CZU: 547.484.22:546.561`131:615.28 https://doi.org/10.59295/mabt2026.24

SYNTHESIS AND EVALUATION OF ANTICANCER PROPERTIES OF PYRUVAMIDE 4-ALLYLTHIOSEMICARBAZONES AND THEIR COPPER(II) CHLORIDE COMPLEXES

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Rezumat

A fost sintetizată o serie de 4-aliltiosemicarbazone ale piruvamidelor (**HL$^{1–6}$**) și compușii cu clorură de cupru(II) (**1**–**6**). Structurile și compozițiile lor au fost caracterizate utilizând analiza elementală, spectroscopia RMN ^{1}H și ^{13}C, și spectroscopia FTIR. Compușii au fost testați pentru activitate antiproliferativă împotriva liniilor celulare de cancer uman THP-1, PC-3 și HT-29, și asupra liniei celulare epiteliale normale hTERT-RPE1. Complecșii de cupru(II) au prezentat o activitate anticancer mai mare comparativ cu tiosemicarbazonele corespunzătoare. Dintre acestea, compușii **1** și **3** au demonstrat cele mai promițătoare efecte, depășind în unele cazuri activitatea medicamentului de referință doxorubicină. Indicii lor de selectivitate pentru celulele de leucemie umană THP-1, în raport cu celulele normale hTERT-RPE1, au fost mai mari de 10. Activitățile biologice studiate au fost puternic influențate de natura substituentului de la atomul de azot în piruvamidă, evidențiind potențialul semnificativ pentru optimizarea suplimentară a piruvamidelor substituite în vederea creșterii eficacității anticancer.

Cuvinte-cheie: Tiosemicarbazone; acidul piruvic; compușii cuprului(II); activitatea anticancer; linie celulară TPH-1; linie celulară PC-3; linie celulară HT-29; linie celulară hTERT-RPE1.

SINTEZA ȘI EVALUAREA PROPRIETĂȚILOR ANTICANCER ALE 4-ALILTIOSEMICARBAZONELOR ALE PIRUVAMIDELOR ȘI ALE COMPLECȘILOR LOR CU CLORURĂ DE CUPRU(II)

Abstract

A series of pyruvamide 4-allylthiosemicarbazones (**HL$^{1–6}$**) and their copper(II) chloride complexes (**1**–**6**) were synthesized. Their structures and compositions were characterized using elemental analysis, ^{1}H and ^{13}C NMR spectroscopy, and FTIR spectroscopy. The compounds were tested for antiproliferative activity against human cancer cell lines TPH-1, PC-3, HT-29, as well as the normal epithelial cell line hTERT-RPE1. The copper(II) complexes showed greater anticancer activity compared to the thiosemicarbazones. Among them, complexes **1** and **3** exhibited the most promising effects, in some cases exceeding the activity of the reference drug doxorubicin. Their selectivity indices for human leukemia THP-1 cells relative to normal hTERT-RPE1 cells were above 10. The observed biological activities were strongly influenced by the nature of the substituent at the nitrogen atom of the pyruvamide moiety, indicating significant potential for further optimization of substituted pyruvamides to enhance anticancer efficacy.

Keywords: Thiosemicarbazone; pyruvic acid; copper(II) complexes; anticancer activity; TPH-1 cell line; PC-3 cell line; HT-29 cell line; hTERT-RPE1 cell line.

Introduction

Thiosemicarbazones and their metal complexes are known for their broad range of applications, spanning from analytical chemistry to pharmacology and even nuclear medicine [1]. Owing to the presence of amide, imine, and thione functional groups, these compounds can act as polydentate ligands, which explains why a large number of their metal complexes have been synthesized and thoroughly characterized [2]. In recent years, increasing interest has also

been directed toward thiosemicarbazones due to their diverse biological activities, including antifungal, antiviral, antibacterial, and anticancer effects [3-5].

Cancer remains a central focus in the life sciences, as it has long been one of the leading causes of death throughout human history. Rather than a single disease, it represents a broad group of disorders characterized by the uncontrolled proliferation and dissemination of abnormal cells. The proper functioning of any organism depends on a finely regulated balance between cell growth and cell death; disruption of this equilibrium can result in abnormal tissue development, ultimately leading to tumor formation and potentially fatal cancer.

The biological activity of thiosemicarbazones has been investigated since 1956, when was first reported the antitumor effects of thiosemicarbazones derived from 2-formylpyridine. It has been established that the nature of the substituent at the 4N position significantly affects biological activity, while the acidic character of the 3NH group enables deprotonation, allowing the ligand to adopt an anionic form and extend conjugation across the thiosemicarbazone framework. This extended conjugation is believed to contribute to enhanced antitumor properties [6-7].

Moreover, metal complexes of thiosemicarbazones have attracted attention as promising anticancer and chemotherapeutic agents. Their activity is often associated with the inhibition of ribonucleotide diphosphate reductase, a key enzyme essential for DNA biosynthesis and cell division, thereby suppressing the growth of various types of cancer [8-9].

