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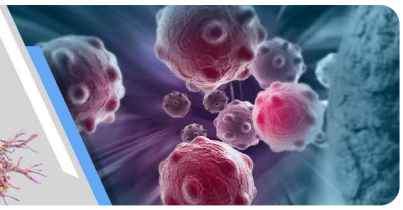

Biological Activity of Copper(II) Complex (2-((2-(Prop-2-En-1-Ylcarbamothioyl)Hydrazinylidene)Methyl)Phenolato)-Chloro-Copper(II) Monohydrate

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Abstract

The ligand 2-(2-hydroxybenzylidene)-*N*-(prop-2-en-1-yl) hydrazinecarbothioamide **(H_2L)** and its copper(II) coordination compound (2-((2-(prop-2-en-1-ylcarbamothioyl)hydrazinylidene)methyl)phenolato)-chloro-copper(II) monohydrate **[Cu(HL)Cl]•H_2O** were synthesized. The antiproliferative activity of the tested compounds was assessed *in vitro* using RD (human muscle rhabdomyosarcoma spindle and large multinucleated cells), HeLa (human cervix adenocarcinoma cells), BxPC-3 (human pancreatic adenoma cells) and MDCK (Madin-Darby canine kidney epithelial normal cells) cell lines, and their selectivity indexes (SIs). The tested complex exhibited superior anticancer activity compared to Doxorubicin (DOXO), with lower IC_{50} values against cancer cells and SIs exceeding those of DOXO. The tested compounds demonstrated high antioxidant effects against $ABTS^{\bullet+}$ radical cation. Furthermore, the toxicity of the compounds was investigated *in vivo* using *Daphnia magna* through bioassay and microanalysis. The results suggest that the tested copper complex has potential as an effective anticancer therapy candidate.

Keywords: Copper(II); Coordination compound; Cell lines; Anticancer agent; Selectivity; Toxicity.

Introduction

In 2022, according to the World Health Organization (WHO), there were an estimated 20 million new cases of cancer and 9.7 million deaths attributed to the disease. The WHO is predicting that more than 35 million new cancer cases will be detected in 2050. That is a 77 percent increase from 2022. Cancer, an intricate array of diseases, is characterized by the unbridled proliferation of cells. This uncontrolled cellular growth not only fosters the formation of tumor tissues but also poses the ominous threat of metastasis to distant organs. Despite the remarkable progress achieved in the field of anticancer therapy, significant challenges such as high systemic toxicity and drug resistance persist, undermining the efficacy of modern medicine's fight against cancer. Chemotherapy, celebrated for its effectiveness, often exhibits toxic effects on both cancerous and healthy cells. These adverse effects frequently necessitate treatment interruptions or even discontinuation. Thus, there is an urgent need for anticancer drugs to target proliferating cancer cells with precision, sparing healthy tissues from harm. Consequently, the pursuit of novel agents that combine efficacy, selectivity, and safety remains the ultimate goal of cancer chemotherapy. Currently, there are numerous anticancer agents available. Among the most well-known are doxorubicin (DOXO),

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cisplatin, fluorouracil, hydroxyurea, and cyclophosphamide. DOXO, one of the leading anticancer drugs approved by the Food and Drug Administration (FDA), stands as one of the most potent chemotherapeutic agents. However, its clinical efficacy is limited, particularly by cardiotoxicity, which often arises and poses a significant constraint in its application. Recent research [1] indicates that the impact of DOXO on cardiomyocyte mitochondria exacerbates oxidative stress. In recent years, there has been a high level of interest in synthetic copper(II) complexes featuring thiosemicarbazone ligands [2] with promising anticancer and cancer-inhibiting potential. These substances have not only demonstrated their effectiveness in the controlled environment of *in vitro* studies but have also shown their efficacy in the complex milieu of *in vivo* models [3-10]. Thiosemicarbazones and their complexes, being a versatile class of organic compounds, boast a wide range of biological activities, with particular emphasis on their antiproliferative capabilities, as evidenced in the literature [11].

