BIOLOGICALLY ACTIVE 3d METAL COMPLEXES WITH 2-FORMYL- AND 2-ACETYLPYRIDINE 4,*S*-DIALLYLISOTHIOSEMICARBAZONES: SYNTHESIS, STRUCTURE, AND PROPERTIES

COMPLECȘII METALELOR 3D BIOLOGIC ACTIVI CU 4,S-DIALILIZOTIOSEMICARBAZONELE 2-FORMILPIRIDINEI ȘI 2-ACETILPIRIDINEI: SINTEZA, STRUCTURA ȘI PROPRIETĂȚILE

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Summary. *Eight coordination compounds of Ni(II), Co(III), and Fe(III) with 2-formylpyridine (HL^1) and 2-acetylpyridine (HL^2) 4,S-diallylisothiosemicarbazones were synthesized: $[Ni(HL^1)_2](NO_3)_2$ (**1**), $[Ni(HL^2)_2](NO_3)_2$ (**2**), $[Ni(HL^1)_2](I)_2$ (**3**), $[Co(L^1)_2]I$ (**4**), $[Co(L^2)_2]I$ (**5**), $[Fe(L^1)_2]NO_3$ (**6**), $[Fe(L^1)_2]Br$ (**7**), $[Fe(L^2)_2]NO_3$ (**8**). The ligands were characterized by*

NMR and FTIR spectroscopy, and the metal complexes were studied using elemental analysis, FTIR spectroscopy, and molar conductivity in methanol. The molecular structures of compounds 1–3 were confirmed via single-crystal X-ray diffraction. The biological activity of complexes was evaluated, revealing notable antibacterial, antifungal, and antioxidant properties. In particular, compounds 1, 2, 4, and 6 demonstrated higher antiradical activity against $ABTS^{\bullet+}$ *cation radicals than Trolox, a commonly used antioxidant standard. These findings highlight the potential of such metal complexes in the development of new bioactive agents.*

Keywords: *antifungal activity; antimicrobial activity; antioxidant activity; Co(III), Fe(III), Ni(II) coordination compounds; isothiosemicarbazones.*

INTRODUCTION

The escalating emergence of multidrug-resistant microbial strains, coupled with the growing recognition of oxidative stress as a key factor in the pathogenesis of numerous diseases, underscores the urgent need for the development of novel antimicrobial and antioxidant agents [1–3]. One of the current strategies in medicinal chemistry focuses on modifying existing biologically active molecules to develop more selective and less toxic therapeutic agents. Among the most promising scaffolds are thiosemicarbazide derivatives—versatile compounds known for their ability to coordinate with transition metals and for exhibiting a wide range of biological effects [4–5]. In particular, thiosemicarbazones based on heterocyclic aldehydes and ketones stand out due to their potential to form stable chelates and their marked pharmacological activity [6–7]. Functionalization of these molecules, for example by introducing allyl groups at the fourth position of the thiosemicarbazone moiety, significantly enhances their biological performance and broadens their therapeutic potential. Metal complexes with such ligands, especially those involving biologically relevant transition metals, have already shown promising results in preclinical models and have been patented as low-toxicity, high-efficacy agents [8–11]. Earlier studies reported the synthesis and biological evaluation of copper(II) complexes with 4,*S*-diallylisothiosemicarbazones of 2-formylpyridine (HL^1) and 2-acetylpyridine (HL^2) [12]. Building on these findings, the present work focuses on designing similar coordination compounds with selected 3*d* metal ions in order to explore how the nature of the metal influences their antimicrobial and antioxidant activities.

MATERIAL AND METHOD

All reagents were of analytical grade and used without further purification unless otherwise stated. The 3*d* metal salts were obtained from Merck (Darmstadt, Germany) and used as supplied. 2-Formylpyridine and 2-acetylpyridine, employed in the synthesis of 4-allyl-*S*-alkylisothiosemicarbazones, were purchased from Sigma-Aldrich and used without additional purification. Solvents were purified and dried according to standard methods [13].