The objective of this study was to synthesize a series of pyruvamide 4-allylthiosemicarbazones (Figure 1) along with their copper(II) chloride complexes, and to evaluate their anticancer activity towards human cancer cell lines TPH-1, PC-3, HT-29, and normal epithelial cell line hTERT-RPE1.

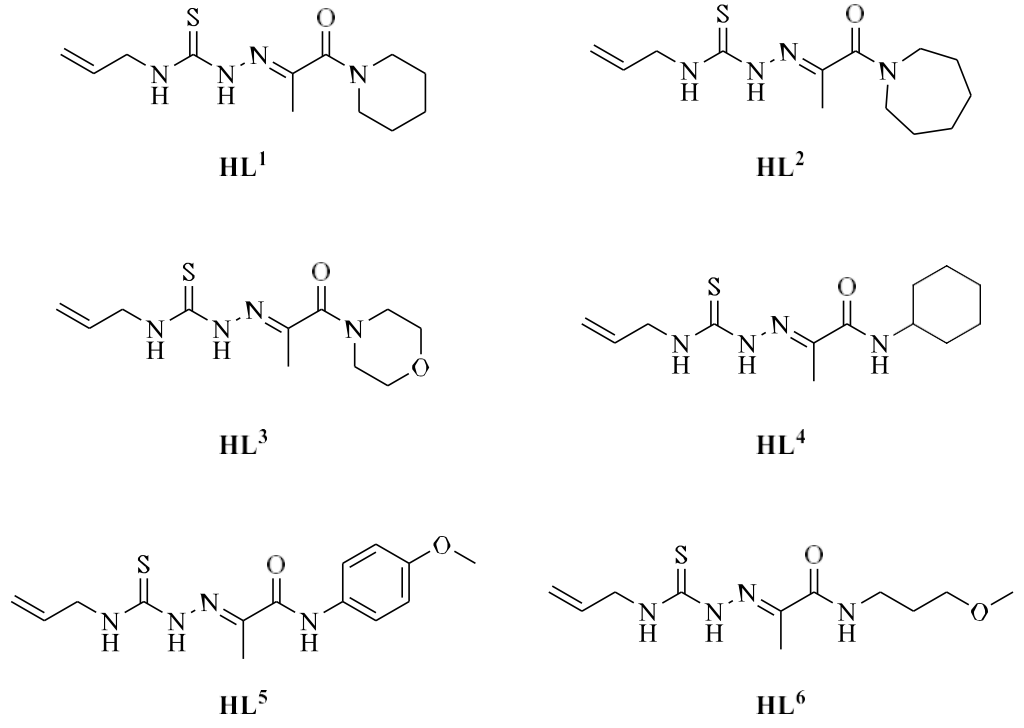


Figure 1. The structural formula of HL^{1-6}

Materials and methods

3-Isothiocyanatoprop-1-ene, hydrazine hydrate, pyruvic acid, oxalyl chloride, sodium carbonate, dichloromethane, dimethylformamide, cyclohexanamine, azepane, 3-methoxypropan-1-amine, piperidine, morpholine, 4-methoxyaniline, tributylamine, acetone, copper(II) chloride dihydrate 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. FTIR spectra were obtained on a Bruker ALPHA FTIR spectrophotometer at room temperature in the range of 4000-400 cm^{-1}. The analysis on copper(II) metal was performed similarly to the literature procedures [10]. The resistance of solutions of complexes in methanol (20°C, c 0.001 M) was measured using an R-38 rheochord bridge.

Synthesis of 4-allylthiosemicarbazones ***HL$^{1-6}$***

General procedure of synthesis ***HL$^{1-6}$***

Initially, pyruvic acid (8.80 g, 0.100 mol) was dissolved in 5 mL of CH_2Cl_2 and transferred into a round-bottom flask. A separate solution of oxalyl chloride (15.24 g, 0.120 mol) in 10 mL CH_2Cl_2 was then added slowly to the cooled mixture (0 °C) under continuous stirring. After addition, a few drops of dimethylformamide were introduced as a catalyst, and the flask was equipped with a condenser fitted with a calcium chloride drying tube. The reaction mixture was kept under heating and stirring for approximately 1.5 hours, resulting in the formation of a yellow oily intermediate.

In a separate vessel, a mixture containing corresponding amine (0.100 mol), sodium carbonate (10.60 g, 0.100 mol) or tributylamine (18.5 g, 0.100 mol) in a case of 4-methoxyaniline, and 10 mL CH_2Cl_2 was cooled to 0 °C with stirring. The freshly prepared 2-oxopropanoyl chloride was added dropwise to this suspension, after which the reaction was allowed to proceed at room temperature for an additional hour. The final mixture was filtered, washed several times with water (3 × 100 mL), and the resulting material was air-dried, yielding the crude product as a yellow, mobile oil. In the case of synthesis with 4-methoxyaniline after filtration, the light gray residue was obtained. For further purification, the substance was recrystallized in acetone to obtain a white solid.

