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IN VITRO MODULATION OF THE THIOL SYSTEM BY Cu–THIOSEMICARBAZONE COMPOUNDS: IMPLICATIONS FOR CELLULAR REDOX HOMEOSTASIS

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Abstract

Introduction. Thiosemicarbazones are organic compounds with antimicrobial, antiviral and antitumor activities. They are capable of modulating redox balance and protecting against oxidative stress. The aim of this study was to investigate the effects of selected thiosemicarbazones and their copper (Cu) complexes on protein thiol groups *in vitro*, in order to elucidate structure-activity relationships and their therapeutic potential.

Materials and Methods. The effects of Cu–thiosemicarbazone compounds on thiol (SH) groups (protein-bound, total, and free) were analyzed at concentrations of 10.0 and 1.0 μmol/L, compared with a negative control and doxorubicin as a positive control.

Results. The compounds CMD-8, CMJ-33, TIA-123, and TIA-160 (10.0 μmol/L) significantly increased SH group levels, indicating a dose-dependent antioxidant effect. In contrast, CMC-34 (1.0 μmol/L) reduced free SH groups, suggesting that both chemical structure and concentration influence redox activity.

Conclusions. Cu-thiosemicarbazones exhibit antioxidant potential by protecting thiol groups and supporting cellular redox homeostasis.

Keywords: Cu-thiosemicarbazones, thiol system, oxidative stress, redox homeostasis.

Rezumat

Introducere. Tiosemicarbazonele reprezintă o clasă de compuși organici cu proprietăți farmacologice bine documentate, incluzând activități antimicrobiene, antivirale și antitumorale, capabili să moduleze echilibrul redox și să protejeze împotriva stresului oxidativ. Scopul studiul a investigheat efectele unor tiosemicarbazone și ale complexelor lor cu Cu asupra grupelor tiolice proteice *in vitro*, pentru a evidenția relația structură–activitate și potențialul terapeutic.

Materiale și metode. Au fost analizate efectele compușilor Cu-tiosemicarbazonă asupra grupelor SH grupe tiolice proteice, totale și libere la 10,0 și 1,0 μmol/L, comparativ cu control negativ, iar doxorubicină s-a utilizat ca control pozitiv.

Rezultate. Compușii CMD-8, CMJ-33, TIA-123 și TIA-160 (10,0 μmol/L) au crescut semnificativ grupele SH, indicând efect antioxidant dependent de doză. CMC-34 (1,0 μmol/L) a redus grupele SH libere, sugerând influența structurii și concentrației asupra activității redox.

Concluzii. Cu-tiosemicarbazonele prezintă potențial antioxidant, protejând grupele tiolice și susținând homeostazia redox celulară.

Cuvinte-cheie: Cu-tiosemicarbazone, sistem tiolic, stres oxidativ, homeostazie redox.

Introduction

Oxidative stress (OS) represents a fundamental biological process involved both in the regulation of normal cellular functions and in the pathogenesis of numerous chronic diseases, including cardiovascular, neurodegenerative disorders, diabetes, and cancer. It occurs when there is an imbalance between the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), and the capacity of endogenous antioxidant systems to neutralize them, leading to oxidative damage of proteins, lipids, and nucleic acids [1].

At low or moderate concentrations, ROS and RNS play essential roles in physiological cellular processes. They contribute to the regulation of cellular responses to various harmful stimuli, participate in host defense mechanisms against infectious agents, are involved in cell

signaling pathways, and can stimulate the initiation of mitogenic responses, i.e., the process by which cells are induced to proliferate.

In contrast, excessive production of ROS/RNS, originating either from the mitochondrial electron transport chain (ETC) or from the overactivation of NADPH oxidases, leads to OS, resulting in cellular damage and contributing to the pathogenesis of chronic diseases, aging, and cancer [2, 3].

The effects of OS arise as a result of peroxidative damage to macromolecules and cellular membranes, as well as disruption of the metabolic activities of cellular components in living organisms. It is well established that organ and tissue pathologies develop when OS becomes excessive within the organism.

