Received: 1 March 2018 | Revised: 12 July 2018 | Accepted: 13 July 2018

DOI: 10.1002/aoc.4544

FULL PAPER

WILEY Applied Organometallic Chemistry

Synthesis, characterization, crystal structure of novel Cu (II), Co (III), Fe (III) and Cr (III) complexes with 2-hydroxybenzaldehyde-4-allyl-S-methylisothiosemicarbazone: Antimicrobial, antioxidant and *in vitro* antiproliferative activity

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2-Hydroxybenzaldehyde-4-allyl-S-methylisothiosemicarbazone and Cu (II), Co (III), Fe (III) and Cr (III) complexes were synthesized and characterized. The new obtained compounds were investigated by elemental analysis, magnetic susceptibility measurements, molar electric conductivity, IR, ^{1}H NMR, ^{13}C NMR, UV–Vis spectroscopy. In addition, the structure of the ligand and six complexes has been determined by single-crystal X-ray diffraction analysis. The Cu atom in complex **3** is penta-coordinated in a distorted square–pyramidal coordination geometry, while the metals in complexes **5–9** are in a distorted octahedral environment. For all compounds the antimicrobial activity was studied on a series of standard strains, such as *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, *Salmonella abony* and *Candida albicans*. The *in vitro* antiproliferative activity of the ligand and complexes was screened on human leukemia HL-60 cells, human cervical epithelial HeLa cells, human epithelial pancreatic adenocarcinoma BxPC-3 cells, human muscle rhabdomyosarcoma spindle and large multinucleated RD cells. The primary screening on a wider series of cancer cells showed that copper coordination compound **2** manifests high activity towards HeLa, BxPC-3 and RD cancer cells, which is three–six times higher than the activity of doxorubicin. The selectivity index that is the ratio between the IC_{50} value for the normal MDCK cells and IC_{50} values for the cancer cells varies in the range 2.44–21.17. This index is 5.5–11 times higher for the copper coordination compound **2** than for the doxorubicin. Cr (III), Fe (III) and Co (III) coordination compounds **6–9** manifest high antioxidant activity towards $ABTS^{\bullet+}$ that exceeds 47–67 times the activity of Trolox.

KEYWORDS
antimicrobial activity, antioxidant activity, antiproliferative activity, metal complexes, X-ray crystal structure

1 | INTRODUCTION

Thiosemicarbazones and their metal complexes attract constant scientific interest due to their antibacterial, antifungal, antioxidant and antitumor activities.[1–7] In recent years, a number of thiosemicarbazone derivatives have been synthesized, and their biological activities were also evaluated.[8–10] Therefore, it is of interest to study the influence of alkylation of thiosemicarbazide on the composition, structure and properties of transition metal complexes with these ligands.

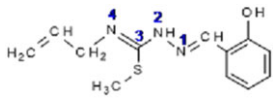
Isothiosemicarbazones have shown different coordination modes and are a potentially biologically active class of ligands because, compared with thiosemicarbazones, the alkylated sulfur atom remains uncoordinated on complexation in many cases,[11–17] with the exception of complexes of platinum metals.[18,19]

The introduction of substituents in the 4th position of thiosemicarbazones leads to a significant growth of their biological activity.[20] These properties make these compounds attractive for the preparation of a variety of new coordination compounds.[21,22]

Isothiosemicarbazones and their metal complexes manifest a wide range of biological properties, such as antifungal, antibacterial, antituberculous and antitumor activities.[23–28] However, there is a limited number of studies on *S*-alkylisothiosemicarbazones, in spite of their importance in selective biological activity.

The introduction of the allyl substituent in the 4th position of the thiosemicarbazide fragment also leads to the growth of the biological activity. Previously it synthesized 2-hydroxybenzaldehyde S-methylisothiosemicarbazone,[29,30] 2-hydroxybenzaldehyde-4-allyl-3-thiosemicarbazone and coordination compounds with some 3d metals, such as copper, nickel, iron, zinc.[31–33]

In continuation of this approach, the present paper describes the chemical synthesis, characterization and *in vitro* biological evaluation of 2-hydroxybenzaldehyde-4-allyl-S-methylisothiosemicarbazone (Scheme 1) and its Cu (II), Co (III), Fe (III) and Cr (III) coordination compounds.



SCHEME 1 Structural formula of the 2-hydroxybenzaldehyde-4-allyl-S-methylisothiosemicarbazone and numbering scheme of the isothiosemicarbazide fragment

2 | EXPERIMENTAL

2.1 | Materials

All the reagents used were chemically pure. $CuCl_2 \cdot 2H_2O$, $CuBr_2$, Cu $(NO_3)_2 \cdot 3H_2O$, $CoCl_2 \cdot 6H_2O$, $CoBr_2 \cdot 6H_2O$, Co $(NO_3)_2 \cdot 6H_2O$, $FeCl_3 \cdot 6H_2O$, Fe $(NO_3)_3 \cdot 6H_2O$ and Cr $(NO_3)_3 \cdot 9H_2O$ (Merck) were used as supplied. 2-Hydroxybenzaldehyde was used as received. N(4)-Allyl-3-thiosemicarbazide was prepared similarly to the literature procedure[34,35] by reaction of the allylisothiocyanate and hydrazine hydrate. The solvents were purified and dried according to standard procedures.[36]

2.2 | Synthesis of ligand (HL)

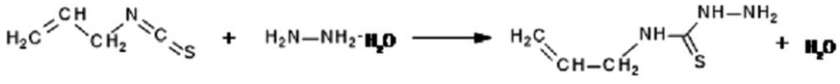
2.2.1 | Synthesis of the 4-allyl-3-thiosemicarbazide

4-Allyl-3-thiosemicarbazide (Scheme 2) was prepared similarly to the literature procedure[34,35].

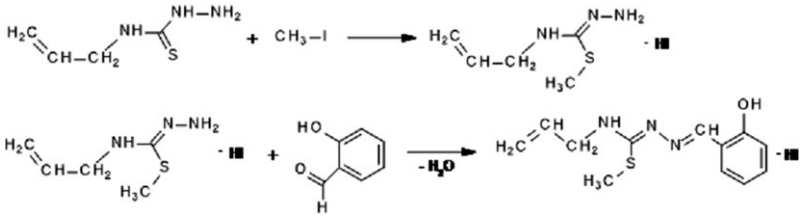
Pale yellow solid; m.p.: 91–93°C; yield (84%); FW: 131.1994 g mol^{-1}. Anal. calcd for $C_4H_9N_3S$: C, 36.62; H, 6.91; N, 32.03; S, 24.44%. Found: C, 36.58; H, 6.88; N, 31.97; S, 24.31%. Main IR peaks (KBr, cm^{-1}): ν (N^1H_2) 3339, 3270; ν (N^2-H), ν (N^4-H) 3189, 3162; ν (C=C allyl) 1641; ν (C=S) 1226. ^{1}H NMR ($CDCl_3$; δ, ppm): 8.71 (br, 1H, NH), 7.54 (br, 1H, NH), 5.73 (m, 1H, CH from allyl moiety), 5.25 (m, 2H, CH_2=C), 4.28 (m, 2H, CH_2-N), 3.78 (br, 2H, NH_2). ^{13}C NMR ($CDCl_3$; δ, ppm): 177.51 (C=S), 132.43 (CH from allyl moiety), 116.15 (CH_2=), 46.77 (CH_2-N).

2.2.2 | Synthesis of 2-hydroxybenzaldehyde-4-allyl-S-methylisothiosemicarbazone (HL)

2-Hydroxybenzaldehyde-4-allyl-S-methylisothiosemicarbazone (**HL**) (Scheme 3) was



SCHEME 2 Synthesis of the 4-allyl-3-thiosemicarbazide



SCHEME 3 Synthesis of 2-hydroxybenzaldehyde 4-allyl-S-methylisothiosemicarbazone hydroiodide

prepared according to a modification of the procedure described in the literature.[11,37,38]

4-Allyl-3-thiosemicarbazide (1.31 g, 10 mmol) was dissolved in 20 ml of ethanol with constant stirring. After that, iodomethane (1.56 g, 11 mmol) was added. The mixture was stirred at room temperature for 2 h, and 2-hydroxybenzaldehyde (1.22 g, 10 mmol) was added. The solution was stirred at 80°C for 30 min. After the reaction mixture was cooled to room temperature, the yellow solid was isolated by filtration, washed with ethanol and dried *in vacuo*.

Yellow solid; m.p.: 142–144°C; yield (83%); FW: 249.332 g mol^{-1}. Anal. calcd for $C_{12}H_{15}N_3OS$: C, 57.81; H, 6.06; N, 16.85; S, 12.86%. Found: C, 57.63; H, 5.82; N, 16.63; S, 12.97%. Main IR peaks (KBr, cm^{-1}): ν (OH) 3450, ν (N^2-H) 3187, ν (C=C allyl) 1641, ν (C=N^1) 1604, ν (C=N^4) 1570, ν (C–O) 1156, ν (CH_3–S) 1087, ν (C–S) 682. ^{1}H NMR ($CDCl_3$; δ, ppm): 11.71 (br, 1H, OH), 8.39 (s, 1H, CH=N), 7.20–7.28 (m, 2H, CH aromatic), 6.98 (d, 1H, CH aromatic), 6.88 (t, 1H, CH aromatic), 5.99 (m, 1H, CH from allyl moiety), 5.25 (m, 2H, CH_2=C), 4.43 (br, 1H, NH), 4.09 (m, 2H, CH_2-N), 2.42 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$; δ, ppm): 161.26 (C-S), 158.74, 155.36, 130.80, 119.08, 118.85, 117.32 (C aromatic), 133.96 (CH=N), 130.77 (CH from allyl moiety), 116.59 (CH_2=), 45.99 (CH_2-N), 13.13 (CH_3).To 2-hydroxybenzaldehyde-4-allyl-S-methylisothiosemicarbazone (3.77 g, 10 mmol) hydroiodide solution was added sodium carbonate (1.06 g, 10 mmol) to pH 7–8. After the reaction mixture was cooled to room temperature, the yellow solid was isolated by filtration, washed with ethanol and dried in *vacuo* (Scheme 4).

^{1}H NMR ($CDCl_3$; δ, ppm): 11.38 (br, 1H, OH), 8.44 (s, 1H, CH=N), 7.20–7.28 (m, 2H, CH aromatic), 6.97(d, 1H, CH aromatic), 6.91(t, 1H, CH aromatic), 5.87 (m, 1H, CH from allyl moiety), 5.67 (br, 1H, NH), 5.23 (m, 2H, CH_2=C), 3.93 (m, 2H, CH_2-N), 2.48 (s, 3H, CH_3).

