CZU: 544.142.3 https://doi.org/10.59295/mabt2026.01

STRUCTURE-ACTIVITY RELATIONSHIP IN THIOSEMICARBAZONE LIGANDS: EFFECT OF ARM NUMBER AND STRUCTURAL FLEXIBILITY

Diana CEBOTARI[1,2], Olga GARBUZ[2,3], Victor TSAPKOV[2],
Aurelian GULEA[2], Sébastien FLOQUET[1]

[1]Institut Lavoisier de Versailles, CNRS UMR 8180, Univ. Versailles Saint Quentin en Yvelines, Université Paris-Saclay, 45 av. des Etats-Unis, 78035 Versailles cedex (France)
[2]State University of Moldova, 60 Alexei Mateevici str., MD-2009 Chisinau, Republic of Moldova.
[3]Institute of Zoology, 1 Academiei str., MD-2028 Chisinau, Republic of Moldova.

Abstract

A series of 18 thiosemicarbazone-type ligands divided into several ligand families depending on whether they are monothiosemicarbazones (HL^1 to HL^5), bis-thiosemicarbazones with flexible linkers (H_2L^6 to H_3L^{10}) or rigid linkers (H_2L^{11} to H_2L^{14}), and phenol-bis-thiosemicarbazones (H_3L^{15} to H_3L^{18}) was synthesised and subsequently investigated with regard to their antioxidant properties.

The antioxidant activity of these ligands was evaluated using the ABTS radical scavenging assay. The obtained IC_{50} values revealed a wide range of antioxidant capacities among the investigated compounds. Several ligands exhibited significant radical scavenging activity compared to the reference antioxidants Trolox and Rutin. In particular, compound H_3L^{16} demonstrated the strongest antioxidant effect with an IC_{50} value of 4.83 μM, followed by HL^3 (7.74 μM), H_2L^{11} (8.60 μM), and H_2L^{12} (9.10 μM). The enhanced antioxidant activity was found to correlate with the presence of phenolic hydroxyl groups, aromatic conjugation, and increased structural rigidity, which facilitate electron or hydrogen transfer processes involved in radical neutralization.

Keywords: mono-thiosemicarbazone, bis-thiosemicarbazone, phenol-bis-thiosemicarbazone, antioxidant activity, ABTS method.

RELAȚIA STRUCTURĂ-ACTIVITATE A TIOSEMICARBAZONELOR: EFECTUL NUMĂRULUI DE BRAȚE ȘI AL FLEXIBILITĂȚII STRUCTURALE

Resumat

A fost sintetizată o serie de 18 tiosemicarbazone, clasificată în mai multe familii de liganzi în funcție de tipul structural: mono-tiosemicarbazone (HL^1 până la HL^5), bis-tiosemicarbazone cu legături flexibile (H_2L^6 până la H_3L^{10}), bis-tiosemicarbazone cu legături rigide (H_2L^{11} până la H_2L^{14}) și fenol-bis-tiosemicarbazone (H_3L^{15} până la H_3L^{18}), iar ulterior au fost investigate proprietățile lor antioxidante. Activitatea antioxidantă a tiosemicarbazonelor sintetizate a fost evaluată prin testul de captare a radicalilor ABTS. Valorile IC_{50} obținute au evidențiat o gamă largă de capacități antioxidante în rândul compușilor studiați. Mai mulți compuși au prezentat o activitate semnificativă de eliminare a radicalilor în comparație cu antioxidanții de referință: Trolox și Rutin. În special, compusul H_3L^{16} a demonstrat cel mai puternic efect antioxidant, cu o valoare IC_{50} de 4,83 μM, urmat de HL^3 (7,74 μM), H_2L^{11} (8,60 μM) și H_2L^{12} (9,10 μM). S-a constatat că activitatea antioxidantă sporită este corelată cu prezența grupărilor hidroxil fenolice, conjugarea aromatică și rigiditatea structurală crescută, care facilitează procesele de transfer de electroni sau hidrogen implicate în neutralizarea radicalilor.