Thiols are recognized as one of the principal protective mechanisms against OS. They have been shown to play important roles in enzymatic reactions, apoptosis, detoxification processes, and the antioxidant defense of the organism. Numerous studies have demonstrated alterations in thiol status and thiol–disulfide homeostasis in various diseases, including those affecting the digestive, respiratory, reproductive, and urinary systems, as well as in metabolic disorders and cancer. This further highlights the critical importance of thiol status in the pathogenesis of oxidative stress-mediated diseases [4].

Thiols are a class of organic compounds containing the sulfhydryl (-SH) functional group, consisting of a sulfur atom bonded to a hydrogen atom and attached to a carbon atom [5]. In the organism, during oxidative processes generated by ROS, excess electrons are transferred to thiols, leading to the formation of disulfide bonds. Due to the dynamic redox balance, electrons within these reversible bonds can be restored to regenerate thiol groups.

The antioxidant capacity of thiol–disulfide homeostasis is essential for numerous biological processes, including enzymatic reactions, signal transduction, detoxification, transcription, regulation of enzyme activation, cellular signaling mechanisms, and apoptosis [6, 7].

Thiol groups (–SH), present in the amino acid cysteine within protein structures and in low-molecular-weight molecules such as glutathione, are considered sensitive markers of cellular redox status. These groups play a critical role in maintaining the three-dimensional structure of proteins and in regulating the activity of antioxidant enzymes. Oxidation of these groups may lead to loss of protein function and disruption of essential biochemical processes [8]. Recent studies emphasize the importance of evaluating thiol-related parameters as indicators of OS and of the efficacy of biologically active compounds on redox balance [9].

In recent years, research has increasingly focused on identifying molecules capable of influencing cellular redox balance through direct interactions with thiol groups, which are key components of antioxidant systems. In this context, thiosemicarbazones (TSCs) have gained particular attention. These compounds represent a class of Schiff bases, typically obtained through the condensation of thiosemicarbazide with aldehydes or ketones, and have attracted the interest of chemists and biologists due to their broad spectrum of pharmacological effects, including their potential to modulate OS and associated cellular functions.

A particularly promising area of application for these effective metal-chelating agents is their investigation as antitumor agents, owing to their ability to modulate redox processes and selectively affect cancer cells. Thiosemicarbazones exhibit a broad clinical antitumor spectrum, demonstrating efficacy in various types of tumors, including leukemia, pancreatic cancer, breast cancer, non-small cell lung cancer, cervical cancer, and prostate cancer [10, 11].

Doxorubicin (DOXO) is an anthracycline chemotherapeutic agent widely used in clinical anticancer regimens. Its mechanisms of action are not fully elucidated. However, DOXO exerts pleiotropic effects, including DNA damage, generation of ROS, and induction of apoptosis, senescence, autophagy, ferroptosis, and pyroptosis, as well as immunomodulatory activity [12]. Through the generation of ROS and the depletion of thiol groups, doxorubicin disrupts cellular

redox balance, impairing protein function and compromising DNA integrity. This mechanism makes it a classical model for OS studies and for investigating oxidative effects at the cellular level [13].

In light of the above, the analysis of the effects of Cu–thiosemicarbazone compounds on protein thiol groups, as well as on total and free thiols, provides important insights into how these compounds influence cellular redox balance. This evaluation enables the identification of both potential antioxidant and pro-oxidant mechanisms of Cu–thiosemicarbazones. Since these effects have not yet been extensively studied and characterized, their investigation contributes to a better understanding of the biological activity of these compounds and facilitates the identification of those with the highest therapeutic potential.

The aim of the present study is to investigate the effects of Cu–thiosemicarbazone compounds on thiol–disulfide homeostasis, an essential system involved in maintaining cellular redox balance. By analyzing changes in thiol groups, the study seeks to evaluate how these compounds influence OS and the functioning of antioxidant systems.