^{13}C NMR ($CDCl_3$; δ, ppm): 161.79 (C-S), 158.54, 157.05, 131.26, 119.51, 118.76, 116.89 (C aromatic), 133.86 (CH=N), 131.18 (CH from allyl moiety), 116.34 (CH_2=), 45.81 (CH_2-N), 29.70 (CH_3).

2.3 | General procedure for the preparation of the metal complexes (1–9)

All the new metal complexes **1**–**9** were prepared by the direct reaction between the ligand and the corresponding metal salts.

2.3.1 | Synthesis of [Cu (L)(H_2O)$_2$] Cl (1)

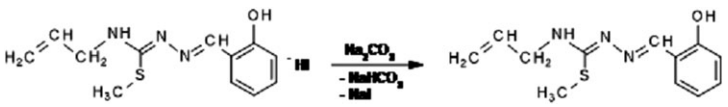
2-Hydroxybenzaldehyde-4-allyl-S-methylisothiosemicarbazone **HL** (0.249 g; 1 mmol) was dissolved in warm ethanol (30 ml), after that $CuCl_2$·$2H_2O$ (0.171 g; 1 mmol) was added. The solution was stirred at 70°C for 60 min. Dark green crystalline powder was obtained on slow evaporation. It was filtered off, washed with cold ethanol, and dried *in vacuo*.

Dark green solid; yield (76%); FW: 383.00 g mol^{-1}. Anal. calcd for $C_{12}H_{18}ClCuN_3O_3S$: C, 37.59; H, 4.69; Cl, 9.26; Cu, 16.57; N, 10.96; S, 8.35; H_2O, 9.39%; Found: C, 37.38; H, 4.45; Cl, 9.11; Cu, 16.30; N, 10.63; S, 8.12; H_2O, 9.12%. Main IR peaks (KBr, cm^{-1}): ν (N^2-H) 3188, ν (C=C allyl) 1638, ν (C=N^1) 1596, ν (C=N^4) 1553, ν (C–O) 1138, ν (CH_3–S) 1085, ν (C–S) 679. μ_{eff} (292 ± 1 K): 1.84 μ_B; χ (CH_3OH): 101 Ω^{-1} cm^2 mol^{-1}.

2.3.2 | Synthesis of [Cu (L)(H_2O)$_2$] Br (2)

The complex was synthesized using $CuBr_2$ (0.224 g; 1 mmol) and **HL** (0.249 g; 1 mmol).

Green solid; yield (75%); FW: 427.50 g mol^{-1}. Anal. calcd for $C_{12}H_{18}BrCuN_3O_3S$: C, 33.68; H, 4.21; Br, 18.71; Cu, 14.85; N, 9.82; S, 7.48; H_2O, 8.42%; Found: C, 33.52; H, 3.98; Br, 18.53; Cu, 14.65; N, 9.69; S, 7.27; H_2O, 8.26%. Main IR peaks (KBr, cm^{-1}): ν (N^2-H) 3187, ν (C=C allyl) 1638, ν (C=N^1) 1591, ν (C=N^4) 1550, ν (C–O) 1144, ν (CH_3–S) 1084, ν (C–S) 678. μ_{eff} (292 ± 1 K): 1.80 μ_B; χ (CH_3OH): 115 Ω^{-1} cm^2 mol^{-1}.



SCHEME 4 Neutralization of 2-hydroxybenzaldehyde-4-allyl-S-methylisothiosemicarbazone hydroiodide

2.3.3 | Synthesis of [Cu (L)$(H_2O)_2$]NO_3 (3)

The complex was synthesized from Cu $(NO_3)_2 \cdot 3H_2O$ (0.242 g; 1 mmol) and **HL** (0.249 g; 1 mmol).

Green solid; yield (78%); FW: 409.91 g mol^{-1}. Anal. calcd for $C_{12}H_{18}CuN_4O_6S$: C, 35.16; H, 4.43; Cu, 15.50; N, 13.67; S, 7.82; H_2O, 8.78%; Found: C, 35.11; H, 4.37; Cu, 15.42; N, 13.60; S, 7.73; H_2O, 8.64%. Main IR peaks (KBr, cm^{-1}): ν (N^2-H) 3186, ν (C=C allyl) 1640, ν (C=N^1) 1593, ν (C=N^4) 1556, ν (NO_3^-) 1372, ν (C–O) 1145, ν (CH_3–S) 1084, ν (C–S) 679. μ_{eff} (292 ± 1 K): 1.79 μ_B; χ (CH_3OH): 108 Ω^{-1} cm^2 mol^{-1}.

2.3.4 | Synthesis of [Co $(L)_2$] Cl (4)

The complex was synthesized from $CoCl_2 \cdot 6H_2O$ (0.238 g; 1 mmol) and **HL** (0.498 g; 2 mmol).

Brown solid; yield (72%); FW: 591.00 g mol^{-1}. Anal. calcd for $C_{24}H_{28}ClCoN_6O_2S_2$: C, 48.77; H, 4.78; Cl, 6.00; Co, 9.97; N, 14.22; S, 10.85; Found: C, 48.69; H, 4.70; Cl, 6.08; Co, 9.89; N, 14.18; S, 10.77. Main IR peaks (KBr, cm^{-1}): ν (N^2-H) 3185, ν (C=C allyl) 1641, ν (C=N^1) 1589, ν (C=N^4) 1552, ν (C–O) 1142, ν (CH_3–S) 1084, ν (C–S) 675. μ_{eff} (292 ± 1 K): 0 μ_B; χ (CH_3OH): 96 Ω^{-1} cm^2 mol^{-1}.

2.3.5 | Synthesis of [Co $(L)_2$] Br (5)

The complex was synthesized from $CoBr_2 \cdot 6H_2O$ (0.327 g; 1 mmol) and **HL** (0.498 g; 2 mmol).

Brown solid; yield (75%); FW: 635.49 g mol^{-1}. Anal. calcd for $C_{24}H_{28}BrCoN_6O_2S_2$: C, 45.36; H, 4.44; Br, 12.57; Co, 9.27; N, 13.22; S, 10.09; Found: C, 45.29; H, 4.38; Br, 12.49; Co, 9.13; N, 13.25; S, 10.16. Main IR peaks (KBr, cm^{-1}): ν (N^2-H) 3186, ν (C=C allyl) 1641, ν (C=N^1) 1590, ν (C=N^4) 1554, ν (C–O) 1139, ν (CH_3–S) 1084, ν (C–S) 675. μ_{eff} (292 ± 1 K): 0 μ_B; χ (CH_3OH): 87 Ω^{-1} cm^2 mol^{-1}.

2.3.6 | Synthesis of [Co $(L)_2$]NO_3 (6)

The complex was synthesized from Co $(NO_3)_2 \cdot 6H_2O$ (0.291 g; 1 mmol) and **HL** (0.498 g; 2 mmol).

Brown solid; yield (79%); FW: 617.59 g mol^{-1}. Anal. calcd for $C_{24}H_{28}CoN_7O_5S_2$: C, 46.67; H, 4.57; Co, 9.54; N, 15.88; S, 10.38; Found: C, 46.70; H, 4.68; Co, 9.68; N, 15.71; S, 10.20. Main IR peaks (KBr, cm^{-1}): ν (N^2-H) 3188, ν (C=C allyl) 1640, ν (C=N^1) 1592, ν (C=N^4) 1555, ν (NO_3^-) 1374, ν (C–O) 1141, ν (CH_3–S) 1085, ν (C–S) 675. μ_{eff} (292 ± 1 K): 0 μ_B; χ (CH_3OH): 90 Ω^{-1} cm^2 mol^{-1}.

2.3.7 | Synthesis of [Fe $(L)_2$] Cl (7)

The complex was synthesized from $FeCl_3 \cdot 6H_2O$ (0.271 g; 1 mmol) and **HL** (0.498 g; 2 mmol).

Brown solid; yield (83%); FW: 587.95 g mol^{-1}. Anal. calcd for $C_{24}H_{28}ClFeN_6O_2S_2$: C, 49.03; H, 4.80; Cl, 6.03; Fe, 9.50; N, 14.29; S, 10.91; Found: C, 48.96; H, 4.74; Cl, 6.07; Fe, 9.45; N, 14.21; S, 10.86. Main IR peaks (KBr, cm^{-1}): ν (N^2-H) 3185, ν (C=C allyl) 1644, ν (C=N^1) 1589, ν (C=N^4) 1560, ν (C–O) 1146, ν (CH_3–S) 1085, ν (C–S) 676. μ_{eff} (292 ± 1 K): 5.74 μ_B; χ (CH_3OH): 109 Ω^{-1} cm^2 mol^{-1}.

2.3.8 | Synthesis of [Fe $(L)_2$]NO_3 (8)

The complex was synthesized from Fe $(NO_3)_3 \cdot 6H_2O$ (0.350 g; 1 mmol) and **HL** (0.498 g; 2 mmol).

Brown solid; yield (78%); FW: 614.50 g mol^{-1}. Anal. calcd for $C_{24}H_{28}FeN_7O_5S_2$ (g·mol^{-1}): C, 46.91; H, 4.59; Fe, 9.09; N, 15.96; S, 10.44; Found: C, 46.84; H, 4.51; Fe, 9.01; N, 15.89; S, 10.38. Main IR peaks (KBr, cm^{-1}): ν (N^2-H) 3187, ν (C=C allyl) 1640, ν (C=N^1) 1593, ν (C=N^4) 1555, ν (NO_3^-) 1373, ν (C–O) 1142, ν (CH_3–S) 1087, ν (C–S) 677. μ_{eff} (292 ± 1 K): 5.93 μ_B; χ (CH_3OH): 113 Ω^{-1} cm^2 mol^{-1}.

2.3.9 | Synthesis of [Cr $(L)_2$]NO_3 (9)

The complex was synthesized from Cr $(NO_3)_3 \cdot 9H_2O$ (0.400 g; 1 mmol) and **HL** (0.498 g; 2 mmol).

Brown solid; yield (76%); FW: 610.65 g mol^{-1}. Anal. calcd for $C_{24}H_{28}CrN_7O_5S_2$: C, 47.20; H, 4.62; Cr, 8.51; N, 16.06; S, 10.50; Found: C, 47.16; H, 4.67; Cr, 8.46; N, 16.01; S, 10.42. Main IR peaks (KBr, cm^{-1}): ν (N^2-H) 3186, ν (C=C allyl) 1644, ν (C=N^1) 1591, ν (C=N^4) 1556, ν (NO_3^-) 1376, ν (C–O) 1145, ν (CH_3–S) 1085, ν (C–S) 677. μ_{eff} (292 ± 1 K): 3.83 μ_B; χ (CH_3OH): 109 Ω^{-1} cm^2 mol^{-1}.

