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Scientific paper

Novel Antioxidants Based on Selected 3*d* Metal Coordination Compounds with 2-Hydroxybenzaldehyde 4,*S*-Diallylisothiosemicarbazone

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Abstract

2-Hydroxybenzaldehyde 4,*S*-diallylisothiosemicarbazone (**HL**) was synthesized and characterized by ^{1}H, ^{13}C NMR and FTIR spectroscopies. It exists in solution in two isomeric forms: *cis* (~25%) and *trans* (~75%). Six stable complexes were obtained by interaction of **HL** with copper(II), nickel(II), cobalt(III) and iron(III) salts: [Cu(L)Cl] (**1**), [Cu(L)NO_3] (**2**), [Cu(3,4-Lut)(L)NO_3] (**3**), [Ni(L)OAc] (**4**), [Co$(L)_2$]Cl (**5**), [Fe$(L)_2$]NO_3 (**6**). The synthesized complexes have been studied by elemental analysis, FTIR, molar electrical conductivity and single crystal X-ray diffraction (**6**). For all compounds the antioxidant activity against cation radicals $ABTS^{\bullet+}$ was studied. All complexes and free ligand are more active than trolox that is used in medicine practice. Complex **4** (IC_{50} = 7.20 μM) is the most active one. The introduction of heterocyclic amine did not improve the antioxidant activity. The introduction of *S*-allyl group into isothiosemicarbazone affected the activity of the synthesized substances, and in some cases the resulting complexes exhibit greater activity than complexes with isothiosemicarbazones with other *S*-radicals.

Keywords: Isothiosemicarbazone, complexes, crystal structure, antioxidant activity.

1. Introduction

Free radical can be defined as an atom or molecule containing one or more unpaired electrons in valency shell or outer orbit and is capable of independent existence. They are formed in the body as by-products of natural processes of electron transfer between metabolites, and as intermediates in reactions catalyzed by enzymes.[1] Free radicals play a key role in the development of many diseases: cancer, brain diseases, immune system disorders, heart diseases, diabetes et al.[2–5] There are also discussions about the participation of free radicals in the aging of the body, due to the derivatization of proteins by free radicals, which leads to the loss of biological function and subsequent degradation of proteins. Thus, the body's natural antioxidants no longer function normally as they did in a young body.[6]

Antioxidant substances could be natural or synthetic. Natural antioxidants are obtained entirely from natural sources and are used in food, cosmetics, and pharmaceutical industries.[7] On the other hand, synthetic antioxidants are more active than natural ones and possess constant antioxidant activity.[8] The content of natural antioxidants in plants is extremely low, therefore, after going through all the stages of extraction, a very small amount of the antioxidant is obtained as a result. While the amount of synthetic antioxidant can be controlled during the synthesis. So, the synthesis of new biologically active compounds is one of the important directions of the modern chemistry.

Thiosemicarbazones are an important class of biologically active substances. They often have promising anticancer, antimicrobial, and antifungal properties, while their antioxidant properties are less studied.[9–13] Much less

attention is paid to the biological properties, especially antioxidant properties, of such thiosemicarbazone derivatives as *S*-substituted isothiosemicarbazones.[14–16] The biological properties of thiosemicarbazones and isothiosemicarbazones often changes upon coordination of metal ions.[17] The isothiosemicarbasones differ from the thiosemicarbazones in the way of coordination to the metal ion, because the alkylation of the sulfur atom in the composition of thiosemicarbazones, leads to the fact that the sulfur atom is not involved in the coordination.[18] The lipophilicity, which controls the rate of penetration into the cell, is modified in the process of coordination. That may lead to the enhance of biological activity of metal complex comparing to free ligand.[19] The complexes of copper, zinc, nickel and cobalt with 2-formylpyridine and 2-acetylpyridine 4-allyl-*S*-methylisothiosemicarbazones show promising anticancer activity but their antioxidant activity is rather moderate.[10,20] Replacement of 2-formylpyridine moiety with 2-hydroxybenzaldehyde moiety in 4-allyl-*S*-methylisothiosemicarbazone led to a significant increase of the antioxidant activity of not only the coordination compounds but also the initial ligands.[21] The complexes of 3*d* metals with 2-hydroxy-3-methoxybenzaldehyde 4-allyl-*S*-methylisothiosemicarbazone still posses a strong antioxidant activity that is superior to such substance as trolox that is used in medical practice, but it does not exceed the corresponding compounds with 2-hydroxybenzaldehyde 4-allyl-*S*-methylisothiosemicarbazone. So, the introduction of the methoxy group 2-hydroxybenzaldehyde fragment did not cause the strengthening of their antioxidant activity.[22]

That is why in this work we replaced the *S*-methyl group in the structure of 2-hydroxybenzaldehyde 4-allyl-*S*-methylisothiosemicarbazone with an *S*-allyl group and synthesized new antioxidants with thus obtained 2-hydroxybenzaldehyde 4,*S*-diallylisothiosemicarbazone (HL) (Fig. 1).

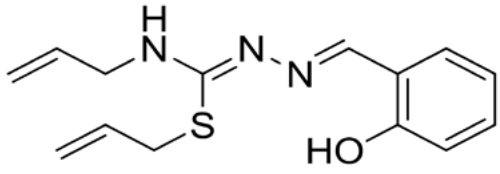


Figure 1. 2-Hydroxybenzaldehyde 4,*S*-diallylisothiosemicarbazone (HL).