Then the obtained pyruvamide (20.0 mmol) was dissolved in 25 mL of ethanol, after which *N*-(prop-2-en-1-yl)hydrazinecarbothioamide (20.0 mmol) was added. The reaction mixture was stirred and heated for 1 hour. The pale-yellow product was separated by filtration, washed with a little ethanol, and left to air-dry.

3-(Piperidin-1-yl)propane-2,3-dione 4-allylthiosemicarbazone ***HL^{1}***

Pale yellow solid. Yield: 83%; mp 137-139 °C. FW: 268.38 g/mol; Anal Calc. for $C_{12}H_{20}N_4OS$: C, 53.70; H, 7.51; N, 20.88; S, 11.95; found: C, 53.60; H, 7.42; N, 20.79; S, 11.87%. FTIR data (cm^{-1}): 3315, 3288 (NH), 1632 (C=O), 1620 (C=N), 1362 (C=S). ^{1}H NMR ($CDCl_3$, 400 MHz) 8.71 (br., 1H; NH); 7.46 (br., 1H; NH); 5.89 (m., 1H; CH); 5.20 (m., 2H; CH_2); 4.30 (m., 2H; NH-CH_2); 3.50 (m., 2H; $CH_{2piperidine}$); 2.11 (s., 3H; CH_3); 1.62 (m. 6H; $CH_{2piperidine}$). ^{13}C NMR ($CDCl_3$, 100 MHz) 178.20 (C=S); 165.33 (C=O); 143.47 (C=N); 132.88 (CH=CH_2), 117.37 (CH_2=), 46.93 (C_{allyl}); 45.54, 45.51, 26.02, 24.44, 20.35 ($C_{piperidine}$); 14.19 (CH_3). The characteristics of the obtained substance correspond with the data reported in the literature [11].

3-(Azepan-1-yl)propane-2,3-dione 4-allylthiosemicarbazone ***HL^{2}***

Pale yellow solid. Yield: 78%; mp 106-107 °C. FW: 282.40 g/mol; Anal Calc. for $C_{13}H_{22}N_4OS$: C, 55.29; H, 7.85; N, 19.84; S, 11.35; found: C, 55.20; H, 7.78; N, 19.76; S, 11.28%. FTIR data (cm^{-1}): ν(N–H) 3286, 3166; ν (C=O) 1643; ν (C=N) 1624; ν (C=S) 1362. ^{1}H NMR ($CDCl_3$, 400 MHz) 8.73 (br s, 1H; NH); 7.42 (br s, 1H; NH); 5.89 (m, 1H; CH); 5.22 (m, 2H; CH_2=C); 4.30 (m, 2H; NH-CH_2); 3.50 (t, 2×2H; $CH_{2(hexamethyleneimine)}$-N); 2.11 (s, 3H; CH_3); 1.8-1.5 (m, 4×2H; $CH_{2(hexamethyleneimine)}$). ^{13}C NMR ($CDCl_3$, 100 MHz) 178.29 (C=S); 166.76

(C=O); 143.93 (C=N); 132.86 (CH=CH_2); 117.44 (CH_2=); 46.98 (C_{allyl}); 47.76, 45.38, 28.30, 27.00, 20.34 ($C_{(hexamethyleneimine)}$); 14.10 (CH_3).

3-(Morpholin-4-yl)propane-2,3-dione 4-allylthiosemicarbazone ***HL³***

Pale yellow solid. Yield: 78%; mp 138-139 °C. FW: 270.35 g/mol; Anal Calc. for $C_{11}H_{18}N_4O_2S$: C, 48.87; H, 6.71; N, 20.72; S, 11.86; found: C, 48.78; H, 6.63; N, 20.63; S, 11.78%. FTIR data (cm^{-1}): ν(NH) 3282, 3200; ν (C=O) 1636; ν (C=N) 1619; ν (C=S) 1360. ^{1}H NMR ($CDCl_3$, 400 MHz) 8.75 (br s, 1H; NH); 7.39 (br s, 1H; NH); 5.89 (m, 1H; CH); 5.21 (m, 2H; CH_2); 4.30 (m, 2H; NH–CH_2); 3.76–3.56 (m, 4×2H; $CH_{2(morpholine)}$); 2.11 (s, 3H; CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) 178.30 (C=S); 165.51 (C=O); 142.32 (C=N); 132.83 ($CH_{(allyl)}$); 117.50 ($CH_{2(allyl,sp^2)}$); 66.72 ($CH_2O_{(morpholine)}$); 46.95 ($CH_{2(allyl,sp^3)}$ and $CH_2N_{(morpholine)}$); 14.13 (CH_3). The characteristics of the obtained substance correspond with the data reported in the literature [12].