Materials and methods

The research was conducted *in vitro* using biological samples collected in accordance with contemporary principles of biological standardization of experimental procedures. The study protocol was approved by the Research Ethics Committee of Nicolae Testemițanu State University of Medicine and Pharmacy, with a favorable opinion issued under No. 5 of protocol No. 38 dated June 20, 2024. The study was carried out in accordance with the Declaration of Helsinki, as amended (Somerset West Amendment, 1996) [14], regarding research involving human subjects, who were included only after providing written informed consent.

The study investigated copper coordination compounds with thiosemicarbazones, synthesized at the Moldova State University within the Research Laboratory of Advanced Materials in Biopharmaceutics [15].

The compounds were classified into the following groups (Table 1):

Benzothiazole derivatives of thiosemicarbazone – CMA-18, CMD-8, MG-22;

Phenyl thiosemicarbazone derivatives – CMC-34, CMJ-33, CMT-67;

Allyl thiosemicarbazone derivatives – CMG-41, TIA -123, TIA-160.

To evaluate the specific effects of locally synthesized Cu–thiosemicarbazone compounds, a series of *in vitro* experiments was performed using peripheral blood samples collected from 10 apparently healthy individuals.

Blood samples were collected in the morning under fasting conditions, by cubital vein puncture, in a volume of 5 mL. Under sterile conditions, the blood was transferred into a flask containing 20 mL of Dulbecco's Modified Eagle Medium (DMEM), supplemented with heparin (2.5 U/mL), gentamicin (100 μg/mL), and L-glutamine (0.6 mg/mL).

To assess the influence of the tested compounds, 0.9 mL of this mixture was dispensed into the wells of a 24-well plate. For the control group, used to determine the baseline levels of the studied parameters, 0.1 mL of 0.9% NaCl solution was added to four parallel wells. In the remaining wells, the tested compounds (CMA-18, CMD-8, MG-22, CMC-34, CMJ-33, CMT-67, CMG-41, TIA-123, TIA-160), diluted in 0.1 mL of physiological saline, were added to achieve final concentrations of 10.0 μmol/L and 1.0 μmol/L. All dilutions were tested in duplicate.

The prepared plates were incubated at 37°C for 48 hours in a thermostatic incubator with 3.5% CO_2.

After incubation, the contents of the wells were transferred into 2.0 mL Eppendorf tubes and centrifuged for 5 minutes at 3000 rpm. The obtained supernatant was then transferred into

1.5 mL Eppendorf tubes and stored at −40°C until biochemical analysis. All samples were coded prior to analysis.

Table 1. Newly studied native copper coordination compounds with thiosemicarbazones [16]

S. No.	Cod	Chemical name of the substance
1	Control	0.1 ml 0.9% saline solution + Dulbecco's Modified Eagle Medium (DMEM)
2	DOXO	Doxorubicin
Benzothiazole derivatives of thiosemicarbazone		
3	CMA-18	Chloro-{1-(1,2-benzothiazol-3-yl)-2-[1-(pyridin-2-yl)ethylidene]diazanido}copper
4	CMD-8	Chloro-{4-ethyl-2-[phenyl(pyridin-2-yl)methylidene]hydrazine-1-carbothioamido} copper
5	MG-22	Di-Chloro-{N'-(4-methoxyphenyl)-N,N-dimethylcarbamimidothioato} copper
Phenyl thiosemicarbazone derivatives		
6	CMC-34	Chloro-{N'-[phenyl(pyridin-2-yl)methylidene]-N-pyridin-2-ylcarbamohydrazonothioato} copper
7	CMJ-33	Chloro-{4-(3-methoxyphenyl)-2-[1-(pyridin-2-yl)ethylidene]hydrazine-1-carbothioamido} copper
8	CMT-67	Nitrato-{N-phenyl-N'-(pyridin-2-ylmethylidene)carbamohydrazonothioato} copper
Allyl thiosemicarbazone derivatives		
9	CMG-41	Nitrato-{N'-[phenyl(pyridin-2-yl)methylidene]-N-prop-2-en-1-ylcarbamohydrazonothioato} copper
10	TIA-123	Di-Chloro-{N'-[phenyl(pyridin-2-yl)methylidene]-N-prop-2-en-1-ylcarbamohydrazonothioato} copper
11	TIA-160	Acetato-{2-({[(methylsulfanhyl)(prop-2-en-1-lamino)ethylidene]hydrazinylidene} methyl) enolato}copper