2. Experimental

2. 1. Materials and Methods

N[4]-Allylthiosemicarbazide was synthesized by the reaction between allyl isothiocyanate and hydrazine hydrate.[23] Allyl isothiocyanate, hydrazine hydrate, 2-hydroxybenzaldehyde, copper(II) chloride dihydrate, copper(II) nitrate trihydrate, nickel(II) acetate tetrahydrate, cobalt(II) chloride hexahydrate, iron(III) nitrate hexahydrate, sodium carbonate anhydrous, 3,4-lutidine were obtained from Sigma-Aldrich.

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-400 using $CDCl_3$ as a solvent. The ^{1}H and ^{13}C NMR chemical shifts are reported in ppm relative to the residual solvent peak (7.26 ppm for ^{1}H NMR; 77.16 ppm for ^{13}C NMR). FT-IR spectra were obtained on a Bruker ALPHA FTIR spectrophotometer at room temperature in the range of 4000–400 cm^{-1}. The elemental analysis was performed similarly to the literature procedures[24] and on the automatic Perkin Elmer 2400 elemental analyzer. The resistance of solutions of complexes in methanol (20 °C, c 0.001 M) was measured using an R-38 rheochord bridge.

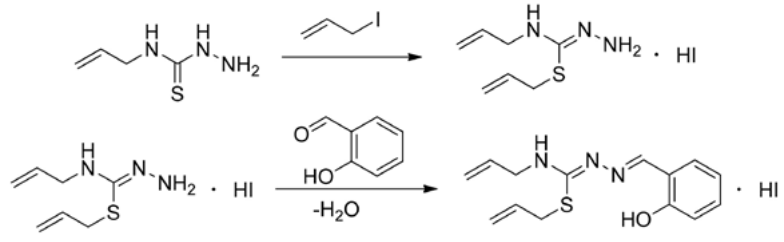
2. 2. Synthesis of 2-hydroxybenzaldehyde 4,*S*-diallylisothiosemicarbazone (HL)

The isothiosemicarbazone HL was obtained as a result of a three-step process.

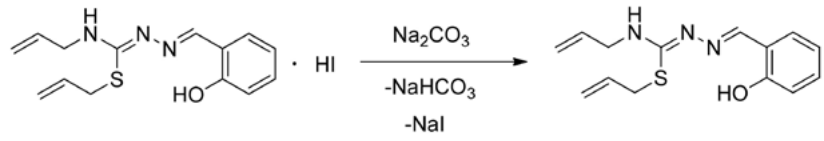
At the first step, the allyl iodide (1.68 g, 10.0 mmol) has been added to the solution of *N*[4]-allylthiosemicarbazide (1.31 g, 10.0 mmol) in ethanol.

After 2 hours of stirring at room temperature 2-hydroxybenzaldehyde (1.22 g, 10.0 mmol) was added. The solution was stirred at 80 °C for 30 min. After cooling to room temperature, a yellow precipitate formed from the solution, which was filtered off, washed with ethanol and dried in air (Scheme 1).

At the third step, the aqua solution of sodium carbonate had been added to the solution of 2-hydroxybenzaldehyde 4,*S*-diallylisothiosemicarbazone hydroiodide (4.03 g, 10.0 mmol) until the pH reached value 7-8. After that, the 2-hydroxybenzaldehyde 4,*S*-diallylisothiosemicarbazone was extracted by chloroform and dried in *vacuo* (Scheme 2).



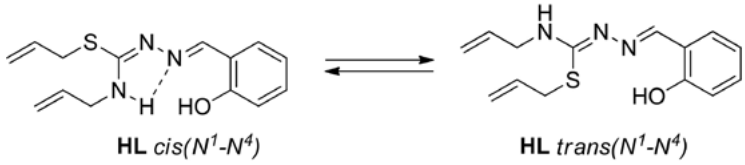
Scheme 1. Synthesis of 2-hydroxybenzaldehyde 4,*S*-diallylisothiosemicarbazone hydroiodide



Scheme 2. Neutralization of 2-hydroxybenzaldehyde 4,*S*-diallylisothiosemicarbazone hydroiodide

Pale yellow solid. Yield: 90%; mp 120-121 °C. FW: 275.369 g/mol; Anal Calc. for $C_{14}H_{17}N_3OS$: C, 61.06; H, 6.22; N, 15.26; S, 11.64; found: C, 60.91; H, 6.00; N, 15.08; S, 11.49%. IR data (cm^{-1}): ν (O–H) 3427; ν (N^4–H) 3150; ν (C=C allyl) 1642; ν (C=N) 1606, 1571; ν (C–O) 1265; ν (CH_2–S) 1152; ν (C–S) 743.

was added. The mixture was stirred for 1 hour at 60 ˚C. The green precipitate was formed during stirring. The resulting precipitate was filtered off, washed with a small amount of ethanol and dried. Yield: 87%. Anal. calc. for $C_{14}H_{16}ClCuN_3OS$: Cu, 17.02; C, 45.04; H, 4.32; N, 11.25; S, 8.59; found: Cu, 16.55; C, 44.89; H, 4.01; N, 11.05; S, 8.30%.



Scheme 3. The isomeric forms of the isothiosemicarbazone **HL**.

