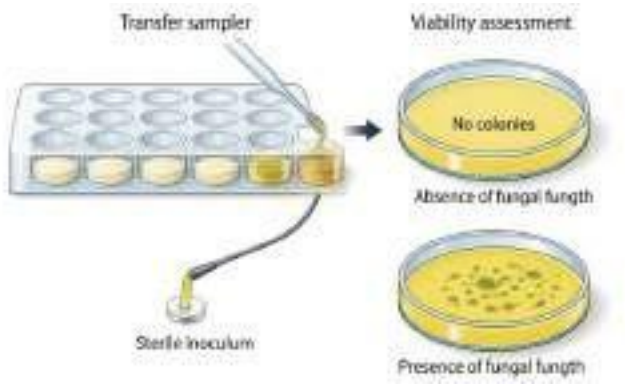


Figure 5. Schematic representation of the determination of the minimum fungicidal concentration (MFC): transfer of samples onto solid medium and evaluation of fungal viability

Ligand coordination reduces polarity and enhances membrane permeability, which explains the higher activity of the complexes compared to the free ligands, as exemplified in Fig. 6.



Figure 6. Comparative representation of antifungal activity: free ligand versus coordination complexes, highlighting the increase in biological efficacy

The figure illustrates the relationship between the inhibition zone diameter and the minimum inhibitory concentration, demonstrating an inverse proportionality between the two variables. Compounds that yield substantial inhibition zones characteristically demonstrate low MIC values, suggesting a high degree of antifungal potency. This correlation is utilised for the validation of screening methods and the interpretation of experimental results in comparative studies.

The results obtained are frequently represented graphically as comparative diagrams, thus allowing the structure-activity relationships to be highlighted. These representations evidently demonstrate that structural modifications and the introduction of a metal centre result in improved biological properties, thereby confirming the significance of coordination chemistry in the development of new antifungal agents [25–26].

MTT assay for cell proliferation

The MTT assay, a colorimetric method, quantifies viable cells in order to evaluate cytotoxic effects on the MCF-7 cell line. It measures the conversion of MTT into insoluble formazan by mitochondrial succinate dehydrogenase in metabolically active cells, thereby reflecting cellular viability. The tested compounds were dissolved in saline solution and DMSO (≤0.1%) and evaluated at concentrations of 0.1, 1, and 10 μM, following the method described by Mosmann [27–28].

Cells were centrifuged, while adherent cells were trypsinized and then diluted to 5×10^4 cells/mL in RPMI 1640 medium supplemented with 10% fetal bovine serum. Subsequently, 5×10^3 cells in 100 μL were seeded into 96-well plates and incubated to allow cell attachment. After compound addition, the cells were incubated for 24 hours at 37 °C and 3% CO_2.

Subsequently, the MTT reagent was added, and the cells were monitored under a microscope for formazan formation. Following the appearance of the purple precipitate, a detergent reagent was added, and the plates were stored in the dark for a period of 4 hours. Absorbance was measured at a wavelength of 540 nm, employing a BioTek plate reader (Agilent, Santa Clara, CA, USA).

The results, expressed as percentage inhibition, were calculated using the following formula:

$$I\,(\%) = 100 - \frac{Abs_{570\,nm\,(sample)} - Abs_{600\,nm\,(sample)}}{Abs_{540\,nm\,(control)} - Abs_{600\,nm\,(control)}} \times 100 \quad (1)$$

The formula is based on the principle of determining cell viability by accounting for the differences in light absorption at 570 nm and 600 nm (Equation 1).

The percentage of inhibition was calculated as follows: The methodology for the anticancer evaluation of thiosemicarbazones and Cu(II) coordination compounds in breast cancer is based on a complex, integrated approach that allows the assessment of both cytotoxic effects and the underlying molecular mechanism of action. The interest in these systems is due to the fact that these semicarbazones and their coordination complexes exhibit significant antiproliferative activity against breast cancer cell lines, such as MCF-7 (estrogen receptor-positive, ER+), due to their ability to interact with DNA, disrupt metal homeostasis, and induce oxidative stress, ultimately leading to programmed cell death [27].

The experimental process begins with the cultivation of tumor cell lines under standardized conditions, using appropriate nutrient media such as RPMI-1640, supplemented with fetal bovine serum and antibiotics, in a controlled atmosphere of 5% CO_2 at 37 °C. Cells are seeded into culture plates, typically 96-well plates, at an optimal density that ensures uniform growth and experimental reproducibility, as exemplified in Fig. 7.