N-cyclohexyl-2-oxopropanamide 4-allylthiosemicarbazone ***HL⁴***

Pale yellow solid. Yield: 80%; mp 193-195 °C. FW: 282.40 g/mol; Anal Calc. for $C_{13}H_{22}N_4OS$: C, 55.29; H, 7.85; N, 19.84; S, 11.35; found: C, 55.20; H, 7.78; N, 19.76; S, 11.28%. FTIR data (cm^{-1}): ν(N–H) 3329, 3288, 3176; ν (C=O) 1640; ν (C=N) 1614; ν (C=S) 1365. ^{1}H NMR ($CDCl_3$, 400 MHz) 8.61 (br s, 1H, NH-CO); 7.49 (br s, 1H, NH); 6.64 (d, 1H, NH); 5.95 (m, 1H, CH_{allyl}); 5.31-5.18 (m, 2H, CH_2=C); 4.35 (t, 2H, CH_2); 3.79 (m, 1H, $CH_{(Cy)}$); 2.11 (s, 3H, CH_3); 1.97-1.07 (m, 10H, 5×$CH_{2(Cy)}$). ^{13}C NMR ($CDCl_3$, 100 MHz) 178.21 (C=S); 162.28 (C=O); 142.95 (C=N); 132.98 (CH=CH_2); 117.30 (CH_2=); 48.69 (CH-NH); 47.11 (CH_2-NH); 33.00, 25.45, 24.89 ($CH_{2(Cy)}$); 10.67 (CH_3).

N-(4-methoxyphenyl)-2-oxopropanamide 4-allylthiosemicarbazone ***HL⁵***

Pale yellow solid. Yield: 80%; mp 154-156 °C. FW: 306.38 g/mol; Anal Calc. for $C_{14}H_{18}N_4O_2S$: C, 54.88; H, 5.92; N, 18.29; S, 10.47; found: C, 54.80; H, 5.86; N, 18.21; S, 10.39%. FTIR data (cm^{-1}): ν(N–H) 3357, 3305, 3198; ν (C=O) 1644; ν (C=N) 1615; ν (C=S) 1361. ^{1}H NMR ($CDCl_3$, 400 MHz) 8.70 (br s, 1H; NH); 8.66 (br s, 1H; NH); 7.84 (br s, 1H; NH); 7.44 (d, 2H, $CH_{(aromatic)}$); 6.86 (d, 2H, $CH_{(aromatic)}$); 5.96 (m, 1H; $CH_{(allyl)}$); 5.32-5.20 (m, 2H, CH_2=C); 4.36 (t, 2H, CH_2); 3.80 (s, 3H, OCH_3); 2.13 (s, 3H, CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) 178.03 (C=S); 161.34 (C=O); 156.85, 129.95, 122.45, 144.20 ($C_{(aromatic)}$); 142.48 (C-CH_3); 132.95 ($CH_{(allyl)}$); 117.56 (CH_2=); 55.53 (CH_3O); 47.29 (CH_2-CH); 10.42 (CH_3-C).

N-(3-methoxypropyl)-2-oxopropanamide 4-allylthiosemicarbazone ***HL⁶***

Pale yellow solid. Yield: 80%; mp 94-93 °C. FW: 272.37 g/mol; Anal Calc. for $C_{11}H_{20}N_4O_2S$: C, 48.51; H, 7.40; N, 20.57; S, 11.77; found: C, 48.42; H, 7.30; N, 20.48; S, 11.68%. FTIR data (cm^{-1}): ν(N–H) 3319, 3270, 3160; ν (C=O) 1644; ν (C=N) 1618; ν (C=S) 1363. ^{1}H NMR ($CDCl_3$, 400 MHz) 8.69 (br s, 1H, NH-CO); 7.67 (br s, 1H, NH); 7.60 (br s, 1H, NH); 5.92 (m, 1H, CH_{allyl}); 5.26 (d, 1H, CH_2=C, J=16.4 Hz); 5.20 (d, 1H, CH_2=C, J=9.8 Hz); 4.35 (m, 2H, CH_2); 3.51 (t, 2H, CH_2); 3.42 (m, 2H, CH_2); 3.31 (s, 3H, OCH_3); 2.10 (s, 3H, CH_3); 1.80 (qnt, 2H, CH_2). ^{13}C NMR ($CDCl_3$, 100 MHz) 178.25 (C=S); 163.21 (C=O); 142.31 (C-CH_3); 132.92 (CH_{allyl}); 117.47 (CH_2=); 72.32 (CH_2-O); 58.89 (CH_3-O); 47.21 (CH_2-CH_{allyl}); 38.72 (CH_2-NH); 28.54 (CH_2); 10.48 (CH_3-C).

Synthesis of copper(II) coordination compounds ***1-6***

General procedure of synthesis

A solution of 4-allylthiosemicarbazone **HL$^{1-6}$** (1.00 mmol) in ethanol was combined with an ethanol solution of copper(II) chloride dihydrate (1.00 mmol). Upon mixing, a green solid gradually appeared. The formed compound was isolated by filtration, rinsed with a small volume of ethanol, and then allowed to dry.

*[Cu(L^1)Cl] (**1**)* Green solid. Yield: 82%. Anal. Calc. for $C_{12}H_{19}ClCuN_4OS$ (366.37 g mol^{-1}): Cu, 17.34; found: Cu, 17.28. Main FTIR peaks (cm^{-1}): ν(NH) 3219, ν(C=O) 1621, ν(C=N) 1574, ν(C–S) 791. χ(CH_3OH): 95 Ω^{-1} cm^{-2} mol^{-1}.