^1H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 spectrometer with $CDCl_3$ as the solvent. FTIR spectra were measured on a Bruker ALPHA FTIR spectro-

photometer in the range of 4000–400 cm^{-1} at ambient temperature. The metal content in the synthesized coordination compounds was determined by titrimetric analysis following procedures analogous to those described in the literature [14-16]. The molar conductivity of methanolic solutions of the complexes (0.001 M, 20°C) was measured using an R-38 Rheochord conductometer.

Prop-2-en-1-yl-N-(prop-2-en-1-yl)-N'-[(pyridin-2-yl)methylene]carbamohydrazonothioate (**HL^1**) was synthesized in three steps. First, allyl iodide (1.68 g, 10.0 mmol) was added to a stirred solution of 4-allylthiosemicarbazide (1.31 g, 10.0 mmol) in ethanol [12]. After stirring for 2 hours at room temperature, 2-formylpyridine (1.07 g, 10.0 mmol) was added, and the reaction mixture was heated at 70 °C for 30 minutes. Upon cooling, a yellow solid precipitated, which was filtered, washed with ethanol, and air-dried. The resulting solid ($[H_2L^1]I$) was dissolved in ethanol, and an aqueous solution of sodium carbonate was added dropwise to adjust the pH to 7–8. The neutral compound was extracted with chloroform and dried under vacuum. Yield: 75%; mp: 62–63 °C. IR (ν, cm^{-1}): 3219 (N–H), 1599, 1575, 1560 (C=N), 1096 (CH_2–S), 766 (C–S).

Prop-2-en-1-yl-N-(prop-2-en-1-yl)-N'-[1-(pyridin-2-yl)ethylidene]carbamohydrazonothioate (**HL^2**) was synthesized following the same procedure as for **HL^1**, using 2-acetylpyridine (1.21 g, 10.0 mmol) in place of 2-formylpyridine. Yield: 80%; mp: 96–97 °C. IR (ν, cm^{-1}): 3215 (N–H), 1601, 1583, 1558 (C=N), 1044 (CH_2–S), 743 (C–S).

General method for synthesis of coordination compounds 1-8. The metal salt was added to an ethanolic solution of the corresponding 4-allyl-*S*-alkylisothiosemicarbazone (HL^1 or HL^2), in a 1:2 molar ratio (metal to ligand). The resulting mixture was stirred at 60 °C using a magnetic stirrer for 40 minutes. Upon cooling, a precipitate formed, which was filtered, washed with a small amount of ethanol, and air-dried.

For the synthesis of coordination compounds of the hydroiodide of 4-allyl-S-alkylisothiosemicarbazones with nickel(II) and cobalt(II) acetates, an ethanolic solution of the hydroiodide of the 4-allyl-*S*-alkylisothiosemicarbazone of the ketone (**HL^1** or **HL^2**) was treated with nickel(II) or cobalt(II) acetate in a 1:2 molar ratio. The resulting mixture was stirred at 50–60 °C using a magnetic stirrer for 40–50 minutes. Upon cooling, a precipitate formed, which was filtered, washed with a small amount of ethanol, and air-dried.

Antibacterial activity

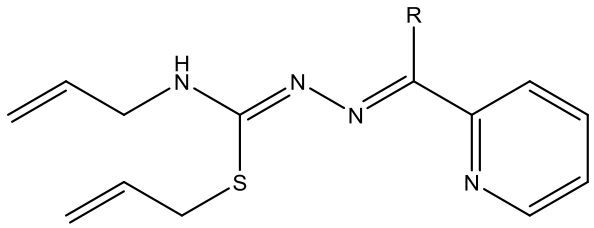
Standard strains of *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Escherichia col*i (ATCC 25922), *Candida albicans* (ATCC 10231), were used to study antibacterial activity of the synthesized compounds. The minimum inhibitory concentrations (MICs, μg mL^{-1}) and minimum bactericidal concentrations (MBCs, μg mL^{-1}) were determined using the method of serial dilutions in liquid broth. The tested substances were dissolved in DMSO at a concentration of 10 mg mL^{-1}. The next dilutions were made using 2% of peptone bullion.