The intricate interplay between thiosemicarbazones and transition metal, the resulting coordination compounds has unveiled a rich tapestry of biological and pharmacological activities, including their potent antiproliferative and antioxidant properties [12-14]. Of particular interest is the pronounced enhancement in biological activity upon coordination of thiosemicarbazones with copper(II) ions, a phenomenon noted in several studies [15]. This paper presents an evaluation of the anticancer activity of the ligand 2-(2-hydroxybenzylidene)-*N*-(prop-2-en-1-yl) hydrazinecarbothioamide **(H_2L)** and its coordination compound (2-((2-(prop-2-en-1-ylcarbamothioyl) hydrazinylidene)methyl)phenolato)-chloro-copper(II) monohydrate **[Cu(HL)Cl]•H_2O**. The anticancer activity of the tested compounds was assessed *in vitro* using RD (human muscle rhabdomyosarcoma spindle and large multinucleated cells), HeLa (human cervix adenocarcinoma cells), BxPC-3 (human pancreatic adenocarcinoma cells) and MDCK (Madin–Darby canine kidney epithelial normal cells) cell lines. The tested compounds were subjected to analysis for their antioxidant activity because compounds capable of inducing oxidative stress can be toxic, leading to pathological changes in living organisms and potentially causing various diseases in the long term. Avoiding prooxidant properties in substances is an important aspect of their characterization. Furthermore, the toxicity of the tested compounds was investigated *in vivo* using *Daphnia magna* through bioassay and microanalysis.

The biological activities of the tested compounds were juxtaposed with those of reference compounds. Doxorubicin (DOXO) (Figure 1), a well-known anticancer compound, was utilized as the reference anticancer agent. Trolox (Figure 1), an established antioxidant compound, served as the reference antioxidant in this study.

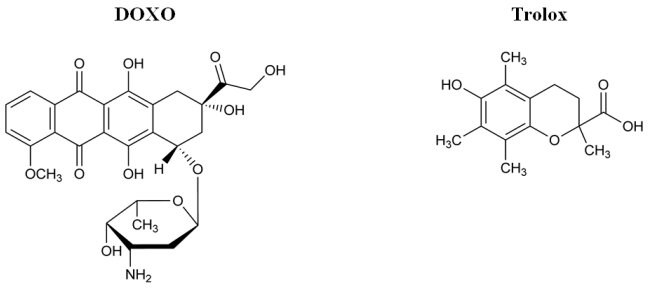

Figure 1: Structural formula of the references compounds: DOXO ((7S,9S)-7-[(2R,4S,5S,6S)-4-amino-5-hydroxy-6-methyloxan-2-yl]oxy-6,9,11-trihydroxy-9-(2-hydroxyacetyl)-4-methoxy-8,10-dihydro-7H-tetracene-5,12- dione), Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid).

Materials and Methods

Characterization of the tested compounds

The 2-(2-hydroxybenzylidene)-*N*-(prop-2-en-1-yl) hydrazinecarbothioamide **(H_2L)**, and (2-((2-(prop-2-en-1-ylcarbamothioyl)hydrazinylidene)methyl)phenolato)-chloro-copper(II) monohydrate **[Cu(HL)Cl]•H_2O** (Figure 2) were synthesized in the Research Laboratory of Advanced Materials in Biopharmaceutics and Technics of the Moldova State University and are described in the literature [16, 17].

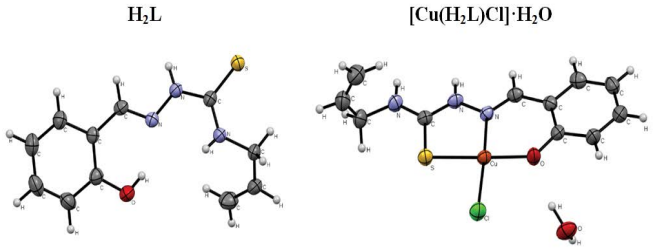

Figure 2: Crystal structures of the tested compounds: 2-(2-hydroxybenzylidene)-N-(prop-2-en1-yl) hydrazinecarbothioamide **(H_2L)**, (2-((2-(prop-2-en-1-ylcarbamothioyl)hydrazinylidene)methyl)phenolato)-chloro-copper(II) monohydrate **([Cu(HL)Cl]•H_2O).**