After stabilization of the cell culture, the investigated compounds are added at various concentrations obtained through serial dilution, allowing the analysis of dose–response relationships and the determination of specific pharmacological parameters [27].

The evaluation of cytotoxic effects is primarily performed using the MTT assay, which is based on the enzymatic reduction of tetrazolium salts to formazan in viable cells. The intensity of the resulting color is proportional to cellular metabolic activity and is measured spectrophotometrically. A decrease in absorbance indicates reduced cell viability, and graphical representation of the data allows determination of the IC_{50} value, corresponding to the concentration required to inhibit 50% of the cell population [27].



Figure 7. Evaluation of cytotoxic effects using the MTT assay in MCF-7 cells

Results and Discussion

Following the synthesis procedures, three ligands and coordination complexes of Zn(II), Cu(II), Co(III), and Ni(II) were obtained, on which a series of antifungal investigations were carried out. The analysis of the experimental data (Fig. 8) shows that the antifungal activity of thiosemicarbazones is strongly dependent both on the structure of the ligand and on the nature of the metal ion involved in the formation of the coordination complexes. It has been demonstrated that Cu(II) thiosemicarbazone complexes exert a strong anticancer effect through a multistep mechanism involving the following processes: cellular uptake, redox cycling, and ROS generation [34]. The produced oxidative stress leads to significant DNA damage, including strand breaks and base oxidation, as well as interference with DNA topology and replication processes. These molecular events activate apoptotic signaling pathways, characterized by caspase cascade activation and PARP cleavage, ultimately resulting in programmed cell death. The MTT assay confirms a concentration-dependent decrease in cell viability, reflected in low IC_{50} values for the tested complexes. Overall, Cu(II)-TSC complexes act as multitarget agents, combining oxidative stress induction, DNA interaction, and apoptosis activation, which explains their enhanced cytotoxic activity compared to free ligands. Additionally, the redox properties of copper favor the generation of reactive oxygen species, which contribute to the damage of cellular components and further enhance the cytotoxic effect.Coordination complexes of copper(II) with thiosemicarbazone-type ligands (Cu(II)-TSC) represent a promising class of non-platinum antitumor agents. Their high biological activity is attributed to a multifactorial mechanism of action that exploits both the redox properties of the copper ion and the ability of the organic ligand to coordinate biometals and interact with cellular targets.

In contrast to the covalent binding of cisplatin to DNA, copper(II) coordination complexes have been observed to exert their effect by the generation of oxidative stress and the interference with essential enzymatic processes. Confirmation of the apoptotic process is achieved through complementary methods such as fluorescent staining, DNA fragmentation analysis, and flow cytometry, which allow the identification of structural and functional changes in treated cells. These techniques demonstrate that treatment with coordination complexes induces chromatin condensation, nuclear fragmentation, and cell accumulation in specific phases of the cell cycle, indicating a clear mechanism of proliferation inhibition.

In the case of copper coordination complexes, anticancer activity is enhanced by the synergistic effect between the ligand and the Cu(II) metal ion. Cu(II) can participate in intracellular redox reactions, generating free radicals that induce selective oxidative stress in tumor cells. This characteristic contributes to the increased selectivity of these compounds toward normal cells and explains the low IC_{50} values observed in experimental studies [29, 31].

This increase in activity can be explained by changes in physicochemical properties induced by metal coordination. Complex formation leads to an increase in lipophilicity, which facilitates passage through the cell membrane, in accordance with Overton's theory. As a result, the coordination compound penetrates tumor cells more efficiently, reaching higher intracellular concentrations and enhancing the cytotoxic effect.

From a mechanistic perspective, the differences between the free ligand and the metal complex are significant. The uncoordinated ligand acts predominantly through limited mechanisms, such as specific interactions with certain enzymes or cellular receptors, leading to a relatively moderate cytotoxic effect. In contrast, Cu(II) coordination complexes exhibit a dual mechanism of action that is much more aggressive toward cancer cells. They are capable of generating reactive oxygen species through Fenton-type redox reactions, inducing intense oxidative stress. The consequences of this process include degradation of cellular macromolecules, DNA fragmentation, and impairment of mitochondrial function, ultimately leading to apoptosis.