*[Cu(L^2)Cl] (**2**)* Green solid. Yield: 88%. Anal. Calc. for $C_{13}H_{21}ClCuN_4OS$ (380.40 g mol^{-1}): Cu, 16.71; found: Cu, 16.61. Main FTIR peaks (cm^{-1}): ν(NH) 3237, ν(C=O) 1626, ν(C=N) 1575, ν(C–S) 775. χ(CH_3OH): 94 Ω^{-1} cm^{-2} mol^{-1}.

*[Cu(L^3)Cl] (**3**)* Green solid. Yield: 72%. Anal. Calc. for $C_{11}H_{17}ClCuN_4O_2S$ (368.34 g mol^{-1}): Cu, 17.25; found: Cu, 17.17. Main FTIR peaks (cm^{-1}): ν(NH) 3310, ν(C=O) 1625, ν(C=N) 1578, ν(C–S) 771. χ(CH_3OH): 79 Ω^{-1} cm^{-2} mol^{-1}.

*[Cu(L^4)Cl] (**4**)* Green solid. Yield: 87%. Anal. Calc. for $C_{13}H_{21}ClCuN_4OS$ (380.40 g mol^{-1}): Cu, 16.71; found: Cu, 16.62. Main FTIR peaks (cm^{-1}): ν(NH) 3364, 3330, ν(C=O) 1613, ν(C=N) 1585, ν(C–S) 774. χ(CH_3OH): 83 Ω^{-1} cm^{-2} mol^{-1}.

*[Cu(L^5)Cl] (**5**)* Green solid. Yield: 78%. Anal. Calc. for $C_{14}H_{17}ClCuN_4O_2S$ (404.37 g mol^{-1}): Cu, 15.71; found: Cu, 15.61. Main FTIR peaks (cm^{-1}): ν(NH) 3314, 3332, ν(C=O) 1614, ν(C=N) 1560, ν(C–S) 772. χ(CH_3OH): 70 Ω^{-1} cm^{-2} mol^{-1}.

*[Cu(L^6)Cl] (**6**)* Green solid. Yield: 89%. Anal. Calc. for $C_{11}H_{19}ClCuN_4O_2S$ (370.36 g mol^{-1}): Cu, 17.16; found: Cu, 17.07. Main FTIR peaks (cm^{-1}): ν(NH) 3311, 3331, ν(C=O) 1617, ν(C=N) 1581, ν(C–S) 769. χ(CH_3OH): 88 Ω^{-1} cm^{-2} mol^{-1}.

Anticancer activity

Cell Culture

Human cancer cell lines TPH-1, PC-3, HT-29, and normal epithelial cell line hTERT-RPE1 were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). Cancer cell lines TPH-1, PC-3, and HT-29 were maintained in Roswell Park Memorial Institute (RPMI)-1640 medium (Sigma, Saint Louis, MO, USA), while the hTERT-RPE1 normal cell line was cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with L-glutamine (2 mM), penicillin (100 IU/mL), streptomycin (100 μg/mL), and 10% (v/v) fetal bovine serum (FBS). The cell cultures were maintained at 37°C in a humidified atmosphere containing 5% CO_2. The culture medium was refreshed every 2–3 days.

MTS-method

IC_{50} values for the tested compounds were determined on HT-29, THP-1, PC-3, and hTERT-RPE1 cell lines. Cells (1×10^4 per well) were seeded in 96-well plates and incubated for 24 h at 37°C (5% CO_2). The compounds, dissolved in DMSO and diluted in culture medium, were added and incubated with the cells for 48 h. Cell viability was assessed using the MTS assay, followed by absorbance measurement at 490 nm. IC_{50} values were calculated using GraphPad Prism 6 software.

Results and discussion

Synthesis and characterization

Six 4-allylthiosemicarbazones of pyruvamide derivatives **HL^{1-6}** were obtained in two stages (Figure 2). In the first stage, pyruvic acid reacted with oxalyl chloride and the corresponding amine in dichloromethane. In the second stage, the resulting pyruvic acid amides reacted with 4-allylthiosemicarbazide in ethanol at a 1:1 molar ratio. The composition and structure of the resultant thiosemicarbazones was proved using ^1H and ^{13}C NMR spectroscopies.

Copper(II) complexes **1-6** were synthesized by reaction between ligands **HL^{1-6}** with copper(II) chloride dihydrate in ethanol at a 1:1 molar ratio (Figure 3). Based on elemental analysis, the following general formulas were proposed for the synthesized copper(II) complexes: [Cu(L^{1-6})Cl] (**1-6**). The molar conductivity values measured for complexes **1-6** are within the range of 70–95 $\Omega^{-1}\cdot cm^2\cdot mol^{-1}$, indicating that in solution these complexes behave as 1:1 electrolyte.