The thiosemicarbazone **H_2L** was characterized by NMR (^1H and ^{13}C) spectroscopy [16]. The complex **[Cu(HL)Cl]•H_2O** was characterized by FT-IR spectroscopy, molar conductivity and magnetic susceptibility measurements, and elemental analysis. Also, the crystal structure of this complex was determined by single-crystal X-ray diffraction analysis. Melting points, FT-IR, and NMR spectra of the tested compounds correspond to the literature data [17]. The tested compounds and the reference controls were dissolved individually in dimethylsulfoxide (DMSO) to prepare 10 mM stock solutions, which were then stored at a specified temperature of 7°C. These stock solutions were subsequently diluted with cell culture medium or physiological solution to obtain solutions with concentrations of 1, 10, 100, and 1000 μM for use in biological testing. The maximum final concentration of DMSO (<0.1%) was ensured not to impact cell proliferation or induce cytotoxicity in the tested cell lines.

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In vitro antiproliferative assay

Cell lines RD (human muscle rhabdomyosarcoma spindle and large multinucleated cells, ATCC CCL-136), HeLa (human cervix adenocarcinoma, ATCC CCL-2), BxPC-3 (human pancreatic adenocarcinoma cells) and MDCK (Madin Darby Canine Kidney epithelial normal cells, ATCC CCL-34) were utilized for analysis following cryopreservation in a freeze medium consisting of complete nutrient medium supplemented with 5% (v/v) DMSO and stored in the vapor phase of liquid nitrogen at temperatures ranging from −180°C to −196°C. To establish a healthy monolayer substrate, cells were cultured for a minimum of 21 days, passaged every 3 days, and provided with fresh nutrient media supplemented with growth factor-inactivated fetal bovine serum upon each passaging. Cells in the logarithmic growth phase were employed for experimentation, and cell viability was assessed using 0.2% trypan blue dye.

The investigation of the antiproliferative activity of the synthesized substances against the RD, HeLa, BxPC-3, and MDCK cell lines was conducted using resazurin analysis. Resazurin, a non-fluorescent indicator dye, undergoes reduction reactions in metabolically active cells, resulting in the production of highly red fluorescent resorufin. The fluorescence intensity generated is directly proportional to the number of viable cells. Resazurin was dissolved in physiological buffers to yield a deep blue-colored solution and added directly to the cell cultures in a homogeneous manner. Subconfluent cultures of cell lines were trypsinized using 3 mL of trypsin-EDTA 0.05% (Invitrogen) per 50 mL of culture flask containing confluent cells, followed by incubation at 37°C with gentle shaking for 5-15 minutes and subsequent cell counting under an inverted microscope. The trypsin reaction was halted by adding 10 mL of appropriate culture medium containing 10% FBS, and the cell suspension was centrifuged at 750 rpm for 10 minutes at 25°C. The resulting cell pellet was resuspended in 2 mL of medium with 10% FBS, carefully mixed, and adjusted to a concentration of $1 \cdot 10^5$ cells/ml. This cell suspension was then seeded into triplicate wells of a 96-well microtiter plate (100 µL/well) and incubated at 37°C with 3% CO_2. After an initial 2-3 hour attachment period, 10 µL of the tested compounds and reference controls, dissolved in DMSO to prepare a 10 mM stock solution, were added to the medium, yielding final concentrations ranging from 0.1 to 100 µM. The plate was further incubated at 37°C with 3% CO_2 for 24 hours. Subsequently, 20 µL of resazurin indicator solution was added to each well, and the plate was incubated at 37°C with 3% CO_2 for an additional 4 hours. Absorbance readings were obtained using a hybrid reader (Synergy H1, BioTek) with 570 nm and 600 nm filters, and the percentage of cell proliferation inhibition was calculated using the formula (1):