In the obtained supernatant, parameters of thiol–disulfide metabolism were quantified using spectrophotometric micromethods, including the content of protein thiol (SH) groups, as well as thiol–disulfide homeostasis parameters (free thiol groups and total thiol groups) [17–18]. All determinations of these biomarkers of lipid peroxidation were performed in the Biochemistry Laboratory of Nicolae Testemițanu State University of Medicine and Pharmacy, using methods modified and adapted by our team for application on the Synergy H1 microplate spectrophotometer (Hybrid Reader) (BioTek Instruments, USA).

The evaluation of the *in vitro* effects of Cu–thiosemicarbazone compounds was carried out according to the standard methodology described by Ryzhikova S. L. et al. [19].

Statistical analysis of the obtained data was performed using the Statistical Package for the Social Sciences (SPSS, version 23; SPSS Inc., Chicago, IL, USA). The main descriptive statistical indicators were determined, including the median, interquartile range (IQR), and median percentage.

Results

The evaluation of the effects of Cu–thiosemicarbazone compounds on cellular redox homeostasis was performed by determining the levels of protein thiol groups, total thiol groups, and free thiol groups. These parameters are important indicators of the redox status of biological systems, as –SH groups play a key role in antioxidant defense mechanisms and in maintaining protein structure and function. Data are presented as median ± interquartile range (IQR), and percentage changes are expressed relative to the negative control (Table 2).

Table 2. Effects of Cu–thiosemicarbazone compounds on thiol–disulfide metabolism *in vitro*

Study groups	SH-protein thiol groups, μmol/g.prot	SH-total thiol groups, μmol/L	SH-free thiol groups, μmol/L
Negative control	4.59 ; IQR 0.38 100%	61.31 ; IQR 2.70 100%	56.18 ; IQR 4.54 100%
Positive control			
DOXO-10.0 μmol/L	4.79 ; IQR 0.25 104%	63.00 ; IQR 1.83 103%	55.42 ; IQR 5.91 99%
DOXO-1.0 μmol/L	4.28 ; IQR 0.37 93%	62.60 ; IQR 2.67 102%	54.81 ; IQR 2.15 98%
Benzothiazole			
CMA-18-10.0 μmol/L	4.20 ; IQR 0.67 91%	64.35 ; IQR 1.94 * 105%	60.16 ; IQR 1.75 107%
CMA-18-1.0 μmol/L	3.95 ; IQR 0.19 ** 86%	57.32 ; IQR 2,83 *** 93%	55.40 ; IQR 3.40 99%
CMD-8-10.0 μmol/L	6.57 ; IQR 1.13*** 143%	65.51 ; IQR 2.09 *** .107%	59.64 ; IQR 1.65 106%
CMD-8-1.0 μmol/L	4.51 ; IQR 0.35 98%	60.52 ; IQR 2.64 99%	56.85 ; IQR 3.51 101%
MG-22-10,0 μmol/L	4.70 ;IQR 0.22 102%	64.91 ; IQR 1.27 ** 106%	58.67 ; IQR 2.33 104%
MG-22-1.0 μmol/L	4.23 ; IQR 0.41 92%	63.64 ; IQR 3.73 104%	57.46 ; IQR 2.19 102%
Phenyl			
CMC-34-10.0 μmol/L	4.20 ; IQR 1.03 91%	58.04 ; IQR 3.73 95%	54.59 ; IQR 3.33 97%
CMC-34-1.0 μmol/L	4.00 ; IQR 0.56 87%	57.26 ; IQR 1.53 *** 93%	40.66 ; IQR 4.03 *** 72%
CMJ-33-10.0 μmol/L	5.34 ; IQR 0.69*** 116%	62.73 ; IQR 3.21 102%	57.72 ; IQR 3.59 103%
CMJ-33-1.0 μmol/L	4.75 ; IQR 0.67 103%	58.68 ; IQR 1.91 * 96%	54.36 ; IQR 2.45 97%
CMT-67-10.0 μmol/L	4.63 ; IQR 0.55 101%	62.74 ; IQR 2.48 102%	57.70 ; IQR 1.26 103%
CMT-67-1.0 μmol/L	4.12 ; IQR 0.22 90%	61.71 ; IQR 2.92 101%	54.58 ; IQR 1.35 97%
Allyl			
CMG-41-10.0 μmol/L	4.85 ; IQR 0.70 * 106%	63.13 ; IQR 1.60 103%	55.09 ; IQR 4.72 98%
CMG-41-1.0 μmol/L	4.44 ; IQR 0.36 97%	62.47 ; IQR 5.79 102%	54.26 ; IQR 3.16 97%
TIA-123-10.0 μmol/L	5.34 ; IQR 0.97 ** 116%	71.06 ; IQR 7.67 116%	57.68 ; IQR 0.99 103%
TIA-123-1.0 μmol/L	4.23 ; IQR 0.43 92%	63.15 ; IQR 3.33 103%	55.20 ; IQR 08,0 98%
TIA-160-10.0 μmol/L	5.25 ; IQR 0.45 ** 114%	72.28 ; IQR 5.37 *** 118%	58.03 ; IQR 1.12 103%
TIA-160-1.0 μmol/L	4.31 ; IQR 0.43 94%	66.10 ; IQR 2.36 *** 108%	57.26 ; IQR 1,97 102%