HL *trans(N^1-N^4)* form (Scheme 3, ~75%): ^{1}H NMR (400 MHz; $CDCl_3$; δ, ppm): 11.73 (br, 1H, OH); 8.40 (s, 1H, CH=N); 7.24 (m, 2H, CH aromatic); 6.98 (d, 8.0Hz, 1H, CH aromatic); 6.88 (t, 7.5 Hz, 1H, CH aromatic); 5.95 (m, 1H, CH from allyl moiety); 5.37 (d, 16.6 Hz, 1H, CH_2=C (*trans*)); 5.27 (d, 10.0 Hz, 1H, CH_2=C (*cis*)); 5.26 (d, 16.6 Hz, 1H, CH_2=C (*trans*)); 5.20 (d, 10.0 Hz, 1H, CH_2=C (*cis*));4.80 (br, 1H, NH); 4.05 (t, 4.8 Hz, 2H, CH_2-N); 3.57 (d, 6.5Hz, 2H, CH_2-S). ^{13}C NMR (100 MHz; $CDCl_3$; δ, ppm): 160.60 (C-S); 158.83 (C-O aromatic); 155.57 (C=N); 134.02, 133.70, 119.05, 116.62 (CH aromatic); 130.85, 130.83 (CH from allyl moieties); 118.86 (C aromatic); 118.82, 117.18 (CH_2=); 45.95 (CH_2-N); 33.80 (CH_2-S).

HL *cis(N^1-N^4)* form (Scheme 3, ~25%): ^{1}H NMR (400 MHz; $CDCl_3$; δ, ppm): 11.28 (br, 1H, OH); 8.47 (s, 1H, CH=N); 7.25 (m, 2H, CH aromatic); 6.97 (d, 7.7 Hz, 1H, CH aromatic); 6.91 (t, 7.4 Hz, 1H, CH aromatic); 5.86 (m, 1H, CH from allyl moiety); 5.77 (br, 1H, NH); 5.31 (d, 1H, 16.6 Hz, CH_2=C (trans)); 5.21 (d, 1H, 10.0 Hz, CH_2=C (cis)); 5.20 (d, 1H, 16.6 Hz, CH_2=C (trans)); 5.16 (d, 10.0 Hz, 1H, CH_2=C (cis)); 3.94 (t, 4.8 Hz, 2H, CH_2-N); 3.80 (d, 6.5 Hz, 2H, CH_2-S). ^{13}C NMR (100 MHz; $CDCl_3$; δ, ppm): 160.70 (C-S); 158.56 (C-O aromatic); 157.12 (C=N); 133.87, 133.16, 119.50, 116.38 (CH aromatic); 131.31, 131.26 (CH from allyl moieties); 118.63 (C aromatic); 118.33, 116.98 (CH_2=); 45.91 (CH_2-N); 33.32 (CH_2-S).

2. 3. Synthesis of Coordination Compounds

2. 3. 1. Synthesis of [Cu(L)Cl] (1)

The 2-hydroxybenzaldehyde 4,*S*-diallylisothiosemicarbazone **HL** (0.275 g, 1.00 mmol) was dissolved in 20 mL of ethanol. After that, $CuCl_2{\cdot}2H_2O$ (0.171 g, 1.00 mmol)

IR data (cm^{-1}): 3125, 1601, 1550, 1202, 757. λ (MeOH, $\Omega^{-1}{\cdot}cm^2{\cdot}mol^{-1}$): 74.

2. 3. 2. Synthesis of [Cu(L)NO_3] (2)

The reaction was carried out as described above, but $Cu(NO_3)_2 \cdot 3H_2O$ was used (0.242 g, 1.00 mmol) instead of copper(II) chloride dihydrate. The green precipitate was formed during stirring. The resulting precipitate was filtered off, washed with a small amount of ethanol and dried. Yield: 90%. Anal. calc. for $C_{14}H_{16}CuN_4O_4S$: Cu, 15.89; C, 42.05; H, 4.03; N, 14.01; S, 8.02; found: Cu, 16.18; C, 41.67; H, 4.38; N, 14.05; S, 8.04%. IR data (cm^{-1}): 3187, 1604, 1548, 1214, 759. λ (MeOH, $\Omega^{-1}{\cdot}cm^2{\cdot}mol^{-1}$): 82.

2. 3. 3. Synthesis of [Cu(3,4-Lut)(L)NO_3] (3)

Complex **2** (0.400 g, 1.00 mmol) was dissolved in 20 mL of ethanol by stirring at 60 °C. After that, 3,4-dimethylpyridine (3,4-Lut) (0.179 g, 1.00 mmol) was added to the solution. The mixture was stirred for 1 hour at 60 °C. The green precipitate was formed after stirring. The resulting precipitate was filtered off, washed with a small amount of ethanol and dried.

Yield: 82%. Anal. calc. for $C_{17}H_{20}CuN_6O_4S$: Cu, 13.58; C, 43.63; H, 4.31; N, 17.96; S, 6.85; found: Cu, 13.74; C, 43.32; H, 4.69; N, 17.75; S, 6.56%. IR data (cm^{-1}): 3111, 1601, 1557, 1198, 755. λ (MeOH, $\Omega^{-1}{\cdot}cm^2{\cdot}mol^{-1}$): 70.

2. 3. 4. Synthesis of [Ni(L)OAc] (4)

The reaction was carried out analogically to the synthesis of complex **1**, but $Ni(CH_3COO)_2{\cdot}4H_2O$ (0.249 g, 1.00 mmol) was used instead of copper(II) chloride dihydrate.

Yield: 81%. Anal. calc. for $C_{16}H_{19}N_3NiO_3S$: Ni, 14.97; C, 49.01; H, 4.88; N, 10.72; S, 8.18; found: Ni, 14.79; C, 49.02; H, 4.93; N, 10.96; S, 8.23%. IR data (cm^{-1}): 3121, 1601, 1565, 1210, 753. λ (MeOH, $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$): 12.