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

Antioxidant assay

The antioxidant activity of the synthesized compounds was evaluated using the ABTS•+ method, as outlined by [18] with slight modifications. ABTS•+ radical cations were generated by mixing a 7 mM solution of ABTS (2,20-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)) (Sigma) with a 2.45 mM solution of potassium persulfate ($K_2S_2O_8$) (Sigma) at 25°C in the absence of light for 16 hours. The resulting solution was then diluted further with acetate-buffered saline (0.02 M, pH 6.5) to achieve an absorbance of 0.7 ± 0.1 at 734 nm. For sample preparation, the synthesized compounds were dissolved in DMSO to create solutions with concentrations of 1, 10, and 100 µM. Subsequently, 20 µL of each solution was added to a 96-well microtiter plate, followed by the addition of 180 µL of the $ABTS^{\bullet+}$ working solution. The mixture was thoroughly mixed, and the decrease in absorbance at 734 nm was precisely measured after a 30-minute incubation at 25°C. All measurements were conducted in triplicate, with DMSO used as the negative control. Blank samples were also included, using solvent without $ABTS^{\bullet+}$.

The percentage inhibition (I, %) of free radical cations $ABTS^{\bullet+}$ was calculated using the following formula (2) :

$$\mathbf{I}(\%) = \frac{\mathbf{Abs(control)} - \mathbf{Abs(sample)}}{\mathbf{Abs(control)}} \times \mathbf{100}\% \quad (2)$$

Where Abs734 nm (control) represents the absorbance of the control solution, and Abs734 nm (sample) denotes the absorbance in the presence of sample solutions or standards for positive controls.

Acute toxicity assay

The overall toxicity of the examined compounds was assessed using *Daphnia magna* (Straus, 1820), sourced from a parthenogenetic culture maintained at the Institute of Zoology, Laboratory of Systematics and Molecular Phylogenetics [19-21]. *Daphnia magna* were cultured in aerated aqueous straw infusion growth media supplemented with $MgSO_4 \cdot 7H_2O$ (4.93 g/L), KCl (0.23 g/L), $NaHCO_3$ (2.59 g/L), and $CaCl_2$ (11.76 g/L).

Juveniles were selected based on size and transferred to fresh medium for a 24-hour acclimation period. The *Daphnia magna* were cultured in 24-well culture transparent sterile plates covered with a lid to prevent contamination and evaporation while facilitating gas exchange between air and culture medium. Each well contained 10 organisms in a final volume of 1 mL of each dilution of the tested compounds. The bioassay was conducted at concentrations of 0.1, 1, 10, and 100 µM to determine the LC_{50} for each compound, including the positive control. The final test solutions contained up to 0.1% DMSO, with a final volume of 1 mL. A solution of

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0.1% DMSO in aerated medium (pH~7.5±0.2; O_2 ≥6.0 mg/L) was added as the negative control, while the test compounds served as positive controls. During the experiment, the juvenile *Daphnia magna* were maintained at 22±2°C under a cycle of 16 hours of light and 8 hours of darkness (500–1000 lx). The mobility or viability of the test organisms was observed after 24 and 48 hours of exposure. *Daphnia magna* were considered immobilized only if they did not exhibit movement during the 15 seconds following gentle mixing of the test and control solutions, even if they were still capable of moving their antennae. The percentage of viability (V (%)) of *Daphnia magna* was calculated using the formula (3):

$$\mathbf{V}(\%) = \frac{\mathbf{N}_{(\mathbf{sample})}}{\mathbf{N}_{(\mathbf{control})}} \times \mathbf{100} \quad (3)$$

Where N - Number of the viability of *Daphnia magna*.

Statistical analysis

The results of the cell proliferation assay were expressed as the percentage inhibition of both the test and control substances. To gauge the effectiveness of the experimental compounds on the proliferation of cell lines, the half-maximal inhibitory concentration (IC_{50}) was employed. According to FDA documentation, IC_{50} serves as an indicator of the concentration of a medicinal substance required for 50% inhibition of the tested reaction *in vitro*.