Cuvinte-cheie: mono-tiosemicarbazonă, bis-tiosemicarbazonă, fenol-bis-tiosemicarbazonă, activitate antioxidantă, metoda ABTS.

Introduction

Reactive oxygen species (ROS), such as the superoxide anion, hydroxyl radicals, and hydrogen peroxide, are continuously generated in biological systems as byproducts of normal metabolic processes.[1] Although ROS play an essential role in cellular signaling and physiological regulation, their excessive accumulation can lead to oxidative stress, a condition

associated with the damage of biomolecules such as lipids, proteins, and nucleic acids.[2] It is well known that oxidative stress is implicated in the development of numerous pathological conditions, including cancer, neurodegenerative diseases, inflammation, and cardiovascular disorders.[3] Consequently, the identification and development of molecules capable of neutralizing reactive oxygen species have become an important objective in medicinal and bioinorganic chemistry.

Biologically active molecules such as thiosemicarbazones have attracted considerable attention due to their versatile coordination chemistry and broad spectrum of pharmacological activities.[4–6] These compounds are typically formed through condensation reactions between thiosemicarbazides and aldehydes or ketones, resulting in molecules containing nitrogen and sulfur donor atoms capable of interacting with metal ions and reactive species. Due to these structural characteristics, thiosemicarbazone derivatives have been extensively studied for their antimicrobial, antiviral, antitumor, and antioxidant properties.[7–12] In particular, Academician Aurelian Gulea has played a major role in the development of this field throughout his career, making highly significant contributions evidenced by hundreds of publications and patents.

The biological activity of thiosemicarbazone derivatives is strongly influenced by their molecular structure, electronic properties, and ability to stabilize radical intermediates. Previous studies have demonstrated that modifications to the ligand structure, such as the introduction of electron-donating or electron-accepting substituents, changes in conjugation, or the incorporation of additional donor groups, can significantly affect their redox behavior and radical-neutralizing capacity.[13] Another important structural factor that can influence the antioxidant properties of these ligands is the flexibility or rigidity of the molecular backbone. Flexible ligands can adopt different conformations that facilitate interactions with reactive species, while rigid backbones can promote electronic delocalization and the stabilization of radical intermediates.[13, 14] Furthermore, the number of thiosemicarbazone arms in a molecule—such as mono- or bis-substituted derivatives—can influence the accessibility of active functional groups and, consequently, alter their antioxidant efficacy.[15, 16]

In this study, particular attention is paid to the antioxidant capacity of three families of thiosemicarbazones: mono-, bis-, and phenol-bis-thiosemicarbazones (Figure 1). The influence of variations in the R1 substituent (R^2–CH=N–NH–C(=S)–NHR^1), as well as the number of thiosemicarbazone arms, on antioxidant activity was investigated to gain a better understanding of the structure–activity relationship.

Methods and materials

Chemicals and syntheses

All chemicals and reagents used were of analytical grade and purchased from Sigma-Aldrich: 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS); 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), potassium persulfate ($K_2S_2O_8$), and ascorbic acid. DMSO (dimethyl sulfoxide), methanol, and other reagents (sodium acetate buffer solution, pH 6.5) were obtained from local suppliers. Deionized water was produced using an Adrona Crystal E HPLC water purification system. The ligands were synthesized via a condensation reaction between aldehydes and thiosemicarbazides in an alcoholic medium under reflux, in the presence of a few drops of glacial acetic acid used as a catalyst.[9, 17–19] As a result, a total of 18 thiosemicarbazone derivatives were obtained and classified into three families: mono-thiosemicarbazones, bis-thiosemicarbazones, and phenol-bis-thiosemicarbazones.[20]