2. 3. 5. Synthesis of $[Co(L)_2]Cl$ (5)

The 2-hydroxybenzaldehyde 4,*S*-diallylisothiosemicarbazone **HL** (0.275 g, 1.00 mmol) was dissolved in 20 mL of ethanol. After that, $CoCl_2\cdot 6H_2O$ (0.119 g, 0.50 mmol) was added. The mixture was stirred for 1 hour at 60 °C. The dark brown precipitate was formed during stirring. The resulting precipitate was filtered off, washed with a small amount of ethanol and dried. Yield: 85%. Anal. calc. for $C_{28}H_{33}ClCoN_6O_2S_2$: Co, 9.15; C, 52.21; H, 5.16; Cl, 5.50; N, 13.05; S, 9.96; found: Co, 8.99; C, 52.35; H, 5.21; N, 13.02; S, 9.89%. IR data (cm^{-1}): 3111, 1599, 1552, 1218, 748. λ (MeOH, $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$): 74.

2. 3. 6. Synthesis of $[Fe(L)_2]NO_3$ (6)

The reaction was carried out analogically to the synthesis of complex **6**, but $Fe(NO_3)_3\cdot 6H_2O$ (0.175 g, 0.50 mmol) was used instead of cobalt(II) chloride hexahydrate. As a result the dark brown single crystals were formed. Yield: 86%. Anal. calc. for $C_{28}H_{33}FeN_7O_5S_2$: Fe, 8.15; C, 49.05; H, 5.15; N, 14.30; S, 9.35; found: Fe, 8.28; C, 48.96; H, 5.20; N, 14.02; S, 9.21%. IR data (cm^{-1}): 3144, 1599, 1532, 1215, 751. λ (MeOH, $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$): 72.

2. 4. X-ray Crystallography

Single crystal X-ray diffraction analysis was performed on an Xcalibur E diffractometer (room temperature, Eos CCD detector, graphite monochromator, MoKα radiation, CrysAlis PRO software[25]). Structure solution and refinement were performed using the SHELX2014[26]. The crystallographic data with refinement details of compound **6** are summarized in Table 1. All non-hydrogen atoms were refined using an anisotropic model and hydrogen atoms isotropicaly. The structure of **6** revealed the disorder of the C_3H_5 groups at N3 over two positions with equal probability. Selected bond lengths, angles and hydrogen bonds are presented in Table 2 and Table 3, respectively. Mercury software [27] was used for visualization of the studied structures.

2. 5. Antioxidant Activity

The antioxidant activity was studied using the ABTS free radical-scavenging assay according to Re et al.[28] The ABTS (7 mM) was mixed (1:1, v/v) with potassium persulfate (4.95 mM) and was incubated at 25 °C overnight in the dark place to prepare the stock solution. The prepared $ABTS^{\bullet+}$ solution was diluted with acetate-buffered saline (0.02 M, pH 6.5) to obtain an absorbance of 0.7 ± 0.01 at 734 nm that is suitable for measurements. The solutions of trolox, **HL** and complexes **1–6** in DMSO were prepared. Then, 20 μL of each dilution was added to a 96-well microtiter plate and 180 μL of prepared solution of $ABTS^{\bullet+}$ was dispensed with the dispense module of hybrid reader

Table 1: Crystal and Structure Refinement Data for **6**

Empirical formula	$C_{28}H_{32}Fe_1N_7O_5S_2$
M	666.57
Crystal system, sp. gr., *Z*	Monoclinic, $P2_1/n$, 4
a, Å	12.0411(10)
b, Å	18.005(2)
c, Å	14.6997(14)
β, deg	102.922(10)
V, Å ^{3}	3106.2(5)
ρ_{calc}, g cm^{-3}	1.425
μ, mm^{-1}	0.669
F(000)	1388
Crystal size, mm	0.36 × 0.12 × 0.10
θ Range, °	3.008 to 25.049
Reflections collected / unique	10768/5460 [R(int) = 0.0493]
Reflections with $I > 2\sigma(I)$	2343
Number of refined parameters	383
Completeness, %	99.3
GOOF	1.002
R (for $I > 2\sigma(I)$)	*R*1 = 0.0740,
*wR*2 = 0.1424	
R (for all reflections)	*R*1 = 0.1793,
*wR*2 = 0.1831	
$\Delta\rho_{max}/\Delta\rho_{min}$, $e\cdot$ Å^{-3}	0.537, –0.438

Table 2: Selected Bond Lengths (Å) and Angles (deg) in Coordination Metal Environment in **6**

Bonds	**(Å)**
Fe(1)-O(1)	1.909(4)
Fe(1)-O(2)	1.894(5)
Fe(1)–N(1)	2.126(4)
Fe(1)–N(3)	2.125(5)
Fe(1)–N(4)	2.122(4)
Fe(1)–N(6)	2.132(6)
Angles	(°)
O(1)Fe(1)O(2)	100.3(2)
O(1)Fe(1)N(1)	83.7(2)
O(1)Fe(1)N(3)	156.3(2)
O(1)Fe(1)N(4)	95.7(1)
O(1)Fe(1)N(6)	93.9(2)
O(2)Fe(1)N(1)	98.6(2)
O(2)Fe(1)N(3)	89.8(2)
O(2)Fe(1)N(4)	82.9(2)
O(2)Fe(1)N(6)	153.5(2)
N(1)Fe(1)N(3)	73.5(2)
N(1)Fe(1)N(4)	178.5(2)
N(1)Fe(1)N(6)	105.1(2)
N(3)Fe(1)N(4)	106.9(2)
N(3)Fe(1)N(6)	85.8(2)
N(4)Fe(1)N(6)	73.6(2)