Antioxidant Activity

The antioxidant activity of the synthesized compounds was evaluated using the ABTS$^{\bullet+}$ assay. The ABTS$^{\bullet+}$ radical cation was generated by mixing a 7 mM solution of ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid), Sigma) with a 2.45 mM solution of potassium persulfate ($K_2S_2O_8$, Sigma) and incubating the mixture in the dark at 25 °C for 12–20 hours. The resulting radical solution was diluted with acetate-buffered saline (0.02 M, pH 6.5) until an absorbance of 0.70 ± 0.01 at 734 nm was achieved. Test compounds were prepared in DMSO at concentrations ranging from 1 to 100 μM. Then, 20 μL aliquots of each dilution were added to wells of a 96-well microtiter plate, followed by 180 μL of the ABTS$^{\bullet+}$ working solution, dispensed using the automated module of the BioTek Synergy H1 hybrid reader. After incubation for 30 minutes at 25 °C, the decrease in absorbance at 734 nm was recorded. All measurements were performed in triplicate. DMSO was used as the negative control, and blanks containing solvent without ABTS$^{\bullet+}$ were included. Each experiment was repeated three times, and the results were averaged.

RESULTS AND DISCUSSIONS

Coordination compounds of nickel(II), cobalt(III), and iron(III) with 2-formylpyridine (HL^1) and 2-acetylpyridine (HL^2) 4,*S*-diallylisothiosemicarbazones and their hydroiodides ($[H_2L^1]I$, $[H_2L^2]I$) were synthesized, characterized, and their physicochemical and biological properties studied.



HL^1 (R=H), HL^2 (R=CH_3)

Heating ethanolic solutions of HL^1 or HL^2 with nickel(II), cobalt(II), and iron(III) salts (2:1 molar ratio) at 50–55 °C yielded microcrystalline brown solids (complexes 1–2, 5–8). Complexes 3–5 were obtained by reacting hydroiodides $[H_2L^1]I$ or $[H_2L^2]I$ with nickel(II) or cobalt(II) acetates (2:1 molar ratio). Eight complexes were obtained: $[Ni(HL^1)_2](NO_3)_2$ (1), $[Ni(HL^2)_2](NO_3)_2$ (2), $[Ni(HL^1)_2](I)_2$ (3), $[Co(L^1)_2]I$ (4), $[Co(L^2)_2]I$ (5), $[Fe(L^1)_2]NO_3$ (6), $[Fe(L^1)_2]Br$ (7), $[Fe(L^2)_2]NO_3$ (8). These compounds are soluble in common organic solvents but sparingly soluble in water. Molar conductivity in methanol showed nickel(II) complexes (1–3) behave as 1:2 electrolytes, while cobalt(III) and iron(III) complexes act as 1:1 electrolytes.

The IR spectra of HL^1 and HL^2 show characteristic bands of pyridine C=N and N4–H groups. In the complexes 1–8, the pyridine C=N band shifts 21–39 cm^{-1} to lower

frequencies, indicating coordination through the pyridine nitrogen. The N4–H band shifts down by 130 cm^{-1} in Ni(II) complexes and disappears in Co(III) and Fe(III) complexes, suggesting deprotonation. The azomethine C=N bands also shift 13–39 cm^{-1} to lower frequencies, confirming coordination via azomethine nitrogen. The C–S bands remain nearly unchanged, showing sulfur is not involved in coordination. New bands appear at 510–415 cm^{-1}, assigned to metal–N, metal–O, and metal–S vibrations.

The *in vitro* antimicrobial activity of isothiosemicarbazones HL^1 and HL^2 and some complexes was evaluated against *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, and *Candida albicans* (Table 1). The free ligands showed moderate activity, with HL^2 being more effective than HL^1. Complexation significantly enhanced their antimicrobial effects. The $[Fe(L^1)_2]Br$ complex (**7**) exhibited the highest activity against Gram-positive bacteria, with MIC values of 1.95–0.98 µg/mL, surpassing the efficacy of furacilin. Cobalt complexes (4-5) also showed notable activity, especially against *B. cereus*.

Complexes containing the HL^2 ligand were more active against *E. coli*; for example, $Ni(HL^2)_2$ (**2**) showed moderate inhibition with an MIC of 125 µg/mL, whereas the corresponding HL^1-based complex was inactive. A similar trend was observed for the iron complexes. All tested complexes demonstrated higher antifungal activity against *Candida albicans* compared to nystatin, with MIC values ranging from 3.91 to 62.5 µg/mL, compared to 80 µg/mL for nystatin.