Another significant finding, as indicated by the data analysis, is the variation in the specificity of the compounds according to the cell line employed. However, complexation with Cu(II) has been shown to help partially overcome this resistance, suggesting an increased therapeutic potential of these coordination systems in the treatment of more aggressive forms of breast cancer. Overall, the comparative analysis shows that transforming the organic ligand into a copper coordination complex not only enhances anticancer activity but also profoundly modifies the mechanism of action, thereby offering significant advantages in the development of new effective therapeutic agents.

Validation of the in vitro results is performed through in vivo studies on experimental models, where tumor progression and systemic effects of the compounds are monitored. Tumor reduction and the absence of severe toxicity confirm the potential of these substances as effective therapeutic agents. The integration of these results allows the establishment of a clear structure–activity relationship, providing important perspectives for the development of new anticancer drugs [30–33].The free ligands exhibit moderate activity, predominantly fungistatic, indicating an intrinsic biological potential but one that remains limited in the absence of metal coordination. The introduction of metal ions leads to significant changes in activity; however, the effect is not uniform, being decisively influenced by the type of metal and the position of the methoxy substituent.

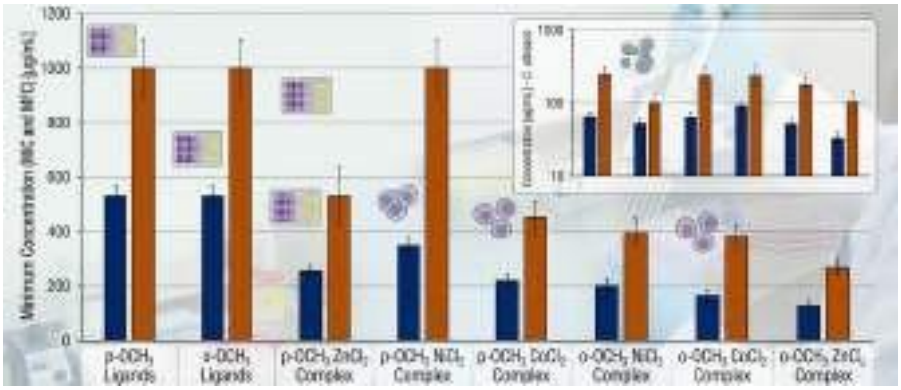


Figure 8. Comparative analysis of antifungal activity (MIC and MFC) for free ligands and metal complexes of Zn(II), Ni(II), and Co(III), highlighting the influence of methoxy substituents (ortho, meta, para) on biological efficacy, including activity against *Candida albicans*

The most significant result is represented by the Ni(II) coordination complexes with the para-methoxy ligand, which exhibit superior antifungal activity, with equal values of minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC), demonstrating a pronounced fungicidal effect. This behaviour suggests the existence of an optimal synergy between the complex geometry, electronic properties, and the ability to interact with fungal biological targets.

In contrast, other coordination complexes, particularly those of Zn(II) and Co(III), have been observed to reduce antifungal activity, indicating that not every complexation process leads to biological enhancement, but only certain structurally favorable combinations.

The outcomes of this study demonstrate that the antifungal efficacy of these systems is determined by a delicate balance between ligand structure and the nature of the metal center. Ni(II) complexes bearing para-methoxy substitution emerge as the most promising candidates for the development of fungicidal antifungal agents.

The comparative analysis of the experimental data allows for the identification of structure-activity relationships, which contributes to a more profound understanding of the mechanisms through which the compounds interact with biological targets. In this context, graphical representation of the results is an essential tool for identifying trends and significant variations among the investigated samples.

The anticancer activity of the ligands (*o-, m-, p-OCH₃*) and their Cu(II) coordination compounds on a breast cancer cell line provides insight into their efficacy (Fig. 9).