Table 3: Hydrogen Bond Distances (Å) and Angles (deg) for **6**

D–H···A	Å			∠(DHA)	Symmetry transformation for A
	D–H	H···A	D···A		
N(2)–H(1N)···O(5)	0.86	2.11	2.946(7)	165	*x*, *y*, *z*
N(5)–H(2N)···O(3)	0.86	2.46	3.201(9)	146	*x*–1, *y*, *z*
C(2)–H(2)···O(3)	0.93	2.45	3.379(9)	175	*x*, *y*, *z*
C(12)–H(12B)···O(5)	0.97	2.36	3.29(1)	160	*x*, *y*, *z*
C(26)–H(26B)···O(3)	0.97	2.19	3.09(1)	154	*x*–1, *y*, *z*

(BioTek) and was shaken for 15 s. The decrease in absorbance was measured at 734 nm after 30 min of incubation. Blank samples do not contain $ABTS^{\bullet+}$.

3. Results and Discussion

3. 1. Synthesis and Characterization

The ligand was synthesized in a good yield and characterized by ^{1}H NMR, ^{13}C NMR and FTIR spectroscopy.

The main feature of the NMR spectra is the fact that all the peaks are doubled, that indicates the presence of two tautomeric (*cis-trans* isomers relative to the C=N^2 bond) forms of isothiosemicarbazone in solution. In the *cis* form, there is a hydrogen bond of the hydrogen atom at N^4, that leads to a shift of the N^4H peak towards a higher chemical shift (to a weaker field). The ratio of the integral intensity of the peaks of the two forms of isothiosemicarbazone HL shows that the *trans* form prevails in the solution (~75%).

Complexes **1**, **2**, **5**-**6** were prepared by the reaction between isothiosemicarbazone **HL** and corresponding metal salts. Coordination compounds of copper(II) (**1**, **2**) and nickel(II) (**4**) were synthesized in 1:1 molar ratio, but cobalt(III) (**5**) and iron(III) (**6**) complexes were synthesized in 2:1 (HL : metal salt) molar ratio. Complex **3** was obtained by reaction between complex **2** and 3,4-dimethylpyridine in 1:1 molar ratio.

The elemental analysis confirms the composition of isothiosemicarbazone **HL** and complexes **1**-**6**. The molar conductivity values of all synthesized complexes, except complex **4**, are in the range 70–75 $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$ that indicates that they are 1:1 electrolytes and dissociate on complex cation and acid residue anion in the process of disso-