Table 1. Minimum inhibitory concentrations (MIC) and bactericidal/fungicidal concentrations (MBC/MFC) of coordination compounds 1, 2, 4, 5, 7, 8 against test bacteria and fungi (in µg/mL)

Compound	*Staphylococcus aureus* ATCC 25923		*Bacillus cereus* ATCC 11778		*Escherichia coli ATCC 25922*		*Candida albicans ATCC 10231*	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MFC
HL^1	125,0	250,0	31,25	62,50	>1000	>1000	15,63	31,25
HL^2	31,25	62,50	62,50	62,50	>1000	>1000	7,81	62,50
1	62,50	125,0	31,25	62,50	>1000	>1000	62,50	125,0
2	7,81	15,63	7,81	7,81	125,0	125,0	7,81	31,25
4	3,91	7,81	7,81	15,63	>1000	>1000	31,25	62,50
5	3,91	3,91	1,95	3,91	-	-	7,81	15,63
7	1,95	3,91	0,98	1,95	>1000	>1000	15,63	62,50
8	3,91	3,91	3,91	3,91	125,0	250,0	7,81	15,63
Furacilin	9,3	9,3	4,7	4,7	18,5	37,5	>1000	>1000
Nystatin	>1000	>1000	>1000	>1000	>1000	>1000	80	80

The study of antioxidant activity revealed that coordination significantly enhances the radical-scavenging properties of the synthesized isothiosemicarbazone-based compounds. Evaluation against ABTS•$^{+}$ cation radicals demonstrated that most nickel, cobalt, and iron complexes exhibit higher antioxidant activity than previously reported copper(II) analogs [12]. Among the investigated compounds, $[Ni(HL^2)_2](NO_3)_2$ (2) showed the highest activity, with an IC_{50} value of 23.2 ± 0.4 μM. Complexes $[Co(L^1)_2]I$ (4), $[Ni(HL^1)_2](NO_3)_2$ (1), and $[Fe(L^1)_2]NO_3$ (6) also demonstrated notable activity (IC_{50} = 25.7 ± 0.5 μM, 27.3 ± 0.7 μM, and 27.4 ± 2.4 μM, respectively), all surpassing the reference antioxidant Trolox (IC_{50} = 33.3 ± 0.2 μM).

Overall, complexes derived from HL^1 generally exhibited stronger antioxidant properties than those based on HL^2, with the exception of the nickel(II) complex (2), which proved to be the most active compound in the series.

Table 2. Antioxidant activity of compounds HL^1, HL^2, 1, 2, 4, 5, 6, 8 with the corresponding isothiosemicarbazones against ABTS$^{\bullet+}$ cation radicals

Compound	IC_{50}, μM
HL^1 [12]	28.5 ± 4.0
HL^2 [12]	80.8 ± 13.4
1	27.3± 0.7
2	23.2 ± 0.4
4	25.7 ± 0.5
5	72.4 ± 4.2
6	27.4± 2.4
8	69.5 ± 1.0
Trolox	33.3 ± 0.2

CONCLUSIONS

The 2-formylpyridine (HL^1) and 2-acetylpyridine (HL^2) 4,*S*-diallylisothiosemicarbazones and their coordination compounds with Ni(II), Co(III), and Fe(III) were synthesized and characterized. Coordination with metal ions significantly enhanced the biological activity of the ligands. Complexes with **HL^2** showed improved activity against *Escherichia coli*, while the $[Fe(L^1)_2]Br$ complex exhibited the highest antibacterial effect against *Gram-positive* strains. All studied complexes demonstrated stronger antifungal

activity against *Candida albicans* compared to nystatin. In antioxidant assays, most Ni, Co, and Fe complexes showed higher radical-scavenging activity than previously reported Cu(II) analogs, with $[Ni(HL^2)_2](NO_3)_2$ being the most active. The biological activity of the complexes is strongly influenced by both the metal center and the nature of the isothiosemicarbazone ligand.

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