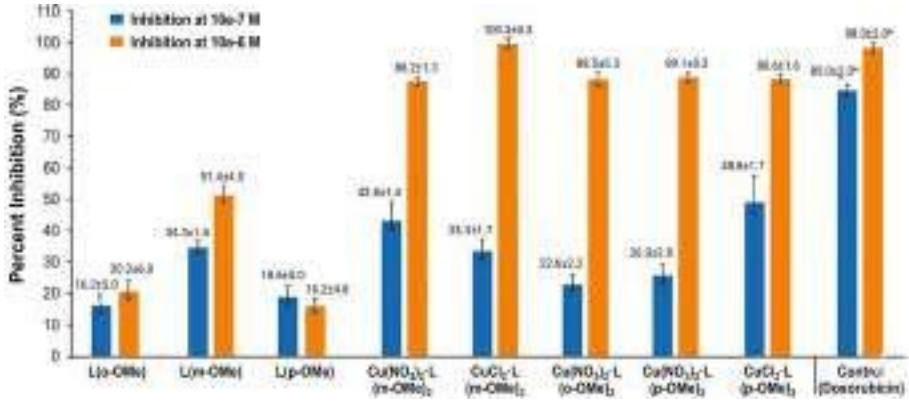


Figure 9. Anticancer activity of the ligands (o-, m-, p-OCH_3) and their corresponding Cu(II) complexes against a breast cancer cell line.

The comparative analysis of the data clearly shows that the cytotoxic activity of the studied compounds is strongly influenced both by concentration and by their chemical nature. First, a general dose - response behavior is observed, with most compounds showing a significant increase in the percentage of inhibition at a concentration of 10^{-6} M compared to 10^{-7} M. This trend confirms that biological efficacy is dose - dependent, although minor deviations are observed in the case of some free ligands.

The most significant discrepancies are evident between the uncomplexed ligands and their coordination compounds. The free ligands demonstrate moderate cytotoxic activity, with relatively low inhibition values, whereas the copper(II) complexes exhibit a marked increase in efficacy, frequently reaching values above 85–90% at higher concentrations. This observation suggests that metal ion incorporation significantly modifies the properties of the compounds, enhancing their interaction with biological targets.

Furthermore, the position of the methoxy substituent on the aromatic nucleus has been demonstrated to clearly influence activity. The meta derivatives demonstrate the highest efficiency, a notable example being the corresponding copper chloride complex, which attains maximal inhibition values slightly above 100%. In contrast, the ortho and para derivatives, although active, show slightly lower values, suggesting the influence of steric and electronic effects on the mechanism of action.

Regarding the comparison with the standard used, most of the metal complexes reach or approach the inhibition level of the reference compound, indicating a high biological potential. Overall, the data support the idea that copper complexes, especially those derived from the meta isomer, represent promising candidates for the development of cytotoxic agents, justifying further in-depth investigations.

Conclusions

The present study highlights the major importance of pyridine-based thiosemicarbazones substituted with N-methoxyphenyl groups in the development of coordination systems with high biological potential. The research demonstrated that the synthetic method based on the condensation reaction between pyridine carbonyl derivatives and substituted thiosemicarbazides is efficient, reproducible, and adaptable, enabling the preparation of ligands with well-defined structural properties.

A key finding confirmed by the results is the decisive role of ligand structure and metal ion nature in modulating biological activity. The complexation of thiosemicarbazones with biometal ions such as Cu(II), Zn(II), Co(III), and Ni(II) leads to significant changes in the physicochemical properties of the coordination compounds.

Biological studies have shown that free ligands exhibit moderate activity, whereas coordination compounds often display enhanced activity, attributed to increased lipophilicity and improved penetration into target cells. In particular, Cu(II) complexes demonstrated significant anticancer potential through complex mechanisms involving DNA interaction and the induction of oxidative stress, leading to cellular apoptosis.

Regarding antifungal activity, the results show that it is strongly influenced by both the nature of the metal and the position of the methoxy substituent. The Ni(II) complex with para-methoxy substitution proved to be the most effective, exhibiting a pronounced fungicidal effect, suggesting an optimal synergy between electronic structure and molecular geometry.

Furthermore, structure - activity relationship analysis confirmed that the introduction of the methoxy group contributes to the optimization of electronic properties and enhances the biological potential of the compounds, while its position on the aromatic nucleus plays a crucial role in determining the final efficacy.

Acknowledgements

We would like to express our gratitude to Dr. habil. Olga Burduniuc, Associate Research Professor, Head of Department at the National Agency for Public Health, Chișinău, Republic of Moldova, for conducting the antifungal investigations.

The experimental material presented in this article was developed within the institutional subprogram “Synthesis and study of new materials based on complex compounds with polyfunctional ligands and useful properties in medicine, biology and engineering”, code: 010602; details at: https://ichem.md/SSMCCLP, Republic of Moldova.