Antioxidant activity

The $ABTS^{\bullet+}$ antioxidant activity was determined according to the method described by Re et al., with modifications.[21] The ABTS radical was generated by reacting a 7 mM ABTS solution with 140 mM potassium persulfate, incubated at 25°C in the dark for 12–16 hours. The

resulting radical solution was then diluted with 0.02 M sodium acetate buffer (pH 6.5). Dilutions of the eighteen experimental compounds were prepared in DMSO. Then, 20 µL of each dilution was transferred into a 96-well microtiter plate, and 180 µL of $ABTS^{\bullet+}$ working solution was added using the dispensing module of the hybrid reader (Synergy H1, Biotek). The mixture was shaken for 15 s. The change in absorbance was measured at 734 nm after 30 minutes of incubation at 25°C. All experiments were performed in triplicate. DMSO was used as the blank. Rutin, Trolox, and ascorbic acid were used as standards. Ascorbic acid (10 mM) was prepared in distilled water as a stock solution and used at concentrations ranging from 6.25 to 50 µM. Trolox (2 mM in methanol) was used as a reference at dilution concentrations ranging from 0.1 to 100 µM.

The percentage inhibition was calculated using the formula:

$$\%\text{Inhibition}=\frac{A0-A1}{A_0}*100$$

where A_0 is the absorbance of the control and A_1 is the absorbance of the sample.

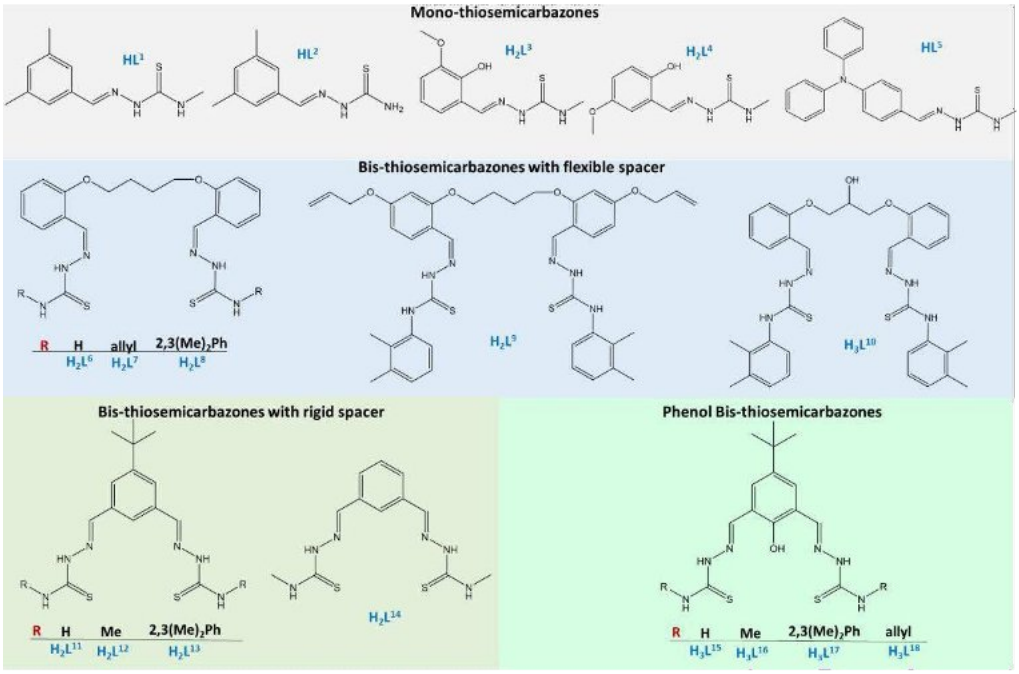


Figure 1. Representation of thiosemicarbazones used in this study

Results and discussion

Antioxidant activity of the thiosemicarbazones

The antioxidant properties of the synthesized thiosemicarbazone ligands were evaluated using the ABTS radical cation decolorization assay. The activity was expressed as IC_{50} values (µM), representing the concentration required to scavenge 50% of the $ABTS^{\bullet+}$ radicals. Lower IC_{50} values indicate stronger antioxidant activity.

For comparison, the well-known antioxidants Trolox and Rutin were used as reference compounds, presenting IC_{50} values of 26.30 µM and 20.70 µM, respectively (Figure 2, Table 1).