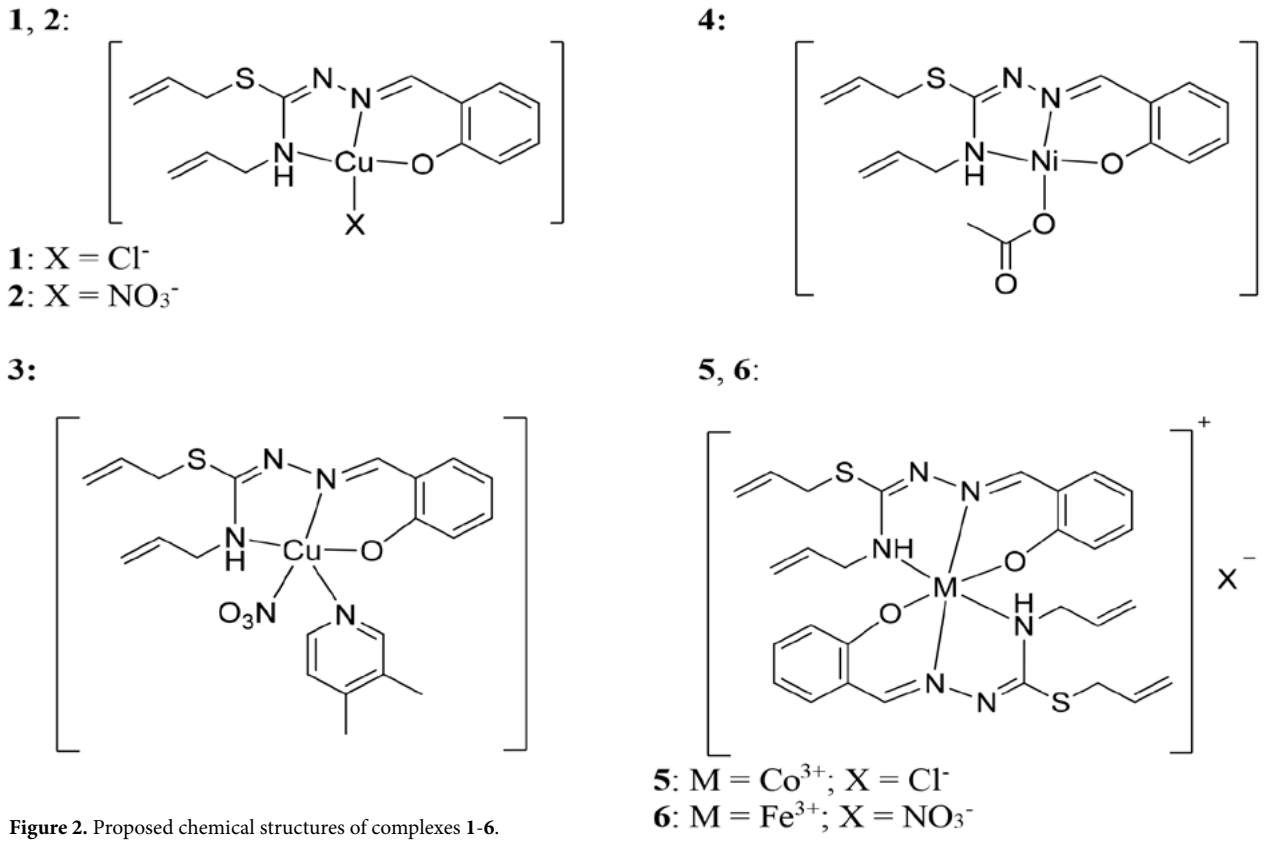


Figure 2. Proposed chemical structures of complexes **1**-**6**.

lution in methanol. The molar conductivity value of nickel complex **4** is 12 $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$, which means that acetate-ion is not washed out from the inner sphere by methanol molecules.

The comparative analysis of the FTIR spectra of the synthesized compounds and the free ligand (**HL**) was made in order to determine the coordination mode of **HL** with metal ions. It was established that the 2-hydroxybenzaldehyde 4,*S*-diallylisothiosemicarbazone behaves as a monodeprotonated tridentate O,N,N-ligand, connected to the central ion by a deprotonated phenolic oxygen atom, azomethine nitrogen and thiocarbamide nitrogen, forming five- and six- members metallacycles. This is confirmed by the disappearance of the ν(O–H) absorption band that was in the region 3427 cm^{-1}. The absorption bands ν(N–H), ν(C=N) and ν(C–O) have shifted to the low-frequency region with corresponding values 3187–3111 cm^{-1}, 1565–1532 cm^{-1}, and 1218–1198 cm^{-1}. The absorption band ν(C-S) has not changed, that confirms that sulfur does not take part in the coordination of isothiosemicarbazone **HL**.

For the complexes **1**-**6** the proposed formula are presented in figure 2.

3. 2. Structure Description of the Complex 6

The X-ray diffraction analysis showed that compound **6** is an ionic compound with formula $[Fe(L)_2]NO_3$ and comprises mononuclear complex cation and an outer-sphere nitrate anion (Fig. 3).

Two tridentate monodeprotonated ligands $\mathbf{L}^-$ are coordinated by O,N,N-sets of donor atoms to the central metal atom in the complex cation. The coordination polyhedron of the Fe(1) atom is a distorted octahedron with the average Fe–O and Fe–N distances 1.902(5) and 2.126(5) Å, respectively, and the angels from 73.5(2) to 106.9(2)° for atoms in *cis*- and 153.5(2) to 178.5(2)° in *trans*-positions (Table 2). These distances are similar to those found in 2-hydroxybenzaldehyde 4-allyl-*S*-methylisothiosemicarbazone (*S*-MeTscH)[21] and 4-allyl-*S*-ethylisothiosemicarbazone (*S*-EtTscH)[29]. A similar way of coordination of $\mathbf{L}^-$, *S*-$MeTsc^-$, and *S*-$EtTsc^-$ is observed in the Fe(III) complexes despite the difference in substituents at the sulfur atom of these isothiosemicarbazones. At the same time, another method of coordination of similar isothiosemicarbazone ligands, that involves the sulfur atom, was reported [14,30,31] for the complexes of Zn(II), Cu(II), Pd(II) transition metals.

The conformation of isothiosemicarbazones ligands with substituents at the terminal nitrogen atom or at both the nitrogen and sulfur atom[32,33] is predisposed for their coordination with O,N,S-set of donor atoms, but their coordination to the transitional metal atoms takes place through O,N,N-set of donor atoms with rearrangement of the isothiosemicarbazone ligand[34]. In the crystal, complex cations and anions alternate and form chains along crys-

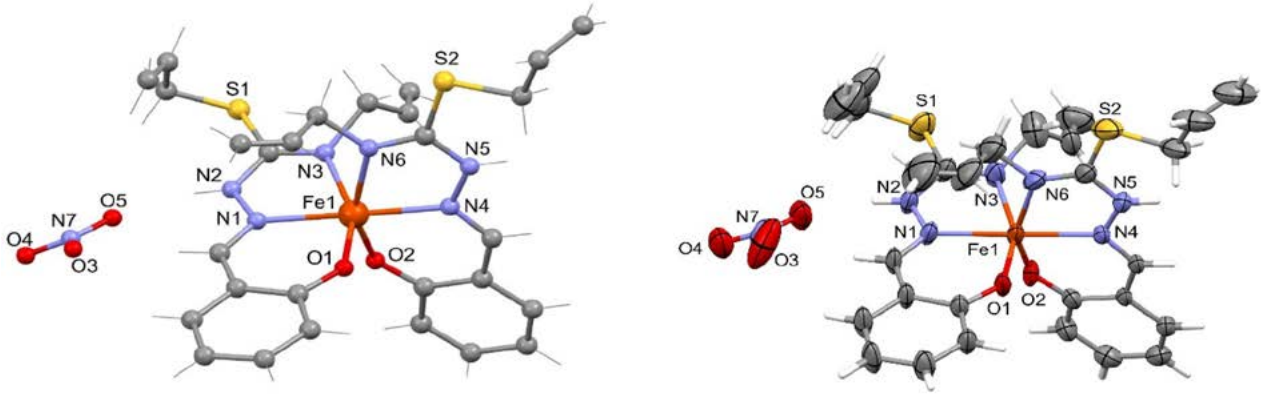


Figure 3. Molecular structure of the compound **6,** ellipsoids are shown at 30% probability level.