References:

1. VAN, Hoang Thi Kim; THANH, Nguyen Dinh. Maltose-Conjugated Thiosemicarbazones from Substituted Benzaldehydes: Synthesis and Inhibitory Activity against Gram-(+) and Gram-(−)

Bacteria. *ACS Medicinal Chemistry Letters*, 2026, 17.3: 670-678. https://doi.org/10.1021/acsmedchemlett.5c00700

2. GULEA, Aurelian. Noi inhibitori de proliferare a celulelor de cancer. *Revista de Ştiinţă, Inovare, Cultură şi Artă „Akademos”*, 2013, 28.1: 102-107. https://ibn.idsi.md/sites/default/files/imag_file/Noi%20inhibitori%20de%20proliferare%20a%20celulelor%20de%20cancer.pdf
3. GULEA Aurelian; RUSNAC Roman; BĂLAN Greta; RUSNAC Anna; NICOLENCO Nicoleta; ŢAPCOV Victor. Utilizarea nitrato-[N-ciclohexil-N'-(piridin-2-ilmetiliden)carbamohidrazontioato]cupru in calitate de inhibitor al proliferării bacteriilor din specia *Acinetobacter baumannii*. Brevet de invenție 4761 (13)B1. Nr. depozit: a 2020 0019; Data depozit: 2020.03.05; Disponibil la: https://db.agepi.md/Inventions/details/a%202020%200019
4. ALY, Ashraf A.; HASSAN, Alaa A.; ABDEL-LATIEF, El-Shaimaa SM. An update of the use of thiocarbohydrazides and thiosemicarbazides in the preparation of heterocycles and their biological importance. *Journal of Heterocyclic Chemistry*, 2018, 55.10: 2196-2223. https://doi.org/10.1002/jhet.3295
5. YADAV, Paras Nath, et al. N(3)-substituted thiophene-2-carboxaldehyde thiosemicarbazones compounds and their copper (II) complexes: Synthesis, characterization, in silico study and anticancer activity. *Polyhedron*, 2026, 118022. https://doi.org/10.1016/j.poly.2026.118022
6. JYOTHI, P. Recent advances in thiosemicarbazone metal complexes: structure–activity relationship and therapeutic prospects. *Discover Chemistry*, 2026, 3.1: 73. https://doi.org/10.1007/s44371-026-00538-3
7. TARAI, Arup, et al. Thiosemicarbazones as ion chelators: Unveiling their coordination and multifaceted applications. *Dyes and Pigments*, 2025, 113419. https://doi.org/10.1016/j.dyepig.2025.113419
8. SINGH, Vipin, et al. Investigation of the anticancer potential of newly synthesized N4-substituted thiosemicarbazones: In silico and in vitro biological approaches. *Journal of Molecular Structure*, 2025, 1324: 140764. https://doi.org/10.1016/j.molstruc.2024.140764
9. BERALDO, Heloisa; GAMBINOB, Dinorah. The wide pharmacological versatility of semicarbazones, thiosemicarbazones and their metal complexes. *Mini reviews in medicinal chemistry*, 2004, 4.1: pp. 31-39. https://doi.org/10.2174/1389557043487484
10. LOBANA, Tarlok S., et al. Bonding and structure trends of thiosemicarbazone derivatives of metals—an overview. *Coordination Chemistry Reviews*, 2009, 253.7-8: pp.977-1055. https://doi.org/10.1016/j.ccr.2008.07.004
11. PAHONTU, Elena, et al. Antibacterial, antifungal and in vitro antileukaemia activity of metal complexes with thiosemicarbazones. *Journal of cellular and molecular medicine*, 2015, 19.4: pp, 865-878. https://doi.org/10.1111/jcmm.12508
12. SCHWITALLA, Kevin, et al. Water-Soluble, Titanocene-Based Prodrugs with Thiosemicarbazones as Cytotoxic Agents. *ACS omega*, 2025, 10.36: 41677-41687. https://doi.org/10.1021/acsomega.5c05481
13. KOGA, Hiroyasu. Antifungal Susceptibility Testing Method for Dermatophytes-Determination of MIC and MFC Values. *Medical Mycology Journal*, 2014, 55.2. DOI: 10.3314/mmj.55.J57
14. SHAHZADI, Anam, et al. Synthesis, Characterization, Anticancer, Antioxidant, and In Silico Evaluation of Semicarbazides and Thiosemicarbazides. *ACS omega*, 2025, 10.30: 33383-33392. https://doi.org/10.1021/acsomega.5c03358
15. PANDEY, Shashank, et al. Zn (II)/Cd (II) thiosemicarbazone complexes: synthesis, structural characterisation, and comparative multifunctional applications. *Inorganica Chimica Acta*, 2026, 123229. https://doi.org/10.1016/j.ica.2026.123229
16. SINGH, Vipin, et al. Exploring the biological potential of N4-substituted thiosemicarbazones derived from 5-bromo salicylaldehyde: In silico and in vitro evaluation. *Journal of Molecular Structure*, 2025, 144676. https://doi.org/10.1016/j.molstruc.2025.144676
17. MAHENDIRAN, Dharmasivam, et al. Copper complexes as prospective anticancer agents: In vitro and in vivo evaluation, selective targeting of cancer cells by DNA damage and S phase arrest. *RSC advances*, 2018, 8.30: pp. 16973-16990. DOI: 10.1039/C8RA00954F