The toxicity of the compounds was assessed by calculating the median lethal concentration values (LC_{50}) from the dose-response equation; determined using the least squares fit method with GraphPad software.

All data are presented as means ± standard deviation (SD).

Results and Discussion

This study presents a series of comparative antiproliferative investigations involving the thiosemicarbazone **H_2L** and the copper(II) complex **[Cu(HL)Cl]•H_2O**, conducted in several cancer cell lines using the spectrophotometric method. The pathological changes in cell morphology were evaluated by microscopy. The cancer cell lines RD, BxPC-3 and HeLa were incubated with the tested compounds **H_2L** and **[Cu(HL)Cl]•H_2O**, to assess their antiproliferative activity (APA). Cytoplasmic vacuolization, a precursor of apoptosis, and cell vacuoles and apoptotic body formation (ApoBDs) were studied by the inverted microscope (LOMO). Pathological changes were notably absent in the cell structure of the negative control. Incubating cancer cells for 24 hours in a nutrient medium containing 10 µM concentrations of complex resulted in significant pathological morphological changes, including intensive cell vacuolization of cytoplasm and plasma membrane, indicating the initial phases of blebbing. The appearance of apoptosis confirmed the progression of apoptosis [22]. However, **H_2L** at a concentration of 10 µM did not induce blebbing in RD, BxPC-3 and HeLa cancer cells. APA experiments revealed a concentration-dependent inhibitory effect of the tested compounds within the micromolar range. Anticancer chemotherapy drugs often exhibit high cytotoxicity towards normal cells, leading to severe side effects that can be fatal. Therefore, we have used normal kidney epithelial cells of the MDCK line to determine the selectivity of the APA of the tested compounds towards cancer cells.

Table 1 presents the IC_{50} values of the tested compounds against various cell lines (human muscle rhabdomyosarcoma cell line RD, human cervical epithelial cell line HeLa, human pancreatic adenocarcinoma cell line BxPC-3, and the model line of normal mammalian cell line MDCK), as well as the corresponding selectivity index (SI).

The study of the APA of the thiosemicarbazone **H_2L** and complex **[Cu(HL)Cl]•H_2O** against RD, HeLa, and BxPC-3 cancer cell lines indicated that the ligand **H_2L** exhibited minimal activity, suppressing the growth and reproduction of cancer cells by only 21.0±1.6%, 34.0±2.1% and 44.3±2.3% at a concentration of 100 µM, respectively. It was impossible to determine the IC_{50} values for **H_2L** because it inhibits less than 50% of cells in the studied concentration range (0.1–100 µM). The tested complex and DOXO exhibit antiproliferative activity toward RD cells with IC_{50} values of 0.6±0.1 µM,

Table 1: The antiproliferative activity of the tested compounds **H_2L**, **[Cu(HL)Cl]•H_2O**, and DOXO towards MDCK, RD, HeLa, BxPC-3 cell lines, and their selectivity index.

Compound	MDCK	HeLa		BxPC-3		RD	
	[a] IC_{50}, µM	[a] IC_{50}, µM	[b] SI	[a] IC_{50}, µM	[b] SI	[a] IC_{50}, µM	[b] SI
DOXO	7.1	10	0.7	3.7	1.9	16.2	0.4
H_2L	≥100	≥100	*N.d.*	≥100	*N.d.*	≥100	*N.d.*
[Cu(HL)Cl]·H_2O	2.5	1.4	1.8	0.5	4.6	0.6	4.2

N.d., not determined.
a IC_{50} value is a concentration of the substance that inhibits cellular proliferation by 50%;
b The SI of each compound was calculated as the ratio of the IC50 towards MDCK epithelial normal cell line to IC_{50} value towards cancer cell line

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16.2±0.3 μM, respectively, and toward BxPC-3 cells with IC50 values of 0.50±0.02 μM and 3.7±0.4 μM, respectively. The complex exhibits the highest activity against BxPC-3 cells. The tested complex and DOXO show antiproliferative activity towards HeLa cells, with IC_{50} values of 1.4±0.2 μM and 10.0±0.6 μM, respectively. The tested complex demonstrates higher activity towards BxPC-3 cells compared to RD and HeLa cells, surpassing the inhibitory activity of DOXO. The SIs values are crucial criteria for assessing the suitability of anticancer drugs for medical use. SI represents the ratio between the half-maximal inhibition concentrations towards normal cell lines and the corresponding cancer cell lines. Compounds with an SI>3 are considered promising for medical applications.