Mono-thiosemicarbazones

The mono-thiosemicarbazone ligands (HL^1–HL^5) showed moderate to good antioxidant activity.

IC_{50} values ranged between 7.74 and 27.88 μM:
HL^1 – 27.88 μM
HL^2 – 21.62 μM
H_2L^3 – 7.74 μM
H_2L^4 – 9.81 μM
HL^5 – 21.17 μM

Among this group, H_2L^3 and H_2L^4 exhibited the strongest radical scavenging ability, displaying significantly lower IC_{50} values than both Trolox and Rutin. The enhanced activity may be attributed to the presence of phenolic hydroxyl groups and conjugated aromatic systems, which facilitate electron or hydrogen donation to neutralize the ABTS radical cation.

Table 1. Antioxidant activity of the ligands

Ligand	IC_{50} (μM)
HL^1	27.88
HL^2	21.62
H_2L^3	7.74
H_2L^4	9.81
HL^5	21.17
H_2L^6	13.00
H_2L^7	66.60
H_2L^8	≥100
H_2L^9	≥100
H_3L^{10}	57.20
H_2L^{11}	8.60
H_2L^{12}	9.10
H_2L^{13}	28.80
H_2L^{14}	11.20
H_3L^{15}	20.31
H_3L^{16}	4.83
H_3L^{17}	24.27
H_3L^{18}	51.13

Bis-thiosemicarbazones with flexible spacer

The bis-thiosemicarbazones containing a flexible spacer (H_2L^6–H_3L^{10}) displayed a wider variation in antioxidant activity. Only H_2L^6 demonstrated relatively good antioxidant activity, while the remaining compounds exhibited moderate to weak radical scavenging ability. The lower activity observed for most ligands in this series may be explained by the flexible linker, which reduces electronic delocalization between the two thiosemicarbazone moieties.

Bis-thiosemicarbazones with rigid spacer

The bis-thiosemicarbazones containing a rigid aromatic spacer (H_2L^{11}–H_2L^{14}, Figure 2 and Table 1) generally exhibited improved antioxidant properties.

The compounds H_2L^{11} and H_2L^{12} showed particularly strong activity, suggesting that the rigid aromatic core promotes electron delocalization and stabilization of the radical species generated during the scavenging process.

Phenol bis-thiosemicarbazones

The phenolic bis-thiosemicarbazones (H_3L^{15}–H_3L^{18}) displayed diverse antioxidant activities. Among all investigated compounds, H_3L^{16} exhibited the strongest antioxidant activity (IC_{50} = 4.83 μM). This remarkable efficiency can be attributed to the presence of phenolic

hydroxyl groups, which act as hydrogen donors and stabilize the resulting radical species through resonance.