2.4 | Physical measurements

Melting points were determined using the capillary method. The determination of metal content in the synthesized coordination compounds, using titration methods, was performed similarly to the literature procedures. The chemical elemental analysis for the determination of C, H and N was done with the Carlo-Erba LA-118 microdosimeter. Molar conductivity values of the coordination compounds were determined in 10^{-3} mol l^{-1} methanol solutions using slide wire bridge R-38. The complexes were studied by thermogravimetry in static air atmosphere, with a sample heating rate of 10°C min^{-1} using a STA 6000 Perkin Elmer. Magnetic susceptibility measurements were performed at room temperature in the polycrystalline state on a Faraday magnetic balance (home-made). Infrared spectra of the compounds were recorded on a Bruker ALPHA FTIR spectrophotometer at room temperature in the range of 4000–400 cm^{-1}. The ^1H and ^{13}C NMR spectra were recorded on a Bruker

DRX-400, using $CDCl_3$ as a solvent. The chemical shifts (δ) in ppm were measured relative to tetramethylsilane.

2.5 | X-ray crystallography

Crystallographic measurements for ligand **HL** and six coordination compounds (**3**), (**5–9**) were carried out with an Oxford-Diffraction XCALIBUR E CCD diffractometer (Santa Clara, CA, USA) equipped with graphite-monochromated Mo K_α radiation. The unit cell determination and data integration were carried out using the CrysAlis package of Oxford Diffraction.[39] All structures were solved by direct methods using SHELXS-97 and refined by full-matrix least-squares on F_o^2 with SHELXL-97.[40] All atomic displacements for non-hydrogen, non-disordered atoms were refined using an anisotropic model. The positions of hydrogen atoms were calculated geometrically. The H-atoms were refined isotropically in the 'rigid body' model with $U_{ef} = 1.2\ U_{equiv}$ or $U_{ef} = 1.5\ U_{equiv}$ of the corresponding O, N and C atoms. The ethanol molecule in **HL**, the allyl moiety, the nitrate ion in **6** were disordered over two positions and refined using the corresponding partial occupancies. The main crystallographic data together with refinement details are summarized in Table 1. Selected bond lengths, angles and hydrogen bonds are presented in Tables 2 and 3, respectively. The hydrogen bonds (D-H...A) were described by the following parameters: d (D...A) < R (D) + R (A) + 0.5 Å, d (H...A) < R (H) + R (A) − 0.12 Å, angle D-H...A > 100^0.[41,42] The geometric parameters were calculated and the figures were drawn with the use of the PLATON program.[43] The hydrogen atoms that are not involved in the hydrogen bonding were omitted from the generation of the packing diagrams.

2.6 | Biological studies

2.6.1 | Antibacterial and antifungal activity

The antibacterial activity of complexes was determined under liquid nutritive environment [2% of peptonate bullion (pH 7.0)] using successive dilutions method. *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *Enterococcus faecalis*, *Salmonella abony* (*GISK 03/03*), standard stems were used as reference culture for *in vitro* experiments. The dissolution of studied substances in dimethylformamide, microorganisms, cultivation, suspension obtaining, determination of minimal inhibition concentration (MIC) and minimal bactericide concentration (MBC) were carried out according to the previously reported method.[44]

Antimycotic properties of the synthesized substances were investigated *in vitro* on laboratory stems of *Candida albicans*. The activity was determined in liquid Sabouroud nutritive environment (pH 6.8). The inoculates were prepared from fungi stems that were harvested during 3–7 days. Their concentration in suspension is (2–4) or 10^6 colonies forming units mh^{-1}. Sowings for levures and micelles were incubated at 37°C during 7 and 14 days, respectively.

2.6.2 | Antiproliferative activity

Cell cultures

Human cervical epithelial cells of line HeLa, human epithelial pancreatic adenocarcinoma cells of line BxPc-3, human muscle rhabdomyosarcoma spindle and large multinucleated cells of line RD, human promyelocytic leukemia cells of line HL-60 and Madin Darby Canine Kidney epithelial normal cells of line MDCK were used in this study. BxPc-3 and HL-60 lines were cultured as monolayer in Roswell Park Memorial Institute (RPMI) 1640 medium containing L-glutamine (2 nM), antibiotics penicillin–streptomycin (final concentration 100 IU ml^{-1} penicillin and 100 μg streptomycin per ml) and supplemented with fetal bovine serum (FBS; 10% v/v). Cell lines HeLa, RD and MDCK were cultured in Dulbecco's Modified Essential Medium (DMEM) with L-glutamine (4 mM), glucose (4.5 g l^{-1}), bovine albumin fraction (0.2% v/v), HEPES buffer (N-2 hydroxyethylpiperazine-N'-2-ethane sulfonic acid; 20 mM), antibiotics penicillin–streptomycin (final concentration 100 U ml^{-1} penicillin and 100 μg streptomycin per ml) and supplemented with FBS (10% v/v). Cells were maintained at 37°C in a 2–5% humidified CO_2 atmosphere in the incubator in 75-cm^2 culture dishes, and used for experiments between passage 5 and 16. The compounds were dissolved at the time of the experiments.

Cell proliferation Resazurin assay

Cells of lines HeLa, BxPc-3, RD, MDCK were trypsinized with trypsin-ethylenediaminetetraacetic acid (trypsin–EDTA) 0.05% (Invitrogen) and counted under an inverted microscope (OLYMPUS). The cell proliferation assay was performed using Resazurin (7-hydroxy-3H-phenoxazin-3-one-10-oxide sodium salt; SIGMA), which allowed us to measure the number of viable cells.

In brief, plate out, in triplicate of $1{\cdot}10^4$ cells in a total of 100 μl medium in 96-well microtiter plates (Becton Dickinson, Lincoln Park, NJ, USA) were incubated at 37°C, 2% CO_2. Compounds were dissolved in dimethyl sulfoxide to prepare the stock solution of 10 mM. These compounds and doxorubicin were diluted at multiple concentrations with culture media, added to each well and incubated for 24 h. Following each treatment, 20 μl Resazurin indicator solution was added to each well and incubated for 4 h. Subsequently, the absorbance was read

TABLE 1 Crystallographic data, details of data collection and structure refinement parameters for compounds **HL**, **3** and **5–9**

Compound	**HL**	**3**	**5**
Chemical formula	$C_{28}H_{33}I_2N_6O_4S_2$	$C_{12}H_{18}N_4O_6SCu$	$C_{24}H_{28}N_6O_2S_2BrCo$
M (g mol^{-1})	835.52	409.90	635.48
Temperature (K)	293(2)	293(2)	293(2)
Wavelength (Å)	0.71073	0.71073	0.71073
Crystal system	monoclinic	triclinic	monoclinic
Space group	$P2_1/c$	P-1	$P2_1/n$
a (Å)	8.5525(3)	7.5980(6)	12.4768(5)
b (Å)	18.3208(8)	9.512(2)	12.3122(5)
c (Å)	11.9705(6)	13.076(3)	18.5988(7)
α (^{0})	90	69.81(2)	90
β (^{0})	91.774(4)	80.304(13)	107.265(4)
γ (^{0})	90	72.143(14)	90
V (Å^3)	1874.74(14)	842.2(3)	2728.34(17)
D_{calc} (g cm^{-3})	1.486	1.616	1.547
μ (mm^{-1})	1.835	1.457	2.280
F(0 0 0)	831	422	1296
Goodness-of-fit on F^2	1.108	1.044	1.018
Final R_1, wR_2 [$I > 2\sigma(I)$]	0.0506, 0.1209	0.0649, 0.1338	0.0514, 0.0993
R_1, wR_2 (all data)	0.0696, 0.1330	0.2121, 0.1795	0.0879, 0.1129
Largest difference in peak and hole (e Å^{-3})	0.622, −1.633	0.571, −0.519	0.586, −0.398

Compound	**6**	**7**	**8**	**9**
Chemical formula	$C_{24}H_{22}N_7O_5S_2Co$	$C_{24}H_{28}N_6O_2S_2ClFe$	$C_{24}H_{28}N_7O_5S_2Fe$	$C_{24}H_{28}N_7O_5S_2Cr$
M (g mol^{-1})	611.54	279.62	614.50	610.65
Temperature (K)	293(2)	293(2)	293(2)	293(2)
Wavelength (Å)	0.71073	0.71073	0.71073	0.71073
Crystal system	monoclinic	monoclinic	monoclinic	monoclinic
Space group	$P2_1/c$	$P2_1/c$	$P2_1/c$	$P2_1/c$
a (Å)	8.4111(9)	8.0707(6)	8.0797(3)	8.1146(4)
b (Å)	17.0575(12)	16.1057(7)	16.3746(8)	16.4518(10)
c (Å)	21.3922(16)	21.0139(11)	21.0875(7	20.7130(12)
α (^{0})	90	90	90	90
β (^{0})	113.153(3)	94.156(5)	96.459(3)	95.365(5)
γ (^{0})	90	90	90	90
V (Å^3)	2822.0(4)	2724.3(3)	2772.22(19)	2753.1(3)
D_{calc} (g cm^{-3})	1.442	1.433	1.472	1.473
μ (mm^{-1})	0.803	0.838	0.743	0.616
F(0 0 0)	1260	1220	1276	1268
Goodness-of-fit on F^2	1.008	1.052	1.025	1.026
Final R_1, wR_2 [$I > 2\sigma(I)$]	0.0515, 0.1231	0.0588, 0.1121	0.0477, 0.1052	0.0650, 0.1098
R_1, wR_2 (all data)	0.0689, 0.1323	0.1101, 0.1298	0.0728, 0.1179	0.1238, 0.1292
Largest difference in peak and hole (e Å^{-3})	0.569, −0.375	0.406, −0.339	0.324, −0.296	0.304, −0.285