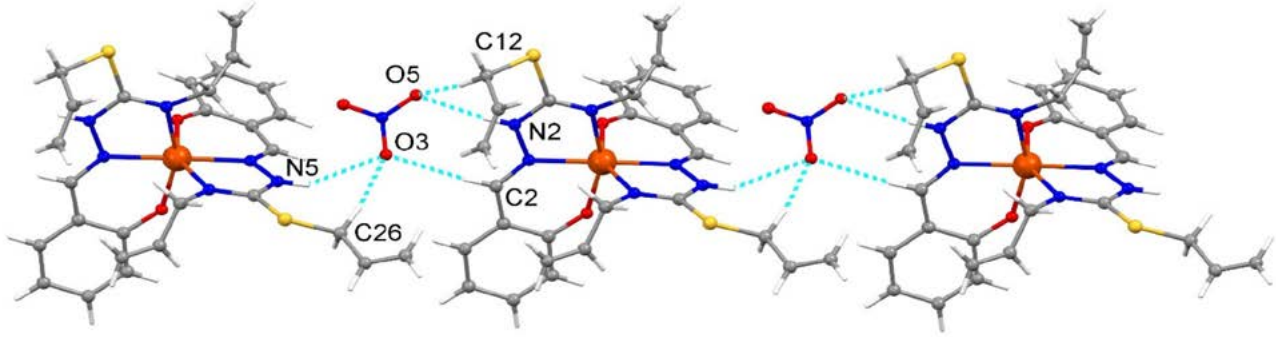


Figure 4. H-bonded chain in the crystal of complex **6**

tallographic *a* axis due to N–H···O and C–H···O hydrogen bonds (Fig. 4, Table 3).

3. 3. Antioxidant Activity

The antioxidant activity of the compounds was studied and determined using the $ABTS^{\bullet+}$ method. All studied substances (Table 4) are more active than trolox that is used in medicine to reduce oxidative stress or damage.

The isothiosemicarbazone **HL** exhibits antioxidant activity that is comparable with trolox. Coordination of **HL** to the copper(II) ions (complexes **1** and **2**) leads to a 2.1–2.6 times increase of antioxidant activity. The acid residue anion in the composition of complex does not have a major influence on the concentration of semi-maximal inhibition (IC_{50}) of $ABTS^{\bullet+}$.

The introduction of heterocyclic amine (3,4-dimethylpyridine) into the inner sphere of copper(II) nitrate complex has a negative effect on the antioxidant activity of the obtained mixed-ligand complex and leads to a 1.4 times decrease of antioxidant activity comparing to copper(II) nitrate complex **2**, but it still remains 1.9 times more active than **HL** and 2.3 times more active than trolox.

Nickel(II), cobalt(III) and iron(III) complexes manifest better antioxidant properties than copper(II) complexes. Their IC_{50} values are in the range of 7.20–9.37 μM, so they are 2.9–3.7 times more active than initial isothiosemicarbazone **HL**, 1.1–1.4 times more active than the most active copper(II) complex **2** of this series of substances and 3.6–4.6 times more active than trolox, a water soluble analog of vitamin E that is used as standard antioxidant in medical practice.

For the studied series of coordination compounds the activity of complexes substantially depends on the nature of central atom in the following way: Ni≈Fe>Co>Cu.

Table 4. Antioxidant activity of ligand and compounds **1**-**6** against cation radicals $ABTS^{\bullet+}$.

Compound	IC_{50}, μM
HL	26.8 ± 0.2
[Cu(L)Cl] (**1**)	12.5 ± 0.1
[Cu(L)NO_3] (**2**)	10.3 ± 0.1
[Cu(3,4-Lut)(L)NO_3] (**3**)	14.4 ± 0.1
[Ni(L)OAc] (**4**)	7.20 ± 0.03
[Co$(L)_2$]Cl (**5**)	9.37 ± 0.05
[Fe$(L)_2$]NO_3 (**6**)	7.33 ± 0.11
Trolox	33.3 ± 0.2

Previously, coordination compounds of some 3*d* metals with 2-hydroxybenzaldehyde 4-allylthiosemicarbazone (TscH)[35], 2-hydroxybenzaldehyde 4-allyl-*S*-methylisothiosemicarbazone (*S*-MeTscH)[21], 2-hydroxybenzaldehyde 4-allyl-*S*-ethylisothiosemicarbazone (*S*-EtTscH)[29] have been described.

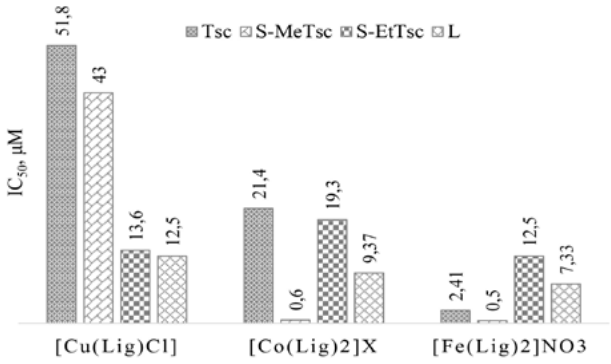


Figure 5. Comparison of the IC_{50} values of analogous copper(II), cobalt(III) and iron(III) complexes with 2-hydroxybenzaldehyde 4-allylthiosemicarbazone and its *S*-substituted derivatives. (TscH – 2-hydroxybenzaldehyde 4-allylthiosemicarbazone; *S*-MeTscH – 2-hydroxybenzaldehyde 4-allyl-*S*-methylisothiosemicarbazone; *S*-EtTscH – 2-hydroxybenzaldehyde 4-allyl-*S*-ethylisothiosemicarbazone; Lig = Tsc^-, *S*-$MeTsc^-$, *S*-$EtTsc^-$, L^-; X= Cl^-, NO_3^-)