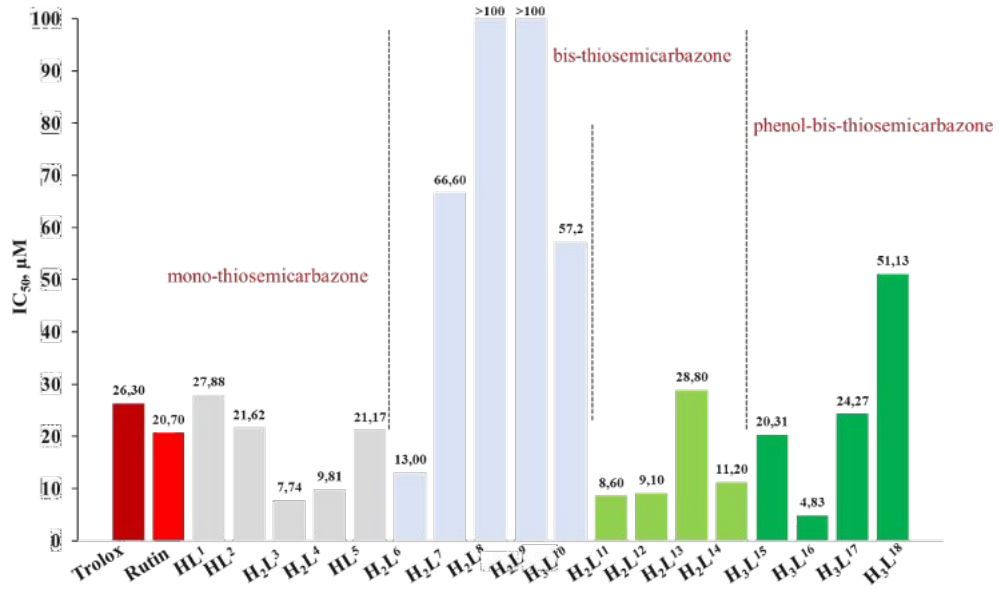


Figure 2. Antioxidant activity of three families of thiosemicarbazones: mono-thiosemicarbazones (gray); flexible bis-thiosemicarbazones (light blue), rigid bis-thiosemicarbazones (light green), and phenol-bis-thiosemicarbazones (dark green)

Structure–activity relationship

A comparative analysis of the obtained results suggests several structural factors influencing antioxidant activity:

a. Presence of phenolic OH groups: Phenolic moieties significantly enhance radical scavenging ability due to their hydrogen-donating capacity.

b. Aromatic conjugation: Extended π-conjugation facilitates stabilization of the radical species formed during the reaction with ABTS•$^{+}$.

c. Molecular rigidity: Ligands containing rigid aromatic spacers exhibit stronger activity than those with flexible linkers, likely due to improved electronic delocalization.

d. Electron-donating substituents: Substituents such as methyl groups may increase electron density and enhance antioxidant capacity.

Proposed ABTS scavenging mechanism

The antioxidant activity observed for the thiosemicarbazone ligands can be explained by two possible mechanisms:

Hydrogen atom transfer: thiosemicarbazone donates a hydrogen atom to the ABTS radical:

Thiosemicarbazone–OH + ABTS•$^{+}$ → Thiosemicarbazone–O• + ABTS

The resulting phenoxyl radical is stabilized through resonance within the aromatic system.

Single electron transfer: thiosemicarbazone transfers an electron to the radical cation:

Thiosemicarbazone + ABTS•$^{+}$ → Thiosemicarbazone•$^{+}$ + ABTS

The formed radical cation is stabilized by the conjugated structure of the thiosemicarbazone.

Conclusions

The ABTS assay revealed that several synthesized thiosemicarbazone ligands possess significant antioxidant activity, in some cases surpassing the reference antioxidants Trolox and Rutin.

The most potent compounds identified were:

H_3L^{16} (IC_{50} = 4.83 μM)

H_2L^{3} (IC_{50} = 7.74 μM)

H_2L^{11} (IC_{50} = 8.60 μM)

H_2L^{12} (IC_{50} = 9.10 μM)

These findings highlight the importance of phenolic groups, aromatic conjugation, and structural rigidity in enhancing the antioxidant properties of thiosemicarbazone derivatives.

Acknowledgments

This work is supported by the "ADI 2019" project funded by the IDEX Paris-Saclay, ANR-11-IDEX-0003-02. DC gratefully acknowledges Campus France for Excellence Eiffel grant as well as University State of Moldova for funding her PhD thesis.

References:

1. Snezhkina, A. V., Kudryavtseva, A. V., Kardymon, O. L., Savvateeva, M. V., Melnikova, N. V., Krasnov, G. S., Dmitriev, A. A. ROS Generation and Antioxidant Defense Systems in Normal and Malignant Cells. Oxid. Med. Cell. Longev., 2019, 2019, 6175804. DOI: 10.1155/2019/6175804.
2. Schieber, M., Chandel, N. S. ROS Function in Redox Signaling and Oxidative Stress. *Curr. Biol.*, 2014, 24(10), R453–R462. DOI: 10.1016/j.cub.2014.03.034.
3. Thanan, R., Oikawa, S., Hiraku, Y., Ohnishi, S., Ma, N., Pinlaor, S., Yongvanit, P., Kawanishi, S., Murata, M. Oxidative Stress and Its Significant Roles in Neurodegenerative Diseases and Cancer. *Int. J. Mol. Sci.*, 2014, 16(1), 193–217. DOI: 10.3390/ijms16010193.
4. Casas, J. S., García-Tasende, M. S., Sordo, J. Main group metal complexes of semicarbazones and thiosemicarbazones. A structural review. *Coord. Chem. Rev.*, 2000, 209(1), 197–261. DOI: 10.1016/S0010-8545(00)00363-5.
5. Pantea, V., Pavlovschi, E., Stratulat, S., Gulea, A., Tagadiuc, O., Gudumac, V. Targeting redox balance: antioxidant effects of thiosemicarbazones on human peripheral blood. *Moldovan Journal of Health Sciences*, 2025, 12(3), 101–109. DOI: 10.52645/MJHS.2025.3.16.
6. Richardson, D. R., Kalinowski, D. S., Richardson, V., Sharpe, P. C., Lovejoy, D. B., Islam, M., Bernhardt, P. V. 2-Acetylpyridine Thiosemicarbazones are Potent Iron Chelators and Antiproliferative Agents: Redox Activity, Iron Complexation and Characterization of their Antitumor Activity. *J. Med. Chem.*, 2009, 52(5), 1459–1470. DOI: 10.1021/jm801585u.
7. Fuior, A. *Synthesis and characterization of coordination compounds based on [Mo2O2E2]2+ (E = O or S) fragments for applications in biology*. Doctoral dissertation, Université Paris-Saclay, 2019. Available at: http://www.theses.fr/2019SACLV094.
8. Fuior, A., Hijazi, A., Garbuz, O., Bulimaga, V., Zosim, L., Cebotari, D., Haouas, M., Toderaş, I., Gulea, A., Floquet, S. Screening of biological properties of MoV2O2S2- and MoV2O4-based coordination complexes: Investigation of antibacterial, antifungal, antioxidative and antitumoral activities versus growing of Spirulina platensis biomass. *J. Inorg. Biochem.*, 2022, 226, 111627. DOI: 10.1016/j.jinorgbio.2021.111627.
9. [9Cebotari, D., Calancea, S., Garbuz, O., Balan, G., Marrot, J., Shova, S., Guérineau, V., Touboul, D., Tsapkov, V., Gulea, A., Floquet, S. Synthesis, structure and biological properties of a series of dicopper(bis-thiosemicarbazone) complexes. *New J. Chem.*, 2024, 48(26), 12043–12053. DOI: 10.1039/D4NJ00342J.
10. [10] Pahontu, E., Julea, F., Rosu, T., Purcarea, V., Chumakov, Y., Petrenco, P., Gulea, A. Antibacterial, antifungal and in vitro antileukaemia activity of metal complexes with thiosemicarbazones. *J. Cell. Mol. Med.*, 2015, 19(4), 865–878. DOI: 10.1111/jcmm.12508.
11. Anitha, P., Chitrapriya, N., Jang, Y. J., Viswanathamurthi, P. Synthesis, characterization, DNA interaction, antioxidant and anticancer activity of new ruthenium(II) complexes of