Note: *Statistical significance compared to the control group: * – $p < 0.05$; ** – $p < 0.01$; *** – $p < 0.001$.*

The *in vitro* analysis of protein thiol groups, as well as total and free thiol (SH) groups, revealed changes dependent on both the type of compound tested and the concentration applied. In the negative control group, the median values were 4.59 μmol/g protein for protein thiol groups, 61.31 μmol/L for total thiol groups, and 56.18 μmol/L for free thiol groups.

Administration of DOXO (positive control) at concentrations of 10.0 μmol/L and 1.0 μmol/L did not induce significant changes in these parameters, with values remaining close to those of the negative control. Overall, DOXO caused only minor alterations in the analyzed parameters. At a concentration of 10.0 μmol/L, protein thiol levels showed a slight increase of 4%, and total thiol groups increased by 3%, while free thiol groups remained comparable to control values. At 1.0 μmol/L, DOXO induced a slight decrease in protein thiol groups (−7% vs. control), suggesting a possible partial oxidation of SH groups.

The *in vitro* analysis of the effects of phenyl Cu–thiosemicarbazone compounds revealed significant variations in thiol-related parameters depending on the specific compound and the concentration used. In the case of CMA-18, a reduction in protein thiol group levels was observed, particularly at the concentration of 1.0 μmol/L, where the value decreased by 14% compared to the control, indicating a possible interaction with thiol groups or an increase in oxidative processes. At the same time, at the higher concentration, an increase in total thiol groups by 5% ($p < 0.05$) was observed, suggesting a possible compensatory activation of antioxidant mechanisms.

A pronounced effect on protein thiol groups was observed for the compound CMD-8, especially at the concentration of 10.0 μmol/L, where the value increased by up to +43% ($p < 0.001$) compared to the control. This significant increase may indicate a potential protective effect on proteins or stimulation of the cellular antioxidant system. Concurrently, total thiol groups increased by +7% ($p < 0.001$), and free thiol groups by +6% ($p > 0.05$), confirming the positive influence of this compound on redox homeostasis.

Within the same group, the compound MG-22 exhibited moderate effects on the analyzed parameters. At a concentration of 10.0 μmol/L, an increase in total thiol groups of +6% ($p < 0.01$) was observed, while protein-bound and free thiol groups increased by +2–4% ($p > 0.05$). These results suggest a relatively stable influence on the redox balance (Table 2).