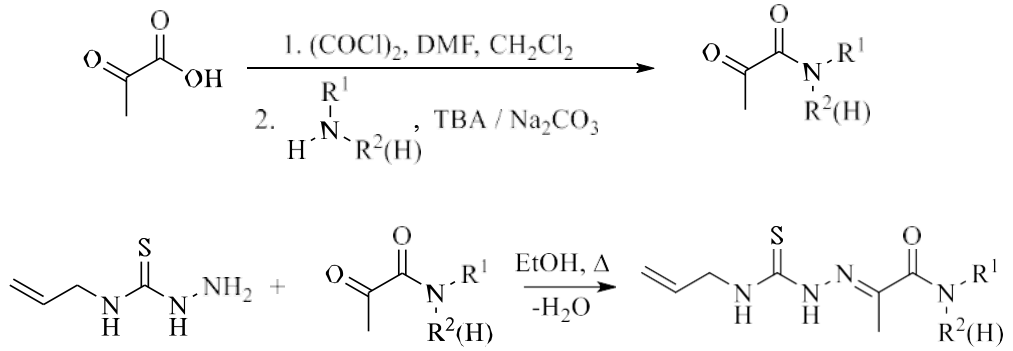


Figure 2. Synthesis of pyruvamide 4-allylthiosemicarbazones HL^{1-6}

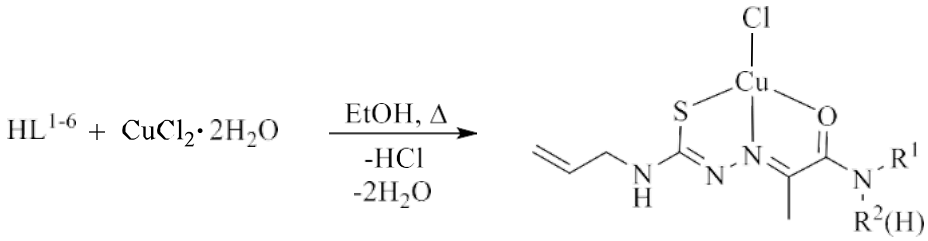


Figure 3. General scheme of the synthesis of copper(II) chloride complexes 1-6

Spectroscopic studies

To confirm the coordination of the ligand to the copper atom in the complexes and to determine the coordination mode, a comparative analysis of the FTIR spectra of 4-allylthiosemicarbazones **HL^{1-6}** and their copper(II) complexes **1-6** was performed. In the spectra of the complexes, the disappearance of one absorption band characteristic of the NH group is observed, indicating deprotonation of the ligand upon complex formation. The absorption bands corresponding to the remaining NH groups are shifted to the lower-frequency region. In addition, the absorption bands characteristic of the C=O and C=N groups are also shifted to lower frequencies. In the FTIR spectra of the copper(II) complexes **1-6**, the disappearance of absorption bands characteristic of the ν(C=S) group and the appearance of a new ν(C–S) absorption band are observed, which confirms the transition of the ligands to the thiol form in the complexes.

Anticancer activity

The antiproliferative activity of the studied substances was tested on the human leukemia THP-1 cell line at concentrations of 10, 1, and 0.1 μM. The results, expressed as the percentage of cell proliferation inhibition at these concentrations, as well as the calculated half-maximal inhibitory concentration values (IC_{50}), are presented in Table 1.

The antiproliferative activity of thiosemicarbazones is influenced by the substituent at the nitrogen atom of the pyruvamide fragment, increasing in the order: **HL^5** < **HL^4** < **HL^6** < **HL^1** < **HL^2** ≈ **HL^3**. Compounds **HL^2** and **HL^3** inhibit cell proliferation by more than 50% (IC_{50} = 3.85 ± 0.36 μM and 2.79 ± 0.28 μM, respectively). In all cases, copper(II) complexes are more active than the corresponding ligands. All complexes except **4** reduce THP-1 cell proliferation by over 50% at 10 μM, with activity increasing as follows: **4** < **5** < **1** < **6** < **2** < **3**. Complexes **2** and **3** are the most active, with complex **3** (IC_{50} = 0.14 ± 0.01 μM) showing 2.3 fold higher activity than doxorubicin (IC_{50} = 0.32 ± 0.06 μM).

Table 1. Antiproliferative activities of the synthesized compounds against human leukemia THP-1 cell line at three concentrations.

Compound	Inhibition of cell proliferation, (%)			IC_{50}, μM
	10 μM	1 μM	0.1 μM	
HL^1	39.9±0.6	24.0±0.4	3.9±0.9	>10
1	87.8±0.1	39.9±0.2	29.7±0.9	0.95±0.02
HL^2	53.8±1.3	48.5±0.1	10.5±0.1	3.85±0.36
2	79.8±1.0	49.3±1.2	33.8±1.0	0.66±0.08
HL^3	53.7±1.0	51.0±0.1	28.8±0.2	2.79±0.28
3	88.6±0.2	62.0±0.4	49.6±0.9	0.14±0.01
HL^4	29.9±1.6	28.7±3.0	26.2±7.9	>10
4	37.2±3.7	16.8±1.3	9.0±2.5	>10
HL^5	24.7±1.0	3.7±2.1	2.2±0.2	>10
5	53.0±6.0	35.9±0.5	42.3±2.4	5.64±1.50
HL^6	37.2±1.9	31.1±2.3	9.6±0.1	>10
6	71.9±1.0	43.1±1.6	42.7±0.1	0.69±0.04
Doxorubicin	98.3±0.4	75.0±6.7	24.7±0.8	0.32±0.06

Activity was also tested against PC-3 cells, which were generally less sensitive to the compounds (Figure 4).