TABLE 2 Selected bond lengths (Å) and angles (°) for **HL**, **3**, **5–9**

	d, Å			
Bond lengths	**HL**	**3**	**5**	**6**
Cu(1) [Co]-O(1)		1.895(8)	1.885(3)	1.893(3)
Co-O(1A)			1.883(3)	1.893(2)
Cu(1) [Co]-N(1)		1.935(10)	1.894(3)	1.874(3)
Co-N(1A)			1.877(3)	1.876(3)
Cu(1) [Co]-N(3)		1.964(9)	1.954(3)	1.934(3)
Co-N(3A)			1.947(3)	1.944(3)
Cu(1)-O(1 W)		2.406(7)		
Cu(1)-O(2 W)		1.990(8)		
S(1)-C(1)	1.695(2)	1.759(13)	1.761(5)	1.741(4)
S(1)-C(12)	1.778(6)	1.764(14)	1.797(5)	1.797(5)
N(1)-N(2)	1.384(6)	1.381(11)	1.381(5)	1.384(4)
N(1)-C(2)	1.278(7)	1.266(13)	1.325(6)	1.279(5)
N(2)-C(1)	1.325(6)	1.379(14)	1.349(7)	1.346(5)
N(3)-C(1)	1.314(7)	1.264(14)	1.289(5)	1.307(5)
N(3)-C(5)	1.466(7)	1.439(15)	1.449(5)	1.438(5)
C(3)-C(2)	1.442(7)	1.447(15)	1.413(6)	1.418(6)
O(1)-C(4)		1.315(14)	1.311(5)	1.298(4)
Bond angles	*ω*, deg.			
N(1)-Cu(1) [Co]-O(1)		92.4(4)	94.39(16)	94.36(12)
O(1)-Cu(1) [Co]-N(3)		173.4(5)	93.83(14)	176.94(12)
N(1)-Cu(1) [Co]-N(3)		81.7(4)	82.71(15)	82.61(13)
N(1)-Cu(1) [Co]-O(2 W) [N1A]		167.2(3)	178.91(16)	179.16(14)
O(1)-Cu(1) [Co]-O(2 W) [N1A]		91.5(3)	87.26(14)	86.48(12)
N(3)-Cu(1) [Co]-O(2 W) [N1A]		93.6(4)	96.19(15)	96.55(13)
O(1)-Cu(1) [Co]-O(1 W) [N3A]		91.9(3)	88.88(14)	89.59(12)
N(1)-Cu(1) [Co]-O(1 W) [N3A]		104.0(3)	97.77(15)	97.32(13)
N(3)-Cu(1) [Co]-O(1 W) [O(1A)]		92.4(4)	89.71(14)	89.86(12)
O(2 W) [O(1)]-Cu(1) [Co]-O(1 W) [O(1A)]		88.0(3)	90.65(13)	90.31(11)
N(1A)-Co-O(1A)			93.83(14)	94.40(11)
N(1A)-Co-N(3A)			82.28(15)	82.69(13)
O(1A)-Co-N(1)			86.13(13)	85.58(12)
O(1A)-Co-N(3A)			176.10(14)	177.09(11)
N(3A)-Co-N(3)			91.00(14)	90.39(13)

	d, Å		
Bond lengths	**7**	**8**	**9**
Fe(1) [Cr1]-O(1)	1.900(3)	1.905(2)	1.925(3)
Fe(1) [Cr1]-O(1A)	1.921(3)	1.919(2)	1.916(3)
Fe(1) [Cr1]-N(1)	2.135(3)	2.130(2)	2.012(3)
Fe(1) [Cr1]-N(1A)	2.135(3)	2.136(2)	2.008(3)
Fe(1) [Cr1]-N(3)	2.124(4)	2.120(3)	2.057(3)

(Continues)

TABLE 2 (Continued)

	d, Å		
Bond lengths	**7**	**8**	**9**
Fe(1) [Cr1]-N(3A)	2.125(3)	2.116(3)	2.057(4)
S(1)-C(1)	1.753(4)	1.747(3)	1.753(4)
S(1)-C(12)	1.787(4)	1.789(4)	1.774(5)
N(1)-N(2)	1.392(4)	1.396(3)	1.380(4)
N(1)-C(2)	1.291(5)	1.290(4)	1.295(5)
N(2)-C(1)	1.366(5)	1.368(4)	1.356(5)
N(3)-C(1)	1.285(5)	1.292(4)	1.288(5)
N(3)-C(5)	1.465(5)	1.469(4)	1.472(5)
C(3)-C(2)	1.418(6)	1.424(5)	1.421(5)
O(1)-C(4)	1.310(4)	1.308(3)	1.312(4)
Bond angles	*ω*, deg.		
N(1)-Fe(1) [Cr]-O(1)	84.21(12)	84.21(9)	89.10(12)
O(1)-Fe(1) [Cr]-N(3)	157.48(12)	157.72(9)	165.65(12)
N(1)-Fe(1) [Cr]-N(3)	73.39(13)	73.61(10)	77.82(13)
N(1)-Fe(1) [Cr]-N(1A)	172.48(13)	172.19(10)	176.13(14)
O(1)-Fe(1) [Cr]-N(1A)	96.38(12)	96.35(9)	94.67(12)
N(3)-Fe(1) [Cr]-N(1A)	105.51(12)	105.35(10)	98.33(13)
O(1)-Fe(1) [Cr]-N(3A)	95.65(13)	95.24(10)	89.06(13)
N(1)-Fe(1) [Cr]-N(3A)	98.81(12)	98.38(9)	101.75(14)
N(3)-Fe(1) [Cr]-O(1A)	89.23(13)	89.39(10)	92.59(13)
O(1)-Fe(1) [Cr]-O(1A)	98.62(14)	98.05(10)	93.49(13)
N(1A)-Fe(1)-O(1A)	83.42(11)	83.74(9)	89.80(13)
N(1A)-Fe(1)-N(3A)	73.67(12)	73.81(9)	77.48(14)
O(1A)-Fe(1)-N(1)	103.92(12)	103.91(9)	90.85(13)
O(1A)-Fe(1)-N(3A)	154.19(12)	155.02(9)	167.19(13)
N(3A)-Fe(1)-N(3)	85.67(13)	86.18(10)	87.88(14)

with 570 nm and 600 nm filters. The measurement was made by imaging hybrid reader (Synergy H1, Biotek).

The percentage inhibition was calculated according to the formula:

$$100 - \left(\left(\text{Abs570nm}_{\text{sample}} - \text{Abs 600nm}_{\text{sample}}\right) / \left(\text{Abs570nm}_{\text{control}} - \text{Abs 600nm}_{\text{control}}\right) \times 100\right)$$

The IC_{50} values were evaluated bystatistical software.

Cell proliferation MTS assay

The cell proliferation assay was performed using 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS; Cell Titer 96 Aqueous, Promega, USA), which allowed us to measure the number of viable HL-60 cells. In brief, triplicate cultures of $1{\cdot}10^4$ cells in a total of 100 μl medium in 96-well microtiter plates (Becton Dickinson, Lincoln Park, NJ, USA) were incubated at 37°C, 5% CO_2. Compounds were dissolved in ethanol to prepare the stock solution of $1{\cdot}10^{-2}$ M. These compounds and doxorubicin (Novapharm, Toronto, Canada) were diluted at multiple concentrations with culture media, added to each well and incubated for 3 days. Following each treatment, 20 μl MTS was added to each well and incubated for 4 h. MTS is converted to water-soluble colored formazan by a dehydrogenase enzyme present in metabolically active cells. Subsequently, the plates were read at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

TABLE 3 Hydrogen bonds in X-ray structures for (**HL**), **3**, **5–9**

D –H···A	d (D···H), Å	d (H···A), Å	d (D···A), Å	∠(DHA), deg.	Symmetry transformation for H-acceptor
HL					
O1-H1...O1M	0.82	1.84	2.6561	169	$x, y, -1 + z$
O1M-H1M...I1	0.83	2.62	3.4501	178	$x, 1/2 - y, 1/2 + z$
N2-H2...I1	0.86	2.98	3.7236	147	x, y, z
N3-H3...I1	0.6	2.92	3.6178	139	$-1 + x, y, z$
N3-H3...N1	0.86	2.25	2.6261	106	x, y, z
C2-H2A...O1	0.93	2.41	2.7335	100	x, y, z
C5-H5A...S1	0.97	2.62	2.9777	102	x, y, z
C12-H12B...I1	0.96	2.99	3.8812	154	x, y, z
C12-H12C...O1	0.96	2.52	3.2145	130	$1 - x, -y, -z$
3					
O2W-H2WA...O2N	0.90	2.16	2.8954	139	$1 - x, -y, 1 - z$
O2W-H2WB...O1	0.89	1.86	2.6879	154	$1 - x, -y, 1 - z$
N2-H2A...O3N	0.86	2.05	2.8694	158	$-x, 1 - y, 1 - z$
O1W-H1WA...O2N	0.88	2.59	3.2326	131	x, y, z
O1W-H1WA...O3N	0.88	2.18	3.0477	168	x, y, z
O1W-H1WB...O2N	0.88	2.14	2.8357	136	$-x, -y, 1 - z$
C2-H2... O3N	0.93	2.53	3.3086	141	$-x, 1 - y, 1 - z$
C5-H5B... S1	0.97	2.55	2.9615	106	x, y, z
C12-H12B... O1N	0.96	2.44	3.3654	161	$-x, 1 - y, 1 - z$
5					
N2A-H1AA...Br1	0.86	2.54	3.2589	142	$-1 + x, y, z$
N2-H4AC...Br1	0.93	2.57	3.3777	157	$-1/2 + x, 1/2 - y, 1/2 + z$
C5-H4AA...S1	0.97	2.59	3.0234	107	x, y, z
C2-H9AA...Br1	0.93	2.51	3.2717	139	$-1/2 + x, 1/2 - y, 1/2 + z$
C5A-H4BB...S1A	0.97	2.63	3.0143	104	x, y, z
C9A-H5BA...O1	0.93	2.49	3.2075	134	$1/2 - x, -1/2 + y, 1/2 - z$
C12-H9BB...Br1	0.96	2.85	3.7562	158	$-1/2 + x, 1/2 - y, 1/2 + z$
C7A-H1CB...Br1	0.93	2.92	3.8387	170	$1 - x, 1 - y, 1 - z$
6					
N2-H2...O1N	0.86	2.11	2.8216	140	x, y, z
N2A-H2A...O2N	0.86	2.06	2.8820	159	$x, 1/2 - y, 1/2 + z$
C2A-H2AA... O2N	0.93	2.55	3.3279	142	$x, 1/2 - y, 1/2 + z$
C5A-H5AA... S1A	0.97	2.53	2.9504	106	x, y, z
C2-H2B... O2N	0.93	2.32	3.2355	167	x, y, z
C5-H5A... S1	0.97	2.62	2.9904	103	x, y, z
C5-H5A... N1A	0.97	2.62	3.1163	112	x, y, z
C12-H12C... O1N	0.96	2.52	3.3700	148	x, y, z
7					
N2A-H9...C11	0.86	2.51	3.2303	141	x, y, z