In the case of phenyl Cu–thiosemicarbazone compounds, particularly CMC-34, the effects were more pronounced on free thiol groups. At a concentration of 1.0 μmol/L, their levels decreased significantly by −28% ($p < 0.001$) compared to the control, which may reflect oxidation of SH groups and the formation of disulfide bonds. In parallel, total thiol groups decreased by −7% ($p < 0.001$), while protein thiol groups decreased by −13% ($p > 0.05$), suggesting a possible pro-oxidant effect of this compound at lower concentrations.

The compounds CMJ-33 and CMT-67 exhibited moderate effects on the analyzed parameters, with most values remaining close to control levels. However, for CMJ-33 at 10.0 μmol/L, a significant increase in protein thiol groups of +16% ($p < 0.001$) was observed, which may indicate a protective effect against protein oxidation.

The evaluation of *in vitro* results for the allyl Cu–thiosemicarbazone group revealed both increases and decreases in thiol group levels. A significant increase in protein thiol groups was observed particularly for CMG-41 at 10.0 μmol/L, showing a moderate rise of +6% ($p < 0.05$) compared to the control group, suggesting a possible enhancement of antioxidant capacity.

Notably, compounds from the TIA series demonstrated some of the most pronounced effects on the thiol system. TIA-123 at 10.0 μmol/L induced an increase in protein thiol groups up to +16% ($p < 0.01$) and in total thiol groups up to +16% ($p > 0.05$). An even stronger effect was observed for TIA-160, where total thiol groups reached +18% ($p < 0.001$) compared to control, and protein thiol groups increased up to +14% ($p < 0.01$). These results suggest that

TIA-series compounds may exhibit significant antioxidant properties and contribute to the maintenance of redox homeostasis.

Overall, the obtained results indicate that certain Cu–thiosemicarbazone compounds, particularly CMD-8, CMJ-33, CMG-41, TIA-123, and TIA-160 at a concentration of 10.0 µmol/L, significantly increase thiol group levels, suggesting a potential antioxidant effect and possible protection of proteins against oxidative modifications.

Thus, the analysis highlights that the effects of Cu–thiosemicarbazone compounds on the thiol system are dependent on both the chemical structure of the compound and the concentration used. While some compounds demonstrated potential antioxidant effects by increasing thiol group levels, others led to decreases in these parameters, suggesting possible pro-oxidant effects or direct interactions with SH groups of biomolecules. These observations underline the importance of further investigation to clarify the mechanisms of action and pharmacological potential of these compounds.

Discussion

The *in vitro* results obtained in the present study demonstrate that the analyzed Cu–thiosemicarbazone compounds exert variable effects on redox homeostasis, reflected by changes in the levels of protein thiol groups, total thiol groups, and free thiol groups. Thiol groups represent essential components of the cellular antioxidant system, playing a key role in maintaining protein structure and function, regulating enzymatic activity, and protecting cells against oxidative stress. Therefore, the observed alterations in these parameters may indicate the influence of the tested compounds on cellular redox balance [20].

Analysis of the obtained results indicates that the effects of Cu–thiosemicarbazone compounds are dependent on both their chemical structure and the concentration used. In some cases, significant increases in thiol group levels were observed, suggesting a potential antioxidant or protective effect on biomolecules, while in other cases, decreases in these parameters were recorded, which may indicate oxidative processes or direct interactions with protein –SH groups.

One of the most notable compounds in terms of its effect on the thiol system is the derivative CMD-8, which at a concentration of 10.0 µmol/L induced a significant increase in protein thiol groups by +43% compared to the control. At the same time, increases in total and free thiol groups were also observed. These findings suggest that this compound may contribute to the stabilization of the cellular antioxidant system and to the protection of thiol groups against oxidation. The increase in thiol group levels may reflect either activation of antioxidant mechanisms or prevention of cysteine oxidation within protein structures [21].

Similar results have been reported in the scientific literature, where thiosemicarbazone derivatives have demonstrated the ability to act as antioxidant agents by scavenging free radicals or by modulating cellular redox systems. Studies conducted by Beraldo and Gambino have shown that numerous thiosemicarbazone derivatives can interact with free radicals and contribute to the maintenance of cellular redox balance. This activity is mainly attributed to the presence of the sulfur atom and the conjugated double-bond system within the molecular structure, which facilitates electron transfer and participation in redox reactions [22].