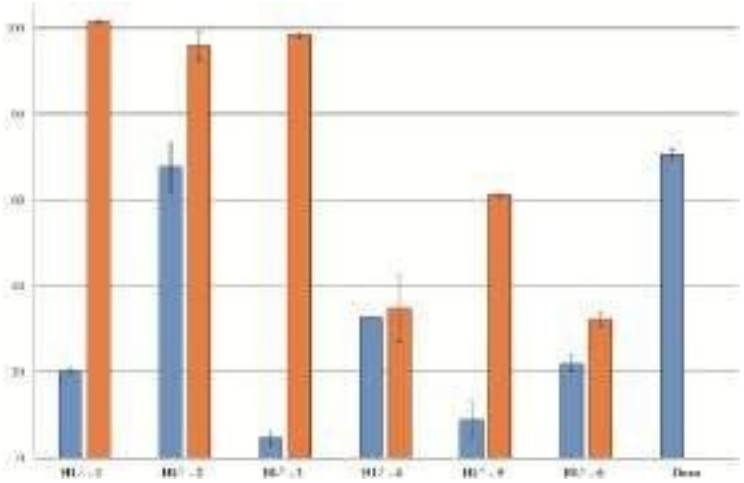


Figure 4. Antiproliferative activity of the synthesized thiosemicarbazones and their copper(II) complexes against the human prostate cancer PC-3 cell line at 10 μM

Similar to the results for the THP-1 cell line, the copper(II) complexes show greater antiproliferative activity against PC-3 cells than the corresponding thiosemicarbazones. At 10 μM, only five compounds **HL^2** and complexes **1**, **2**, **3**, and **5**—reduce cell proliferation by more than 50%. Their inhibition data and IC_{50} values are presented in Table 2.

Among the tested compounds, complex **1** is the most active one against the PC-3 cell line; however, it is nearly twofold less active than doxorubicin, which exhibits an IC_{50} value of 0.83±0.66 μM.

The antiproliferative activity was further investigated against the human colorectal cancer HT-29 cell line. The percentages of inhibition at a concentration of 10 μM are shown in Figure 5.

Table 2. Antiproliferative activities of selected synthesized compounds against the human prostate cancer PC-3 cell line at three concentrations.

Compound	Inhibition of cell proliferation, (%)			IC_{50}, μM
	10 μM	1 μM	0.1 μM	
1	101.5±0.4	24.3±3.4	25.8±1.5	1.58±0.06
HL^2	67.7±5.6	36.6±9.2	33.9±5.2	2.17±1.28
2	95.9±3.4	28.3±3.0	24.8±2.0	1.61±0.22
3	98.4±0.7	5.0±6.1	3.7±1.7	2.95±1.08
5	61.0±0.5	7.7±5.0	5.4±2.3	7.02±0.68

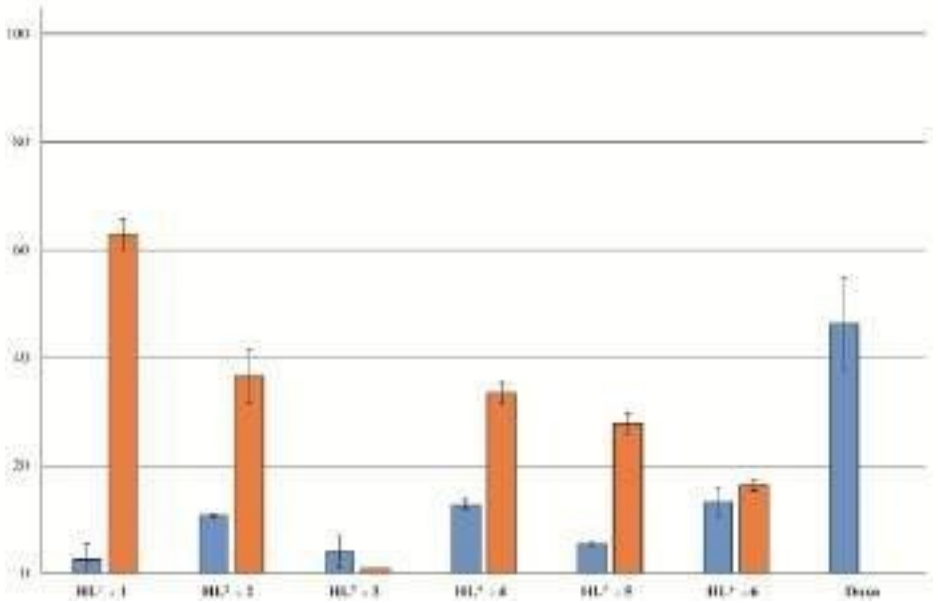


Figure 5. Antiproliferative activity of the synthesized thiosemicarbazones and their copper(II) complexes against the human colorectal cancer HT-29 cell line at 10 μM