(Continues)

TABLE 3 (Continued)

D —H···A	d (D···H), Å	d (H···A), Å	d (D···A), Å	∠(DHA), deg.	Symmetry transformation for H-acceptor
N2-H12...C11	0.86	2.51	3.1446	131	$x, 1/2 - y, 1/2 + z$
C5A-H1AB... S1A	0.97	2.52	2.9692	108	x, y, z
C12-H3B...C11	0.96	2.76	3.5198	136	x, y, z
C2A-H13...C11	0.93	2.67	3.4564	143	x, y, z
C5-H26B... S1	0.97	2.62	3.0247	105	x, y, z
C12-H28B...C11	0.96	2.76	3.6891	162	$x, 1/2 - y, 1/2 + z$
8					
N2A-H4...O1N	0.86	2.10	2.9242	161	x, y, z
N2-H10...O3N	0.86	2.33	2.9817	132	$x, 1/2 - y, 1/2 + z$
C2-H17...O3N	0.93	2.58	3.2863	133	$x, 1/2 - y, 1/2 + z$
C2A-H18...O1N	0.93	2.54	3.3452	145	x, y, z
C5A-H21B...S1A	0.97	2.52	2.9652	108	x, y, z
C5-H25B...S1	0.97	2.61	3.0219	106	x, y, z
9					
N2-H7...O1N	0.86	2.08	2.8980	160	x, y, z
N2A-H10...O2N	0.86	2.37	2.9947	130	$x, 1/2 - y, 1/2 + z$
C5-H5B...S1	0.97	2.54	2.9705	107	x, y, z
C2-H16...O1N	0.93	2.51	3.3060	144	x, y, z
C5A-H26A...S1A	0.97	2.58	3.0219	108	x, y, z

2.6.3 | Antioxidant activity

The antioxidant activity by the $ABTS^{\bullet+}$ method was assessed according to the method described by Re et al.[45] with modifications. The $ABTS^{\bullet+}$ radical cation was formed through the reaction of ABTS solution 7 mM with potassium persulfate solution 140 mM, incubated at 25°C in the dark for 12–16 h. Once formed, the $ABTS^{\bullet+}$ solution was diluted with acetate-buffered saline (0.02 M, pH 6.5) to give an absorbance of 0.7 ± 0.01 at 734 nm.

Dilutions of Trolox, **HL**, **HL·HI·C_2H_5OH**, **1**, **6–9** were prepared in dimethylsulfoxide (DMSO). After that, 20 μl of each dilution was transferred to a 96-well microtiter plate and 180 μl of working solution of $ABTS^{\bullet+}$ was dispensed with the dispense module of hybrid reader (BioTek) and shaken for 15 s. The decrease in absorbance at 734 nm was measured exactly after 30 min of incubation at 25°C. Blank samples were run by solvent without $ABTS^{\bullet+}$.

The decrease in absorbance is expressed as % inhibition, which is calculated from the following formula:

$$\left(\left(\mathrm{Abs}_{\mathrm{control}} - \mathrm{Abs}_{\mathrm{sample}}\right)/\mathrm{Abs}_{\mathrm{control}}\right)\cdot 100.$$

3 | RESULTS AND DISCUSSION

The free ligand **HL** and nine new metal complexes, [Cu (L)(H_2O)]Cl (**1**), [Cu (L)(H_2O)]Br (**2**), [Cu (L)(H_2O)$_2$] NO_3 (**3**), [Co (L)$_2$] Cl (**4**), [Co (L)$_2$] Br (**5**), **[**Co (L)$_2$]NO_3 (**6**), [Fe (L)$_2$] Cl (**7**), **[**Fe (L)$_2$]NO_3 (**8**), [Cr (L)$_2$]NO_3 (**9**) were synthesized in ethanol in good yield.

Single crystals were obtained for six coordination compounds (**3**), (**5–9**) and ligand **HL·HI·C_2H_5OH** with recrystallization from various solvents, such as DMSO, DMF, methanol, ethanol, chloroform, and their structures found using X-ray analysis.

All complexes were prepared by the direct reaction between the ligand **HL** and the corresponding metal salts. Complexes of **HL** with the corresponding copper salts were synthesized in 1:1 molar ratio [Cu:**HL**], but cobalt, iron and chromium coordination compounds were synthesized similarly, but in 1:2 molar ratio [M:**HL**]. The synthesis of the complexes is reproducible and the isothiosemicarbazone **HL** coordinates as a mononegative tridentate ligand. The obtained compounds are microcrystalline solids that are stable in air and decompose above 250°C.

Thermal analyses data reveal that compounds **1–3** are hydrated (Figure S1).[46] The molar conductivity values of the complexes **1–9** are in the range 80–115 Ω^{-1} cm^2 mol

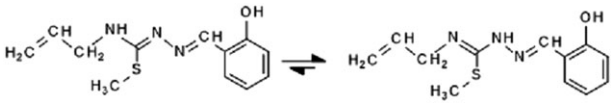
$^{-1}$ in methanol that indicates that all complexes represent 1:1 electrolytes.[47] The elemental analyses data of the ligand and complexes (reported in Section 2) are in agreement with the structure of the ligand and the structures of the complexes, respectively.

3.1 | NMR spectra

The purity and structure of isothiosemicarbazone **HL** was determined using ^{1}H and ^{13}C NMR spectroscopy. The alkylation of sulfur atom is proved by the comparative analysis of 2-hydroxybenzaldehyde-4-allylthiosemicarbazone and 4-allyl-S-methylisothiosemicarbazone NMR spectra (Figure S2). A peak in the range of 177–179 ppm that is common for C=S of thiosemicarbazones disappears in the ^{13}C NMR spectrum of the **HL**. Also peaks of carbon atoms in the methyl group appear in the range 13–30 ppm that are not found in the spectra of corresponding thiosemicarbazone. Peaks of methyl protons appear in the ^{1}H NMR spectrum of **HL** in the range of 2.42–2.48 ppm.[48] All peaks in the spectra of isothiosemicarbazone **HL** are double.[38] This indicates the presence of tautomeric forms of isothiosemicarbazone in solution. The integral ratio between two tautomeric forms is 1:2.7 [**HL** (A):**HL** (B)]. The presence of tautomeric forms can be caused by syn/anti-isomerism around the C=N1 double bond, and *cis/trans* (Z/E) isomerism around the C=N^{4} double bond (Scheme 5).[11,49]

3.2 | Infrared spectra

The type of ligand coordination with the central ions was elucidated from comparative analysis of IR spectra of complexes **1–9** and the free ligand **HL**.[50,51] In the IR spectrum of **HL**, medium absorption bands at 3200–3500 cm^{-1} are due to stretching vibrations of the OH group. In the IR spectra of complexes **1–3**, a considerable peak observed in the 3430–3440 cm^{-1} range supports the presence of ν (H_2O) in the complexes.[52] The phenolic υ (C–O) stretching vibration in the free ligand is observed at 1156 cm^{-1}, which is shifted by 10–18 cm^{-1} towards lower wavenumbers in the complexes, thus indicating coordination of the phenolic oxygen to the metal ion.[53] The IR spectrum of ligand exhibited strong bands at 1604 and 1570 cm^{-1} assignable to ν (C=N). In the spectra of the respective complexes this bands is shifted to lower frequency by about 15–20 cm^{-1},[54] suggesting the coordination of the azomethine nitrogen to the metal center. The absorption band of υ (C=S) in the spectrum of **HL** is absent because the sulfur atom was alkylated with iodomethane, which leads to the presence in the spectrum of a new absorption band at 682 cm^{-1}, corresponding to υ (C-S).[55] This absorption band is not shifted, indicating that the sulfor atom is not involved in coordination to the metal ion.



SCHEME 5 The tautomeric forms of the ligand **HL**

Besides, in the IR spectra of the nitrate complexes **3**, **6**, **8**, **9** there is a broadened intense absorption band at 1373 cm^{-1}. It is attributable to ionic NO_3^-.[56]

3.3 | Electronic spectra and magnetic moments

The electronic spectra in the polycrystalline state of **HL** show the intraligand absorption maxima corresponding to $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transitions: 39.210 and 28.570 cm^{-1}. In the spectra of the complexes these bands are shifted to lower energies (Figure S3). The electronic spectra of complexes **1–3** showed a single absorption band at 17.850, 16.520 and 15.030 cm^{-1}, respectively, which may be assigned to $d_{x^2-y^2}/d_{z^2}$ transition, for their square–pyramidal geometry. A second $d_{x^2-y^2}/d_{xz}$; d_{yz} transition appeared as a weak shoulder on the intraligand and charge transfer bands. The magnetochemical research showed that effective magnetic moments for the synthesized copper complexes **1–3** vary in the range of 1.79–1.84 B.M., which are close to the spin value for one unpaired electron.[57]

The cobalt complexes **4–6** are diamagnetic, which indicates that cobalt (II) is oxidized by oxygen from air to cobalt (III) during the synthesis. The electronic spectra of complexes **4–6** show d–d bands in the regions 15.150–16.120, 19.230–19.607 and 23.800–25.000 cm^{-1}, which may be assigned to $^{1}A_{1g} \rightarrow {}^{3}T_{2g}$, $^{1}A_{1g} \rightarrow {}^{1}T_{1g}$, $^{1}A_{1g} \rightarrow {}^{1}T_{2g}$ transitions, respectively, due to the octahedral geometry.[57]

The iron complexes **7**, **8** are paramagnetic with effective magnetic moments values 5.74–5.93 B.M. The electronic spectra exhibited three bands at 17.090–17.850, 20.830–21.500 and 25.970–25.260 cm^{-1} due to the $^{6}A_{1g} \rightarrow {}^{4}T_{1g}$, $^{6}A_{1g} \rightarrow {}^{4}A_{1g}$ and $^{6}A_{1g} \rightarrow {}^{4}T_{2g}$(D) transitions, respectively, in an octahedral geometry.[58]

The chromium atom is in the +3 oxidation state (μ_{eff} = 3.83 B.M.). The electronic spectrum of chromium complex **9** show two bands at 18.690 and 23.520 cm^{-1} attributable to $^{4}A_{2g}(F) \rightarrow {}^{4}T_{2g}(F)$ and $^{4}A_{2g}(F) \rightarrow {}^{4}T_{1g}(F)$ transitions, respectively, suggesting an octahedral structure.[59]

3.4 | Structural characterization of (HL), [Cu (L)$(H_2O)_2$]NO_3 (3), [Co $(L)_2$] Br (5), [Co $(L)_2$]NO_3 (6), [Fe $(L)_2$] Cl (7), [Fe $(L)_2$]NO_3 (8) and [Cr $(L)_2NO_3$ (9)

The single crystal X-ray study has revealed that the ligand and complexes **3**, **5–9** have molecular structure as depicted in Figure 1.