Another group of compounds that demonstrated significant effects on the analyzed parameters is represented by the TIA series, particularly TIA-123 and TIA-160. In the case of these compounds, significant increases in total thiol groups and protein thiol groups were observed, especially at the concentration of 10.0 µmol/L. For TIA-160, an increase in total thiol groups of +18% compared to control was recorded, suggesting a possible stimulation of cellular antioxidant mechanisms.

These observations are consistent with data reported in the contemporary literature, indicating that Cu–thiosemicarbazone compounds can influence redox balance through multiple mechanisms, including chelation of metal ions involved in free radical-generating reactions or modulation of intracellular glutathione levels. Glutathione represents one of the most important intracellular antioxidant molecules, and its level is closely correlated with the total thiol content of cells [23].

In most experimental groups, the values of protein thiol (SH) groups remained close to those of the negative control or showed moderate increases. Significant increases were observed particularly for the compounds CMD-8, CMJ-33, TIA-123, and TIA-160 at a concentration of 10.0 µmol/L, suggesting a possible stimulation of antioxidant mechanisms or protection of thiol groups against oxidative processes. These findings indicate that certain Cu–thiosemicarbazone compounds may contribute to maintaining protein integrity by protecting thiol groups.

Regarding total thiol groups, most tested compounds produced values comparable to or slightly higher than those of the negative control. The highest increases were observed for TIA-123 and TIA-160 at 10.0 µmol/L, as well as for CMD-8, suggesting a possible activation of cellular systems involved in maintaining redox balance. These changes may reflect the ability of certain Cu–thiosemicarbazone compounds to positively influence cellular antioxidant status.

Analysis of free thiol groups revealed, in most cases, values close to those of the negative control, indicating that the majority of compounds did not significantly affect the availability of free thiol groups. However, a significant decrease was observed for the compound CMC-34 at a concentration of 1.0 µmol/L, which may suggest a direct interaction with thiol groups or an enhancement of oxidative processes at the cellular level.

Such pro-oxidant effects have also been described in other studies, where certain thiosemicarbazone derivatives were shown to generate reactive oxygen species or interfere with mitochondrial function. Under specific conditions, these properties may be therapeutically beneficial, particularly in tumor cells, as the induction of oxidative stress can lead to inhibition of cell proliferation and activation of apoptotic pathways [24–25].

Overall, the obtained results are consistent with data reported in the scientific literature, suggesting that Cu–thiosemicarbazone compounds can influence cellular redox balance and exert antioxidant effects by preserving the integrity of protein thiol groups. These findings support the biological potential of these compounds and highlight the need for further studies to elucidate the underlying molecular mechanisms and to evaluate the structure–activity relationship.

Conclusions

The results of the present study demonstrated that the analyzed Cu–thiosemicarbazone compounds exert differential effects on redox homeostasis, reflected by changes in the levels of protein thiol groups, total thiol groups, and free thiol groups. These parameters represent important indicators of the redox status of biological systems and allow the evaluation of the influence of the tested compounds on cellular antioxidant mechanisms.

Compared to the negative control, some compounds induced significant increases in thiol group levels, suggesting a potential protective effect on the antioxidant system. In particular, the compounds CMD-8, CMJ-33, TIA-123, and TIA-160 at a concentration of 10.0 µmol/L demonstrated significant increases in total and protein thiol groups, indicating a potential antioxidant effect and a possible capacity to stabilize the cellular redox system.

Overall, the obtained results demonstrate that the effects of Cu–thiosemicarbazone compounds on thiol homeostasis are dependent on both the chemical structure of the compound and the concentration used. Some of these substances may exhibit antioxidant potential,

contributing to the maintenance of cellular redox balance, while others may induce oxidative modifications of biomolecules.

Further investigation of the relationship between chemical structure, concentration, and biological activity of these compounds may lead to the identification of molecules with significant therapeutic potential, particularly in the context of diseases associated with oxidative stress or in anticancer therapy.

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