18. VEG, Ekhlakh, et al. Some Biological Applications and Mechanistic Insights of Benzaldehyde-Substituted Thiosemicarbazones and Their Metal Complexes: A Review. *Natural Sciences*, 2025, 5.1-2: e70005. https://doi.org/10.1002/ntls.70005
19. PALIWAL, Kumudini, et al. Targeting Triple Negative Breast Cancer with a Dinuclear Copper (II) Thiocarbohydrazone Complex: Efficacy Evaluation and Cellular Response. *ACS omega*, 2025, 10.36: 41342-41358. https://doi.org/10.1021/acsomega.5c04277
20. ABDOU, Achraf, et al. Design, synthesis, and antibacterial evaluation of novel thiosemicarbazone derivatives from estragole. *Natural Product Research*, 2025, 1-9. https://doi.org/10.1080/14786419.2025.2608710
21. LANERO, Francesco, et al. Non-covalent interactions in thiosemicarbazone coordination chemistry: From crystal engineering to functional materials. *Coordination Chemistry Reviews*, 2026, 558: 217722. https://doi.org/10.1016/j.ccr.2026.217722
22. PALIWAL, Kumudini, et al. Targeting Triple Negative Breast Cancer with a Dinuclear Copper (II) Thiocarbohydrazone Complex: Efficacy Evaluation and Cellular Response. *ACS omega*, 2025, 10.36: pp. 41342-41358. https://doi.org/10.1021/acsomega.5c04277
23. FUIOR, Arcadie, et al. Synthesis, Structures, and Solution Studies of a New Class of [Mo2O2S2]-Based Thiosemicarbazone Coordination Complexes. *ACS omega*, 2022, 7.19: 16547-16560. https://doi.org/10.1021/acsomega.2c00705
24. RUSNAC, Roman, et al. Synthesis, Characterization, and Biological Properties of the Copper (II) Complexes with Novel Ligand: N-[4-({2-[1-(pyridin-2-yl) ethylidene] hydrazinecarbothioyl} amino) phenyl] acetamide. *Inorganics*, 2023, 11.10: 408. https://doi.org/10.3390/inorganics11100408
25. SHAO, Jia, et al. Thiosemicarbazone Cu (II) and Zn (II) complexes as potential anticancer agents: Syntheses, crystal structure, DNA cleavage, cytotoxicity and apoptosis induction activity. *Journal of Inorganic Biochemistry*, 2014, 136: pp. 13-23, https://doi.org/10.1016/j.jinorgbio.2014.03.004.
26. MACIJEWSKA, N., RISTIĆ, P., KALLINGAL, A. et al. Multifaceted biological and computational assessment of aromatic and N-heteroaromatic non-substituted thiosemicarbazones. *Sci. Rep.* (2026). https://doi.org/10.1038/s41598-026-44568-4
27. RUSNAC, Roman, et al. Novel Copper (II) Coordination Compounds Containing Pyridine Derivatives of N 4-Methoxyphenyl-Thiosemicarbazones with Selective Anticancer Activity. *Molecules*, 2024, 29.24: 6002. https://doi.org/10.3390/molecules29246002
28. GULEA Aurelian; RUSNAC Roman; RUSNAC Anna; ȚAPCOV Victov. Etil-4-{2-[(piridin-2-ilmetiliden)hidrazinocarbotioil]amino}benzoat monohidrat care manifestă proprietăți de inhibitor al proliferării celulelor HL-60 ale leucemiei mieloide umane. Brevet de invenție 4613 (13) C1. Nr. depozit: a 2017 0078; Data depozit: 2017.09.11. Detalii la: https://db.agepi.md/Inventions/details/a%202017%200078/LinkTitluElib
29. GRAUR Vasilii, GULEA Aurelian. Novel biological agents based on metal complexes of N4-allylthiosemicarbazones and their derivatives. ISBN 978-9975-62-716-0. Chișinău 2024. Disponibil la: https://ichem.md/sites/default/files/2025-01/Monograph%20Graur%20Gulea%202024.pdf
30. DOS REIS, Camilla Moretto, et al. Microwave-assisted synthesis of new N1,N4-substituted thiosemicarbazones. *Molecules*, 2011, 16.12: 10668-10684. https://doi.org/10.3390/molecules161210668
31. KUVAKIN, Alexander S., et al. Benzalacetone-Based β-Isothiocyanatoketones: Synthesis and Transformation to Diaryl-Substituted 14-Membered Cyclic Thiosemicarbazones and Related Heterocycles. *The Journal of Organic Chemistry*, 2025, 90.50: 17814-17838. https://doi.org/10.1021/acs.joc.5c02318
32. AYIKKODAN VEETTIL, Ashwathi; KUMAR, Namadari Sai; BASHEER, Sabeel Mohammed. Synthesis and Photoluminescence Properties of Quinoline-Derived Thiosemicarbazone Lanthanide Complexes and Their Polymer-Embedded Stability. *ACS Applied Optical Materials*, 2025, 3.12: 2857-2869. https://doi.org/10.1021/acsaom.5c00436
33. CASTIÑEIRAS, Alfonso, et al. Reactivity of Acetylbarbituric Thiosemicarbazone Derivatives with Silver (I) Nitrate and the Influence of Substituents at Nitrogen Atom 4N on the Bonding and Nuclearity of the Resulting Silver (I) Complexes. *ACS omega*, 2025, 10.37: 42862-42881. https://doi.org/10.1021/acsomega.5c05539