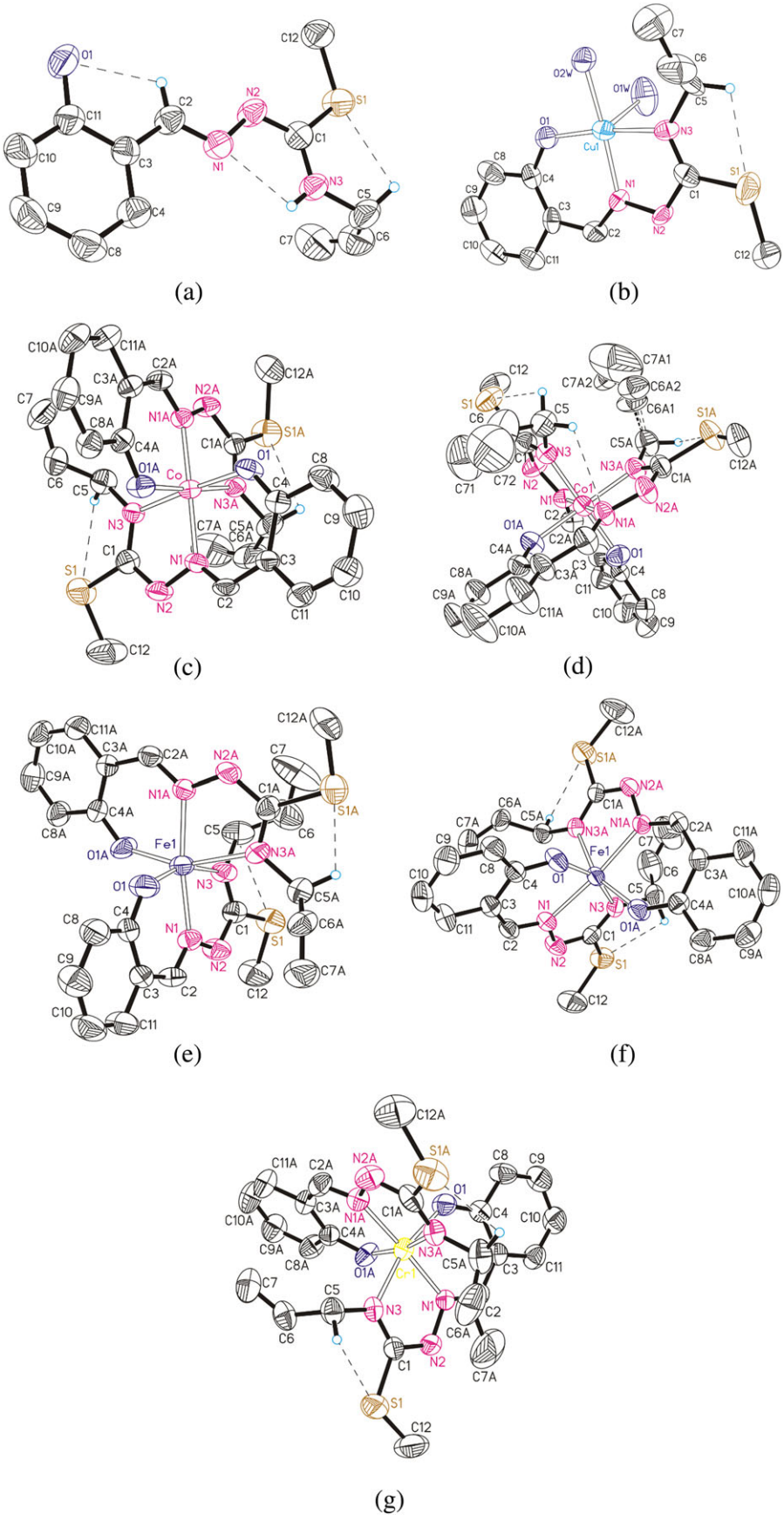


FIGURE 1 Perspective view of the (**HL**), (**3**) and (**5–9**) compounds, along with atom numbering scheme. Thermal ellipsoids are drawn at 50% probability level

In the organic ligand the substituents at the N(2)–C(1) bond are in the *E* position. However, the presence of the phenyl cycle in Z-salicylaldehyde S-methyl-4-phenylisothiosemicarbazone (**I**) has led to formation of *Z* position of substituents with respect to N(2)–C(1) bond.[60] The *A* [S(1)-N(1)-N(2)-N(3)-C(1)-C(2)] core is practically planar within 0.012 Å, and the dihedral angle between the given core and phenyl ring [C(3)–C(11)] is equal to 2.4°, for **I** this angle is equal to 12.4°. But the studied molecule is nonplanar due to the presence of the C_3H_5 substituent in the thiosemicarbazone moiety, the dihedral angle between the best planes of *A* and [C(5)–C(7)] fragment is equal to 85.9°. In the crystal the ligand HL·HI·C_2H_5OH the HLs forms the dimers where the molecules are linked by C(12)-H ... O(1) $(1-x,-y,-z)$ hydrogen bonds (HB; Figure 2a; Table 3). The dimers are joined by hydrogen iodide and ethanol molecules to form three-dimensional framework of HB (Figure 2b).

In complexes **3**, **5–9**, the ligand **HL** acts as mononegative tridentate, around the metallic ion, through an ONN set of donor atoms. But the composition of the coordination polyhedron of the central atom in these compounds is different. The Cu(1) atom in **3** is penta-coordinated in a distorted square–pyramidal coordination geometry. Its basal plane includes three donor atoms of the **L** and oxygen atom O(1 W) of water molecule (Figure 1b; Table 2). The apex of the metal's coordination pyramid in **3** is occupied by oxygen atom O(2 W) of another H_2O molecule with a distance of 2.312(5) Å. The polyhedral volume is related to distortion of coordination polyhedral and its value for the copper atom is 6.251 $Å^3$. In a similar five-coordinated complex of aqua-(E-salicylaldehyde S-methyl-4-phenylisothiosemicarbazone-N,N′,O)-copper (II) nitrate,[60] where the fifth apical position is occupied by the nitro group and the phenyl ring is a substituent on the N(3) atom, the corresponding Cu-O distance is 2.418 Å and the polyhedral volume is 6.289 $Å^3$. The structural characterization of **5–9** revealed that the metals in these complexes are in a distorted octahedral environment, being surrounded by two **L** ligands (Figure 1c–g; Table 2). The octahedral volumes of Co atoms in **5** and **6** are equal to 9.151 and 9.076 $Å^3$, whereas for Fe (**7**, **8**) and Cr (**9**) atoms the corresponding values are 10.994, 10.982, and 10.364 $Å^3$, respectively.

In the crystals the complexes **3** are joined by water molecules in the basal plane into centrosymmetric dimers (Figure 3a). The dimers are related by bridging nitrate groups through the hydrogen bonds to form the polymeric chains along the [100] direction (Figure 3b; Table 3), and the chains are linked via van der Waals interactions.

In addition, according to the criterion proposed in[41] (Cg*I*···Cg*J* < 6.0 Å, β < 60.0°, where β is the angle between the Cg*I*-Cg*J* vector and the normal to the aromatic ring Cg*I*), in chains there is a π–π stacking interaction between the six-member metallocycles and phenyl rings of different dimers related by an inversion center. The Cg*I*···Cg*J* $(1-x,1-y,1-z)$ distance between the centroids of these groups is 3.548 Å, and β = 17.3°. In **5–9** the octahedral complexes are related by outer sphere anions into the chains through the hydrogen bonds (Figures 4 and 5; Table 3).

The crystal structures of **7–9** are practically isostructural (Table 1), therefore the packing diagrams are presented only for **7** and **8** having different anions. In the crystals of **5** and **6** the complexes are linked by bromide ions and NO_3^- groups forming the chains along [1 0–1] and [0 0 1] directions, respectively. In the crystal structures of **7–9** the complexes are joined into the chains aligned along [0 0 1] direction by chlorine ions and NO_3^- groups. Between the chains in **5–9** the van der Waals interaction occurs.

The intramolecular non-bonding interactions involving sulfur atoms were observed for compounds of **HL**, **5–9**. The S···H distances range from 2.52 to 2.63 Å, whereas the S...C distances are in the interval of 2.97–3.02 Å. The intramolecular C–H···S interactions have been also discovered in the single crystal X-ray structure of sodium di (isopropyl) dithiocarbamate pentahydrate.[61] The S···H intramolecular interaction has a distance of 2.39 Å, and the S...C distance is equal to 3.03 Å. It was found that the ubiquitous nature of C–H···S intramolecular interactions in this class of compound is evident in the structures of other di-(isopropyl)

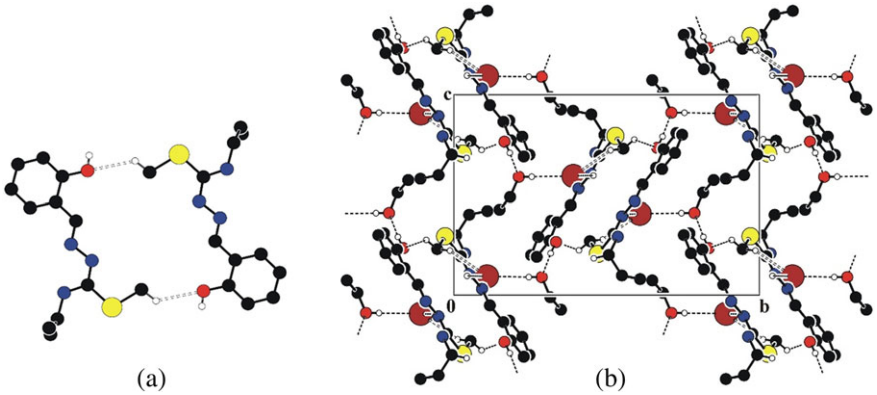


FIGURE 2 The crystal packing of the ligand **HL**, showing (a) the formation of centrosymmetric dimers, where the molecules are linked by C(12)-H ... O(1) hydrogen bond, and (b) 3D molecular network