thiosemicarbazone/semicarbazone bearing 9,10-phenanthrenequinone. *J. Photochem. Photobiol. B*, 2013, 129, 17–26. DOI: 10.1016/j.jphotobiol.2013.09.005.
12. Balan, G., Burduniuc, O., Usataia, I., Graur, V., Chumakov, Y., Petrenko, P., Gudumac, V., Gulea, A., Pahontu, E. Novel 2-formylpyridine 4-allyl-S-methylisothiosemicarbazone and Zn(II), Cu(II), Ni(II) and Co(III) complexes: Synthesis, characterization, crystal structure, antioxidant, antimicrobial and antiproliferative activity. *Appl. Organomet. Chem.*, 2020, 34(3), e5423. DOI: 10.1002/aoc.5423.
13. Yang, L., Liu, H., Xia, D., Wang, S. Antioxidant Properties of Camphene-Based Thiosemicarbazones: Experimental and Theoretical Evaluation. *Molecules*, 2020, 25(5), 1192. DOI: 10.3390/molecules25051192.
14. Garbuz, O., Ceban, E., Istrati, D., Railean, N., Toderas, I., Gulea, A. Thiosemicarbazone-Based Compounds: Cancer Cell Inhibitors with Antioxidant Properties. *Molecules*, 2025, 30(9), 2077. DOI: 10.3390/molecules30092077.
15. Padhyé, S., Kauffman, G. B. Transition metal complexes of semicarbazones and thiosemicarbazones. *Coord. Chem. Rev.*, 1985, 63, 127–160. DOI: 10.1016/0010-8545(85)80022-9.
16. Beraldo, H., Gambino, D. The Wide Pharmacological Versatility of Semicarbazones, Thiosemicarbazones and Their Metal Complexes. *Mini-Rev. Med. Chem.*, 2004, 4(1), 31–39. DOI: 10.2174/1389557043487484.
17. Cebotari, D., Haouas, M., Floquet, S., Gulea, A. Synthesis and characterisations of six new bis-thiosemicarbazone ligands. *Studia Universitatis Moldaviae (Seria Ştiinţe Reale şi ale Naturii)*, 2021, 136(6), 101–112. DOI: 10.5281/ZENODO.4431691.
18. Cebotari, D., Builș, J., Garbuz, O., Balan, G., Marrot, J., Guérineau, V., Touboul, D., Haouas, M., Segado-Centelles, M., Bo, C., Gulea, A., Floquet, S. A new series of bioactive Mo(V)2O2S2-based thiosemicarbazone complexes: Solution and DFT studies, and antifungal and antioxidant activities. *J. Inorg. Biochem.*, 2023, 245, 112258. DOI: 10.1016/j.jinorgbio.2023.112258.
19. Cebotari, D., Calancea, S., Marrot, J., Guillot, R., Falaise, C., Guérineau, V., Touboul, D., Haouas, M., Gulea, A., Floquet, S. Tuning the nuclearity of [Mo2O2S2]2+-based assemblies by playing with the degree of flexibility of bis-thiosemicarbazone ligands. *Dalton Trans.*, 2023, 52(10), 3059–3071. DOI: 10.1039/D2DT03760B.
20. Cebotari, D. *Synthèses et caractérisations de complexes à base du fragment [Mo2O2S2]2+ pour des applications en biologie*. Doctoral dissertation, Université Paris-Saclay; Universitatea de Stat din Moldova, 2022. Available at: https://theses.hal.science/tel-04301336.
21. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.*, 1999, 26(9), 1231–1237. DOI: 10.1016/S0891-5849(98)00315-3.

Authors:



Diana CEBOTARI, Ph.D. in Chemistry, scientific researcher, State University of Moldova, Institut Lavoisier de Versailles, Univ. Versailles Saint Quentin en Yvelines, Université Paris-Saclay, France.
e-mail: cebotaridiana1995@gmail.com
ORCID: 0000-0002-0305-9548



Olga Garbuz, Dr., Laboratory of Systematics and Molecular Phylogenetics, Institute of Zoology, Moldova State University, 1 Academiei Street, MD-2028 Chisinau, Moldova
e-mail: olga.garbuz@sti.usm.md
ORCID: 0000-0001-8783-892X



Victor TSAPKOV, Ph.D. in Chemical Sciences, Associate Professor, Moldova State University.
e-mail: victor.tapcov@usm.md; vtsapkov@gmail.com
ORCID: 0000-0003-1732-3116



Aurelian GULEA, doctor habilitate, university professor, academician; head of LCȘ Advanced Materials in Biopharmaceuticals and Technology, Moldova State University.
e-mail: guleaaurelian@gmail.com
ORCID: 0000-0003-2010-7959



Sébastien FLOQUET, professor- researcher, Institut Lavoisier de Versailles, Univ. Versailles Saint Quentin en Yvelines, Université Paris-Saclay, France.
e-mail: sebastien.floquet@uvsq.fr
ORCID: 0000-0003-2433-1771