34. HANGAN, ADRIANA CORINA, et al. Copper Complexes: Main Mechanisms as Anticancer Agents. *Molecules*, 2026, 31.5: 874. https://doi.org/10.3390/molecules31050874

Authors:



Aliona PÎNTEA, PhD student / junior research scientist, Laboratory of Advanced Materials in Biopharmaceutics and Technics, Institute of Chemistry, State University of Moldova, Chișinău, Republic of Moldova.
e-mail: pintea.aliona@usm.md
ORCID: 0009-0008-1713-9195



Roman RUSNAC, Doctor in Chemical Sciences, Senior Research Scientist / University Lecturer, Laboratory of Advanced Materials in Biopharmaceutics and Technics, Institute of Chemistry, State University of Moldova, Chișinău, Republic of Moldova.
e-mail: roman.rusnac@usm.md
ORCID: 0000-0002-5713-5251



Viorica RAILEAN, PhD in Chemical Sciences, Department of Infectious and Invasive Diseases and Veterinary Administration, Institute of Veterinary Medicine, Nicolaus Copernicus University in Toruń, 13 Szosa Bydgoska Street, 87-100 Toruń, Poland. BioColl Team, Center for Modern Interdisciplinary Technologies, Institute for Advanced Studies, Nicolaus Copernicus University in Toruń, 4 Wileńska Street, 87-100 Toruń, Poland.
e-mail: viorica.railean@umk.pl
ORCID: 0000-0001-9916-0095



Olga Garbuz, PhD in Biological Sciences, Laboratory of Systematics and Molecular Phylogenetics, Institute of Zoology, State University of Moldova, Chișinău, Republic of Moldova.
e-mail: olga.garbuz@sti.usm.md
ORCID: 0000-0001-8783-892X



Aurelian GULEA, Academician, Doctor Habilitate, University Professor, Laboratory of Advanced Materials in Biopharmaceutics and Technics, Institute of Chemistry, Moldova State University, 60 Mateevici Street, MD-2009 Chisinau, Moldova
E-mail: guleaaurelian@gmail.com
ORCID: 0000-0003-2010-7959