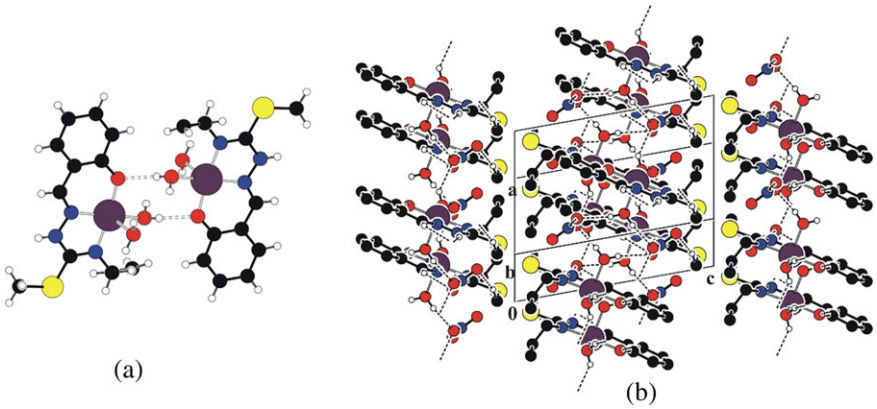


FIGURE 3 View of (a) centrosymmetric dimers in **3**, where the complexes are joined by O(2 W)-H...O(1) hydrogen bond, (b) which are aligned along the [1 0 0] direction forming the chains. The dimers are linked by NO_3^- groups, and between the chains there are van der Waals interactions

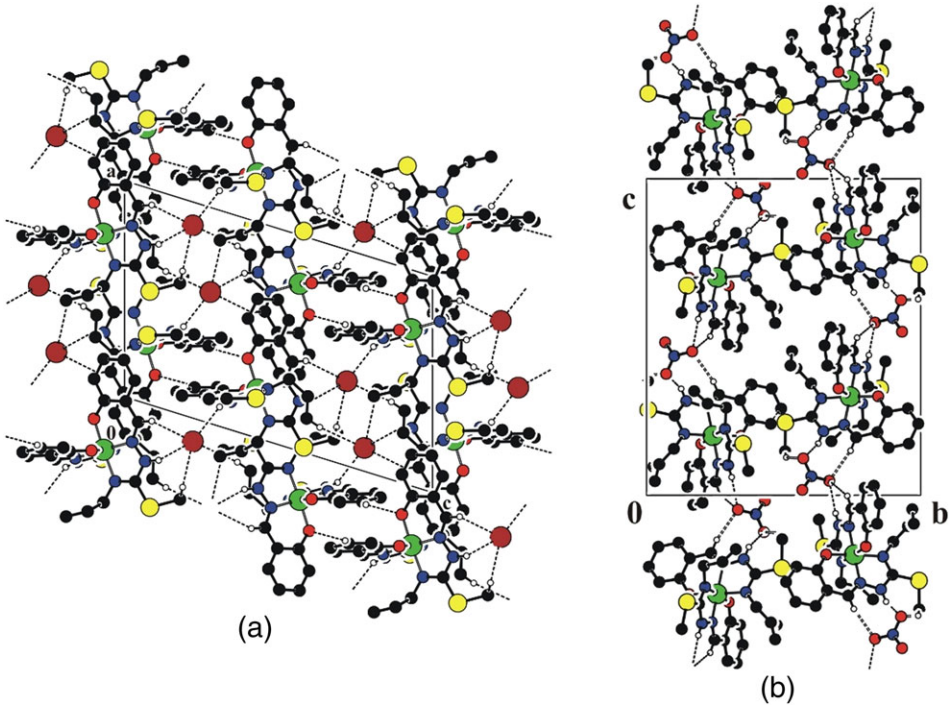


FIGURE 4 The formation of chains in (a) **5** and (b) **6**, which are aligned along [1 0–1] and [0 0 1] directions. The complexes are linked by bromide ions and NO_3^- groups, respectively

dithiocarbamate complexes deposited in the CSD. The C···S distances in these interactions fall within 2.941–3.054 Å (a range of 0.113 Å). It was shown that the restricted rotation in di-(isopropyl) dithiocarbamate complexes can be directly attributed to intramolecular C–H···S interactions. Thus, we may assume that intramolecular C–H···S hydrogen bonds somewhat restrict rotation about the N3–C5 bond in the propyl group.

3.5 | Biological activity

3.5.1 | Antibacterial and antifungal bioassays

The antibacterial and antifungal bioassays were performed according to protocols described previously. The antimicrobial activities of metal complexes were studied against Gram(+) bacteria [*S. aureus* (*ATCC 25923*), *E. faecalis*], Gram(−) bacteria [*E. coli* (*ATCC 25922*), *S. abony* (*GISK 03/03*)], and antifungal activity against *C. albicans*. The study of antibacterial and antifungal activities (Table 4) showed that **HL** and its coordination compounds possess bacteriostatic and bactericidal activities. The activity of the synthesized compounds towards Gram-negative microorganisms is less pronounced than towards Gram-positive bacteria and fungi.

It was found that the 2-hydroxybenzaldehyde 4-allyl-S-methylisothiosemicarbazone does not manifest high antibacterial and antifungal activities in the studied range of concentrations. On the other hand, the coordination compounds show selective antibacterial and antifungal

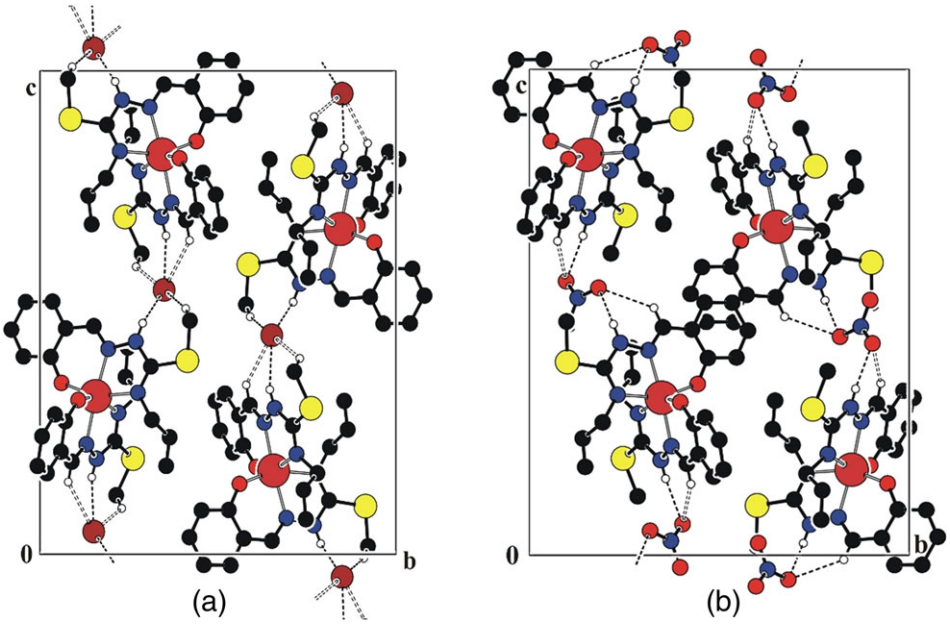


FIGURE 5 The formation of chains in (a) **7** and (b) **8**, which are aligned along the [0 0 1] direction. The complexes are linked by chlorine ions and NO_3^- groups, respectively

TABLE 4 Antibacterial and antifungal activities of ligand **HL** and complexes **1–9** as MIC[a]/MBC[b] values (mg ml^{-1})

	Escherichia coli (G−)		*Salmonella abony* (G−)		*Staphylococcus aureus* (G+)		*Enterococcus faecalis* (G+)		*Candida albicans*	
Compound	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
HL	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
[Cu (L)(H_2O)]Cl (**1**)	0.12	0.12	0.12	0.25	0.12	0.12	0.06	0.25	0.03	0.06
[Cu (L)(H_2O)]Br (**2**)	0.12	0.12	0.12	0.25	0.12	0.12	0.06	0.25	0.12	0.25
[Cu (L)(H_2O)$_2$]NO_3 (**3**)	0.12	0.5	0.25	0.5	0.25	0.25	0.12	0.25	0.25	0.25
[Co (L)$_2$] Cl (**4**)	1.0	1.0	1.0	1.0	0.5	1.0	1.0	1.0	0.5	1.0
[Co (L)$_2$] Br (**5**)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
[Co (L)$_2$]NO_3 (**6**)	1.0	1.0	1.0	1.0	1.0	2.0	1.0	2.0	1.0	2.0
[Fe (L)$_2$] Cl (**7**)	0.5	0.5	0.25	0.5	1.0	1.0	1.0	1.0	0.12	0.5
[Fe (L)$_2$]NO_3 (**8**)	0.5	1.0	0.5	1.0	1.0	1.0	1.0	1.0	0.25	0.5
[Cr (L)$_2$]NO_3 (**9**)	1.0	2.0	0.5	1.0	0.5	1.0	0.5	1.0	0.5	1.0
Furacillinum	0.0185	0.0375	0.075	0.15	0.0093	0.0093	0.0375	0.075		
Nystatine	–	–	–	–	–	–	–	–	0.08	0.08

Escherichia coli (ATCC 25922); *Salmonella abony* (GISK 03/03); *Staphylococcus aureus* (ATCC 25923).

[a]MIC, minimum inhibitory concentration.

[b]MBC, minimum bactericide concentration.

G(−), Gram-negative bacteria; G(+), Gram-positive bacteria.

activities towards a series of standard strains in the range of concentration 30–2000 μg ml^{-1}. It was shown that the nature of the metal ion and acid residue has an influence on the antimicrobial activities of these complexes. It was found that the copper complexes are the most active ones. The comparison of antibacterial and antifungal activities of these compounds against the selected types of bacteria indicates that the activity of the coordination compounds decreases in the following way Cu (II) > Fe (III) > Co (III) ≈ Cr (III). For the homoleptic complexes the minimal bacteriostatic and bactericidal concentrations grow in the following way: $Cl^- < Br^- < NO_3^-$. The complexes of copper halides manifest the highest activity. Nevertheless, the antibacterial and antifungal activities of the

synthesized coordination compounds are lower than activities of furacillinum and nystatine that are used in medical practice.

3.5.2 | Antiproliferative activity

The antitumor activity of synthesized compounds was studied on a series of cancer cells: human leukemia HL-60 cells; human cervical epithelial HeLa cells; human epithelial pancreatic adenocarcinoma BxPC-3 cells; human muscle rhabdomyosarcoma spindle and large multinucleated RD cells.

The 2-hydroxybenzaldehyde-4-allyl-S-methylisothiosemicarbazone and five coordination compounds were tested as inhibitors of proliferation of HL-60 cells. These human promyelocytic leukemia cells were incubated for 3 days in the presence of synthesized compounds (ligand and complexes) and the number of viable cells was measured using the MTS assay. The results are expressed as the percentage of cell growth inhibition at three concentrations.