Complex 1 is the only compound that inhibits the proliferation of this cancer cell line by more than 50%, outperforming doxorubicin in this respect. Its IC50 value toward the HT-29 cell line, calculated from inhibition data at three concentrations (10 μM – 62.9 ± 2.9%; 1 μM – 4.3 ± 2.8%; 0.1 μM – 1.8 ± 0.9%), is 7.20 ± 0.02 μM. This value is approximately three times lower than that of doxorubicin (23.65 ± 5.11 μM), which shows the following inhibition levels: 100 μM – 62.6 ± 3.7%; 10 μM – 46.2 ± 8.5%; 1 μM – 14.6 ± 5.7%. Given that complexes 1 and 3, bearing piperidine and morpholine moieties in the pyruvamide fragment, exhibited the most notable antiproliferative activity, their selectivity toward cancer cells relative to normal cells was further assessed using the hTERT-RPE1 normal cell line. The corresponding results are shown in Figure 6.

Complex **3** reduces the proliferation of the normal cell line by more than 50% at a concentration of 10 μM, suggesting a relatively high level of cytotoxicity toward non-cancerous cells, with an IC_{50} value of 5.59 ± 0.23 μM. Accordingly, its selectivity indices (SI), calculated as the ratio between IC_{50} values for normal and cancer cells, are: SI (THP-1) = 40 and SI (PC-3) = 1.9. In comparison, complex 1 demonstrates a weaker inhibitory effect on normal cells (IC_{50} > 10 μM), with selectivity indices of: SI (THP-1) > 10, SI (PC-3) > 6, and SI (HT-29) > 1.3.

Meanwhile, doxorubicin shows relatively low toxicity toward normal cells, with an IC_{50} value of 55.5 ± 4.9 μM. Its selectivity indices are significantly higher: SI (THP-1) = 170, SI (PC-3) = 66, and SI (HT-29) = 2.3.

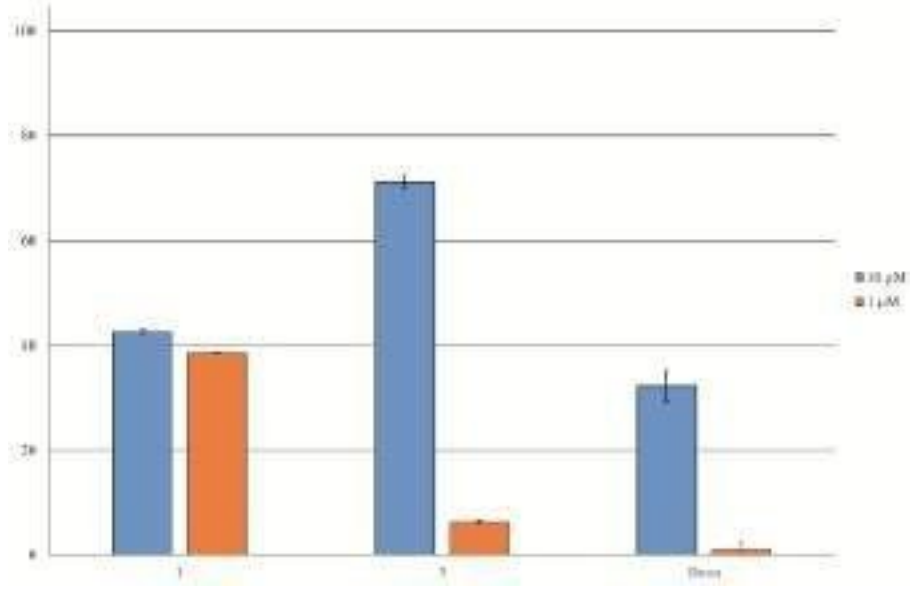


Figure 6. Antiproliferative activity of complexes 1 and 3 against normal hTERT-RPE1 cell line at 10 and 1 μM

Conclusion

This work reports the synthesis and characterization of six 4-allylthiosemicarbazones derived from substituted pyruvamides and their corresponding copper(II) complexes.

Biological evaluation showed that the copper(II) complexes possess higher antiproliferative activity than the free thiosemicarbazones, with activity strongly influenced by the substituent in the pyruvamide fragment. Complexes containing azepane, piperidine, and morpholine groups exhibited the most pronounced effects. Although some compounds surpassed doxorubicin in antiproliferative activity, their selectivity indices remained lower. Overall, the results highlight the key role of substituent variation and support further optimization of pyruvamide derivatives to improve activity and selectivity.

Acknowledgment

This work was fulfilled with the financial support of the subprogram 010602 of the institutional project.

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