The SIs of the complex **[Cu(HL)Cl]•H_2O** towards BxPC-3, RD, and HeLa cell lines are 4.6, 4.2, and 1.8, respectively. The SIs of DOXO towards BxPC-3, RD, and HeLa cell lines are 1.9, 0.4, and 0.7, respectively. Therefore, the complex **[Cu(HL)Cl]•H_2O** is 2.4, 10.5, and 2.6 times more selective than DOXO against BxPC-3, RD, and HeLa cell lines, respectively. To mitigate the possibility of accompanying adverse effects associated with oxidative stress, the tested compounds underwent evaluation for their antioxidant activity [16]. As DOXO causes toxic damage to mitochondria in cardiomyocytes, contributing to increased oxidative stress, it is well known that DOXO-induced cardiomyopathy has a poor prognosis and is often fatal [23]. Figure 3 illustrates the impact of the tested compounds and reference control on $ABTS^{\bullet+}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)) activity. $ABTS^{\bullet+}$ is a commonly used radical cation to evaluate the antioxidant capacity of compounds. The antioxidant activity of these compounds was compared with that of the reference antioxidant control Trolox. The figure likely shows a comparison of various compounds and control in terms of their ability to scavenge $ABTS^{\bullet+}$ radicals. This assay helps in assessing the antioxidant potential of compounds, with higher scavenging activity indicating stronger antioxidant properties. The results of the study on the antioxidant activity of Trolox, **H_2L**, and **[Cu(HL)Cl]•H_2O** against $ABTS^{\bullet+}$ radical cations are presented in Figure 3 in terms of semi-maximal inhibition concentrations (IC_{50}). The thiosemicarbazone **H_2L** exhibits higher activity than Trolox. However, the coordination of H_2L to the copper(II) ion results in a decrease in antioxidant activity. It is noteworthy that both tested compounds demonstrate considerable antioxidant activity.

Toxicity studies are vital in drug development preceding preclinical and clinical trials. Upholding animal protection principles, it is recommended to conduct in vivo studies on invertebrate organisms whenever possible. *Daphnia magna* is commonly used for assessing the cytotoxicity and biological activity of compounds. An immobilization test on *Daphnia magna* was conducted following European Standardized

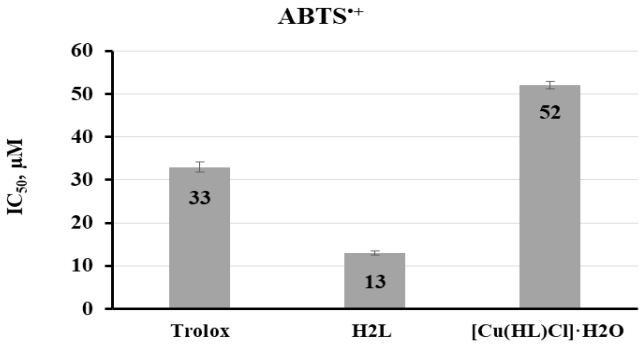

Figure 3: The influence of the tested compounds **H_2L, [Cu(HL)Cl]•H_2O** and reference control Trolox for $ABTS^{\bullet+}$.

Methodology to evaluate compound toxicity, aligning with international animal protection recommendations. [19] This paper presents experiment results assessing compound toxicity through acute toxicity bioassays on aquatic arthropods like *Daphnia magna*. Its transparent structure and survivability under a coverslip facilitate microscopy observation.