The ligand has insignificant inhibitor activity, but some metal complexes selectively act in this biological process (Table 5). The nature, electronic structure and coordination number of the central atom, the geometric configuration of metal complexes and the nature of the ligands (donor atoms) appear to modulate the cell proliferation.

The study of antitumor activity showed that the free ligand and its coordination compounds, except copper coordination compound **2**, possess not very high antiproliferative activity towards cervical cancer HeLa cells (Table 6). The study of proliferation of cervical cancer HeLa cells in the presence of 2-hydroxybenzaldehyde-4-allyl-S-methylisothiosemicarbazone showed that this isothiosemicarbazone inhibits the proliferation of these cells by 47.7% at a concentration of 100 μM, but at a concentration of 10 μM activity disappears. So, the activity of this ligand towards HeLa cells is more pronounced than towards HL-60 cells. The activity of coordination compounds **6**, **8**, **9** at lower concentration (10–0.1 μM) practically disappears. The activity of iron (III) coordination compound surpasses the activity of the ligand **HL**. The copper coordination compound **2** manifests much higher activity than the ligand **HL** and doxorubicin.

As the main drawback for substances with anti-cancer properties is their toxicity, then for synthesized substances it is necessary to determine the selectivity of their action on cancer cells. For this purpose, the cytostatic effect of these substances was studied on normal MDCK cells. The

TABLE 5 Antiproliferative activities of some synthesized compounds on human leukemia (HL-60) cells at three concentrations and their IC_{50} values

Compound	Inhibition of cell proliferation (%)[a]			IC_{50}, μM
	10 μM	1 μM	0.1 μM	
HL	15.28	3.83	0	> 10
$[Cu(L)(H_2O)_2]NO_3$ (**3**)	100	0.75	0	1.45
$[Co(L)_2]NO_3$ (**6**)	19.37	9.99	3.58	> 10
$[Fe(L)_2]Cl$ (**7**)	35.67	0	0	> 10
$[Fe(L)_2]NO_3$ (**8**)	81.92	11.77	8.23	3.69
$[Cr(L)_2]NO_3$ (**9**)	13.99	0	0	> 10

[a]SEM < ±4% of a single experiment in triplicate. The IC_{50} values were calculated using statistical software.

TABLE 6 Antiproliferative activities of some synthesized compounds on cervical cancer (HeLa) cells at four concentrations and their IC_{50} values

Compound	Inhibition of cell proliferation (%)[a]				IC_{50}, μM
	100 μM	10 μM	1 μM	0.1 μM	
HL	47.7	0	0	0	> 100
$[Cu(L)(H_2O)_2]Br$ (**2**)	94.5	93.8	32.3	10.7	1.6
$[Co(L)_2]NO_3$ (**6**)	42.7	2.9	0.3	1.4	> 100
$[Fe(L)_2]NO_3$ (**8**)	110.3	1.5	0.8	0.7	31.6
$[Cr(L)_2]NO_3$ (**9**)	22.5	12.9	7.8	0	> 100
Doxorubicin	–	49.8	12.2	0	10.0

[a]SEM < ±4% of a single experiment in triplicate. The IC_{50} values were calculated using statistical software.

TABLE 7 Inhibition effect of some synthesized compounds on MDCK cells at three concentrations and their IC_{50} values

Compound	Inhibition of cell proliferation (%)[a]				IC_{50}, μM
	100 μM	10 μM	1 μM	0.1 μM	
HL	29.7	0	0	0	> 100
[Cu (L)$(H_2O)_2$] Br (**2**)	100.0	31.6	18.9	11.1	12.7
[Co $(L)_2$]NO_3 (**6**)	38.0	0	0	0	> 100
[Fe $(L)_2$]NO_3 (**8**)	66.0	1.0	0	0	75.2
[Cr $(L)_2$]NO_3 (**9**)	36.0	19.0	19.0	20.0	> 100
Doxorubicin	–	56.0	25.1	19.1	7.1

[a]SEM < ±4% of a single experiment in triplicate.

TABLE 8 IC_{50} values of copper complex **2** towards MDCK, HeLa, BxPC-3 and RD cells

Compound	MDCK	HeLa		BxPC-3		RD	
	IC_{50} μM	IC_{50} μM	$\frac{IC_{50}(MDCK)}{IC_{50}(HeLa)}$	IC_{50} μM	$\frac{IC_{50}(MDCK)}{IC_{50}(BxPC-3)}$	IC_{50} μM	$\frac{IC_{50}(MDCK)}{IC_{50}(RD)}$
[Cu (L)$(H_2O)_2$]Br (**2**)	12.7	1.6	7.94	0.6	21.17	5.2	2.44
Doxorubicin	7.1	10.0	0.71	3.7	1.92	16.2	0.44

TABLE 9 IC_{50} values of some of the synthesized substances towards $ABTS^{\bullet+}$ radical cation

Compound	IC_{50}, μM
HL	31.0
HL·HI·C_2H_5OH	31.0
[Cu (L)$(H_2O)_2$] Cl (**1**)	43.0
[Co $(L)_2$]NO_3 (**6**)	0.5
[Fe $(L)_2$] Cl (**7**)	0.7
[Fe $(L)_2$]NO_3 (**8**)	0.6
[Cr $(L)_2$]NO_3 (**9**)	0.5
Trolox	33.3

experiment showed that **HL** inhibits proliferation of these cells only at 100 μM concentration by 29.7% (Table 7). The free ligand and three coordination compounds (**6**, **8**, **9**) do not represent a danger to normal MDCK cells; their IC_{50} values are > 75 μM for the iron complex and > 100 μM for other complexes. It is worth noting that the iron coordination compound manifests activity toward HeLa cells and does not destroy normal MDCK cells, which improves its properties in comparison with doxorubicin, applied in practice in medicine. On this basis it is presupposed that synthesized compounds have a lower cytotoxic effect on normal cells of human organisms.

In order to determine the selectivity of the antiproliferative activity, the primary screening on a wider series of cancer cells was performed for the copper coordination compound **2**. This compound manifests better activity towards HeLa, BxPC-3 and RD cancer cells, as well as possessing better selectivity comparing antiproliferative activity towards cancer and normal MDCK cells. The selectivity index that is the ratio between the IC_{50} value for the normal MDCK cells and IC_{50} values for the cancer cells varies in the range 2.44–21.17. This index is 5.5–11 times higher for the copper coordination compound **2** than for doxorubicin (Table 8).

3.5.3 | Antioxidant activity

Moreover, the antioxidant properties of **HL**, **HL·HI·C_2H_5OH** and coordination compounds **1** and **6–9** were studied using the $ABTS^{\bullet+}$ method. The experiment showed that proligand **HL** and copper coordination compound **1** do not manifest high antioxidant activity as their IC_{50} values are higher than the IC_{50} value for Trolox[62] that is used in medical practice. On the other hand, the coordination compounds of cobalt (III) (**6**), iron (III) (**7**, **8**) and chromium (III) (**9**) manifest high antioxidant activity that exceeds by 47–67 times the activity of Trolox (Table 9), which shows the perspective for using these substances as synthetic antioxidants.[63]

4 | CONCLUSIONS

New copper (II), cobalt (III), iron (III) and chromium (III) complexes derived from 2-hydroxybenzaldehyde-4-allyl-S-methylisothiosemicarbazone have been synthesized and characterized. Physico-chemical analyses confirmed the

composition and structures of the newly obtained complex combinations. The structure of **HL·HI·C_2H_5OH** and complexes **3**, **5–9** has been determined by single-crystal X-ray diffraction. X-ray crystal studies show the ligand is nonplanar due to the presence of C_3H_5 substituent in the thiosemicarbazone moiety. In all complexes, the isothiosemicarbazone **HL** acts as a mononegative tridentate ligand with O, N, N set of donor atoms. It coordinates to the central ions with deprotonated phenolic oxygen atom, N^1 and N^4 nitrogen atoms, forming five- and six-membered metallacycles.

The Cu(1) atom in complex **3** is penta-coordinated in a distorted square–pyramidal coordination geometry, while the metals in complexes **5–9** are in a distorted octahedral environment.

The ligand and the metal complexes have been screened for their *in vitro* antimicrobial activity against *S. aureus*, *E. faecalis*, *E. coli*, *S. abony* and *C. albicans*, and for antiproliferative activity against HL-60 human leukemia, HeLa cervical cancer and normal MDCK cells.

The quantitative antimicrobial activity test results proved that the metal complexes have specific antimicrobial activity, depending on the microbial species tested, in the range of concentration 30–2000 μg ml^{-1}. The copper coordination compounds **1–3** manifest much greater antimicrobial activity than free ligand, complex **1** being more active than nystatin.

The screening results showed that the synthesized substances inhibit proliferation of the human leukemia HL-60 cells at 10 μM, and cervical cancer HeLa cells at 100 μM. The study of the influence of synthesized compounds on healthy MDCK cells showed that they have lower cytotoxic effect on healthy cells of human organisms. The copper complex **3** and iron complex **8** showed promising antiproliferative activity and low toxicity. The copper complex **2** manifests better anticancer activity towards HeLa, BxPC-3 and RD cells than doxorubicin, and also has much lower antiproliferative activity towards normal MDCK cells. The coordination compounds **6–9** manifest high antioxidant activity that exceeds 47–67 times the activity of Trolox, which shows the perspective for the use of these compounds as synthetic antioxidants. So, copper (II) complexes proved good antimicrobial and antiproliferative activity, while cobalt (III), iron (III) and chromium complexes have demonstrated antioxidant activity.

The biological property of these compounds can be explained on the basis of several factors, involving type of donor atom present in ligand, the metal ion type, metal salt anion used, the total charge on the complex ion and coordination geometry. The results obtained place these metal complexes as candidates for further studies on the elucidation of specific molecular targets and the exact mechanism of cell death.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

1532463, 1532457, 1532458, 1532459, 1532460, 1532461, 1532462 contain the supplementary crystallographic data for **HL**, **3**, **5**–**9**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk.

How to cite this article: Pahontu E, Usataia I, Graur V, et al. Synthesis, characterization, crystal structure of novel Cu (II), Co (III), Fe (III) and Cr (III) complexes with 2-hydroxybenzaldehyde-4-allyl-S-methylisothiosemicarbazone: Antimicrobial, antioxidant and *in vitro* antiproliferative activity. *Appl Organometal Chem*. 2018;32:e4544. https://doi.org/10.1002/aoc.4544