As it can be seen from the diagram (Fig. 5), the alkylation of 4-allylthiosemicarbazone in most cases leads to the increase of antioxidant properties as the corresponding IC_{50} become smaller. In case of copper(II) chloride complexes the antioxidant activity grows in the following order: [Cu(Tsc)Cl]< [Cu(*S*-MeTsc)Cl] < [Cu(*S*-EtTsc)Cl]< [Cu(L)Cl].

In case of cobalt(III) and iron(III) complexes the sequence changes. The complexes with *S*-MeTscH become the most active ones. The complexes with 4,*S*-diallylisothiosemicarbazone **HL** manifest higher activity than corresponding complexes with *S*-EtTscH, but have lower activity than corresponding complexes with *S*-MeTscH. The activity grows in following orders: [Co(*S*-$MeTsc)_2$]NO_3 < [Co$(L)_2$]Cl < [Co(*S*-$EtTsc)_2$]NO_3< [Co$(Tsc)_2$]Cl and [Fe(*S*-$MeTsc)_2$]NO_3< [Fe$(Tsc)_2$]NO_3 < [Fe$(L)_2$]NO_3 < [Fe(*S*-$EtTsc)_2$]NO_3.

4. Conclusion

2-Hydroxybenzaldehyde 4,*S*-diallylisothiosemicarbazone (**HL**) was synthesized and characterized using spectroscopic methods: FTIR, ^1H and ^{13}C NMR. There is a equilibrium between *trans*(N^1-N^4) and *cis*(N^1-N^4) isomeric forms in the solution. The *trans*-from is predominant in the $CDCl_3$ solution. Six new copper(II), nickel(II), cobalt(III) and iron(III) complexes with **HL** were prepared and caracterized using elemental analysis, molar conductivity and FTIR spectrocopy. The structure of the iron(III) complex **6** was confirmed by single crystal X-ray diffraction. Isothiosemicarbazone **HL** coordinates to the 3*d* metal central atoms in monodeprotonated form (L^-) using O,N,N-set of donor atoms in the *cis*(N^1-N^4) isomeric form.

The obtained substances manifest high antioxidant activity against $ABTS^{\bullet+}$ that exceeds the activity of trolox that is used in medical practice. Nickel(II), cobalt(III) and iron(III) complexes manifest higher activity than copper(II) complexes. Modification of the inner sphere of the copper(II) complexes by introduction of heteroaromatic amine did not lead to the amplification of the antioxidant properties. The nickel(II) coordination compound **4** manifests the highest antioxidant activity in this series of synthesized substances.

Introduction of the *S*-allyl group affects the antioxidant properties of the corresponding coordination compounds. Therefore, alkylation is one of the ways to enhance the antioxidant activity of complexes with thiosemicarbazones. The nature of the radical at the sulfur atom affects the antioxidant activity, however, its effect also depends on the nature of the metal, so it is necessary to continue the search for new antioxidants by changing the nature of the substituent at the sulfur atom in *S*-alkylisothiosemicarbazones.

Supporting Information

Crystallographic data for 6 has been deposited with the Cambridge Crystallographic Data Center, CCDC 2218005. Copies of this information may be obtained from The Director, CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK(fax: +44-1233-336033; e-mail: deposit@ccdc.cam.ac.uk or www: http://www.ccdc.cam.ac.uk).

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Povzetek

Sintetizirali smo 2-hidroksibenzaldehid 4,*S*-dialilizotiosemikarbazon (**HL**) in ga karakterizirali z metodami ^{1}H, ^{13}C NMR in FTIR spektroskopijo. Spojina v raztopini obstaja v dveh izomernih oblikah: : *cis* (~25%) in *trans* (~75%). Z reakcijami med **HL** in bakrovimi(II), nikljevimi(II), kobaltovimi(III) in železovimi(III) solmi smo dobili šest stabilnih kompleksov: [Cu(L)Cl] (**1**), [Cu(L)NO_3] (**2**), [Cu(3,4-Lut)(L)NO_3] (**3**), [Ni(L)OAc] (**4**), [Co$(L)_2$]Cl (**5**) in [Fe$(L)_2$]NO_3 (**6**). Komplekse smo karakterizirali z elementno analizo, FTIR, meritvami molske prevodnosti in v primeru spojine (**6**) z monokristalno rentgensko difrakcijo. Za vse spojine smo preučevali antioksidativno aktivnost proti kationskemu radikalu $ABTS^{\bullet+}$. Vsi kompleksi in prosti ligand izkazujejo višjo aktivnost kot Trolox, ki je v uporabi v medicini. Najaktivnejši je kompleks **4** (IC_{50}=7.20μM). Dodatek heterocikličnega amina ni izboljšal antioksidativne aktivnosti. Uvedba *S*-alilne skupine v izotiosemikarbazon je vplivala na aktivnost sintetiziranih spojin, zato v nekaterih primerih takšni kompleksi kažejo večjo aktivnost kot kompleksi, ki vsebujejo izotiosemikarbazone z drugimi *S*-radikali.