The sensitivity of *Daphnia magna* to environmental stressors, including chemical pollutants, makes it valuable for assessing ecological impacts. Its short reproductive cycle and ease of culture enhance practicality in toxicity testing, expediting compound toxicity assessment and regulatory decision-making. The LC_{50} values of the tested compounds were utilized as quantitative indicators of their toxicity and for the comparative evaluation of the results obtained. Figures 4 and 5 illustrate the results of the *Daphnia magna bioassay*. The tested compounds exhibit acute toxicity toward *Daphnia magna* after 24 hours and 48 hours of exposure. The acute toxicity decreases in the following sequences: DOXO ≥ **[Cu(HL)Cl]•H_2O** ≥ **H_2L.** Thus, the complex is less toxic than doxorubicin but more toxic than its ligand.

Microanalysis was employed to assess the impact intensity of compounds at the median lethal concentration on *Daphnia magna* (Figure 5, 6). As expected, microscopic examination of *Daphnia magna* in the control group (without the tested compound) revealed no pathological physiological

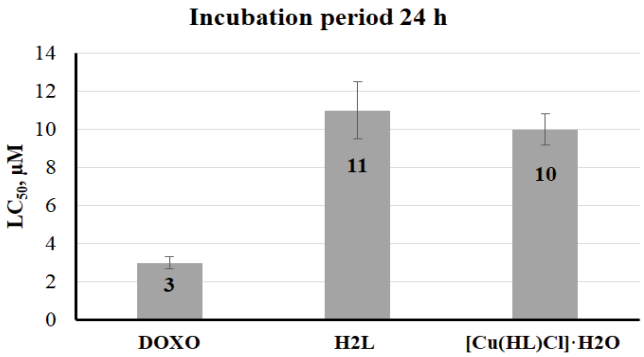

Figure 4: In vivo toxicity on *Daphnia magna* of **H_2L, [Cu(HL)Cl]•H_2O** and DOXO after 24-h incubation.

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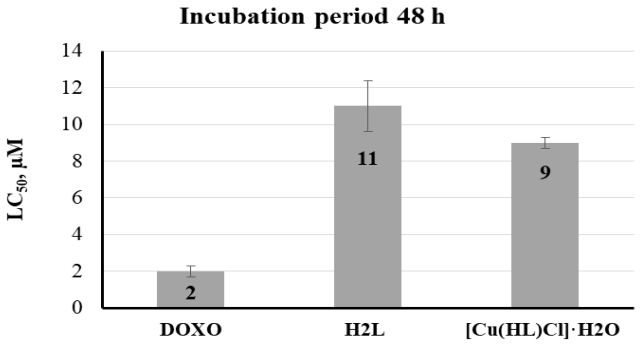

Figure 5: In vivo toxicity on *Daphnia magna* of H_2L, **[Cu(HL)Cl]•H_2O** and DOXO after 48-h incubation.

or morphological changes in Daphnia. Following incubation with the tested compounds, Daphnia settled to the bottom of the plate wells. Under an inverted light microscope, it became evident that some Daphnia were moving slowly, while others of the crustaceans were immobile. Additionally, the limbs and trunk of some Daphnia appeared deformed, with their contents mixed with the nutrient medium (Figure 6). Based on the provided research data, the mechanism of action of the tested complex **[Cu(HL)Cl]•H_2O** involves several crucial aspects governing its antiproliferative activity, selectivity towards tested cell lines, and impact on Daphnia magna. The complex **[Cu(HL)Cl]•H_2O** exhibits heightened activity against cancer cells compared to its ligand H_2L, primarily due to its coordination binding with copper(II), significantly enhancing its antiproliferative properties. Notably, the inhibition of cell proliferation by **[Cu(HL)Cl]•H_2O** is independent of oxidative stress, despite its observed high antioxidant activity. Therefore, further exploration of its therapeutic benefits and safety for potential clinical applications is warranted.

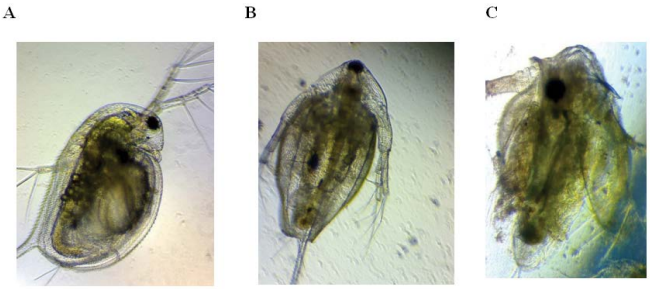

Figure 6: Microscopy pictures of Daphnia magna in control (A) and after incubation with the tested compounds H_2L **(B)** and **[Cu(HL)Cl]•H_2O (C).**

The effects of **[Cu(HL)Cl]•H_2O** on cells appear to involve alternative mechanisms related to cell cycle regulation and apoptosis. This is supported by pathological alterations in cell structure, including cytoplasmic vacuolization and the formation of apoptotic bodies (ApoBDs). Thiosemicarbazone complexes like **[Cu(HL)Cl]•H_2O** may interact with nucleic acids within cells, potentially disrupting DNA or RNA synthesis and impeding the activity of cellular enzymes crucial for replication and transcription of genetic information [24-29].

The observed high selectivity of **[Cu(HL)Cl]•H_2O** towards cancer cells, indicated by elevated selectivity index values, suggests its promise as an anticancer therapy candidate. This selectivity may arise from differences in metabolism and receptor expression on the surface of cancer cells, rendering them more susceptible to the complex compared to normal cells.

In conclusion, this study underscores the considerable potential of the tested copper complex as an effective anticancer therapy candidate. Its demonstrated antiproliferative activity against cancer cells, coupled with minimal toxicity towards *Daphnia magna*, highlights its favorable pharmacological profile. Nonetheless, further extensive preclinical studies are necessary to elucidate the underlying mechanisms of action of this copper complex and assess its efficacy *in vivo*.

Conclusion

In conclusion, our study provides valuable insights for the development of novel antiproliferative agents. Thiosemicarbazone H_2L and its copper complex **[Cu(HL)Cl]•H_2O** were evaluated for their in vitro antiproliferative and antioxidant activities, as well as toxicity profiles. Coordination of H_2L to the copper atom enhanced the activity of the resulting complex, making it more potent than the original ligand. The complex **[Cu(HL)Cl]•H_2O** emerged as a potent inhibitor of cancer cell proliferation, exhibiting high selectivity and low toxicity. Its IC50 values against RD, HeLa, and BxPC-3 cancer cells ranged from 0.5 µM to 1.4 µM, with selectivity indices ranging from 1.8 µM to 4.6 µM, surpassing those of DOXO in both anticancer activity and selectivity. These results suggest that the coordination compound **[Cu(HL)Cl]•H_2O** offers greater efficacy compared to DOXO, highlighting its potential as an anticancer agent.

Additionally, both H_2L and **[Cu(HL)Cl]•H_2O** demonstrated significant antioxidant activity against $ABTS^{\bullet+}$ radical cations. Importantly, the synthesized copper complex exhibited lower toxicity towards Daphnia magna compared to various cancer cell lines (RD, HeLa, BxPC-3, and MDCK), further emphasizing its promising antiproliferative activity against cancer cells and favorable safety profile. Overall, these findings support the potential therapeutic application of the copper complex **[Cu(HL)Cl]•H_2O** as a promising anticancer agent with low toxicity, warranting further investigation for clinical development.

Credit authorship contribution statement

Olga Garbuz: Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. Vasilii Graur:

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Data curation, Validation, Methodology, Visualization. Ianina Graur: Visualization, Investigation. Nadejda Railean: Formal analysis, Methodology, Software. Ion Toderas: Data curation, Funding acquisition, Project administration, Resources. Elena Pahontu, Ilie Ceban, Viorel Jinga, Dorin Istrati, Emil Ceban: Funding acquisition, Investigation, Visualization. Aurelian Gulea: Conceptualization, Data curation, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

The authors declare that they do not have any known financial interests or personal relationships that could have influenced the findings presented in this paper.

Data availability

No data was used for the research described in the article.

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