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***In vitro* antileukemia, antibacterial and antifungal activities of some 3d metal complexes: Chemical synthesis and structure – activity relationships**

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Abstract

The present paper describes the synthesis, characterization and *in vitro* biological evaluation screening of different classes (ammoniacates, dioximates, carboxylates, semi- and thiosemicarbazidates) of Co(II), Co(III), Cu(II), Ni(II), Mn(II), Zn(II) and Fe(III) complexes. Schiff bases were obtained from the reaction of some salicyl aldehydes with, respectively, furoylhydrazine, benzoylhydrazine, semicarbazide, thiosemicarbazide and S-methylthiosemicarbazide to give tridentate ligands containing ONO, ONS or ONN as donor atoms. The synthetic metal complexes are of various geometrical and electronic structures, thermodynamic and thermal stabilities, and magnetic and conductance properties. All complexes, except those of Cu, are octahedral. Some Cu, Co and Mn compounds have a dimeric or a polymeric structure. The composition and structure of complexes were analysed by elemental analysis, IR and ¹H NMR and ¹³C NMR spectroscopies, and magnetochemical, thermoanalytical and molar conductance measurements. All ligands and metal complexes were tested as inhibitors of human leukemia (HL-60) cells growth, and the most potent, the Cu(II) complexes, have been also tested for their *in vitro* antibacterial and antifungal activities. Structure-activity relationships were carried out.

Keywords: metal complexes, organic ligand, complex, leukemia, antibacterial, antifungal

Introduction

Twenty-eight years after the first approval of cisplatin in the clinic against a number of cancer diseases, cisplatin and related compounds continue to be among the most efficient anticancer drugs used so far. Efforts are now focused to develop novel platinum- and non-platinum-based antitumor drugs to improve clinical effectiveness, to reduce general toxicity and to broaden the spectrum of activity [1]. DNA is a main target for the therapeutic treatment of various disorders and diseases. It can interact with many biomolecules and synthetic compounds including

organometallic compounds and metal complexes. Therefore, investigations of the interaction of some ligands with transition metals can further provide and/or improve our understanding about rational metal based inhibitors design [2–7]. We have started a program directed toward the synthesis of different classes of anticancer, antibacterial and antifungal agents designed with complexes of a transition metal and an organic ligand [8–12]. Thiosemicarbazones and their transition metal (Cu and Co) complexes demonstrated potent cytotoxic activities against a series of murine and human tumor cells in culture

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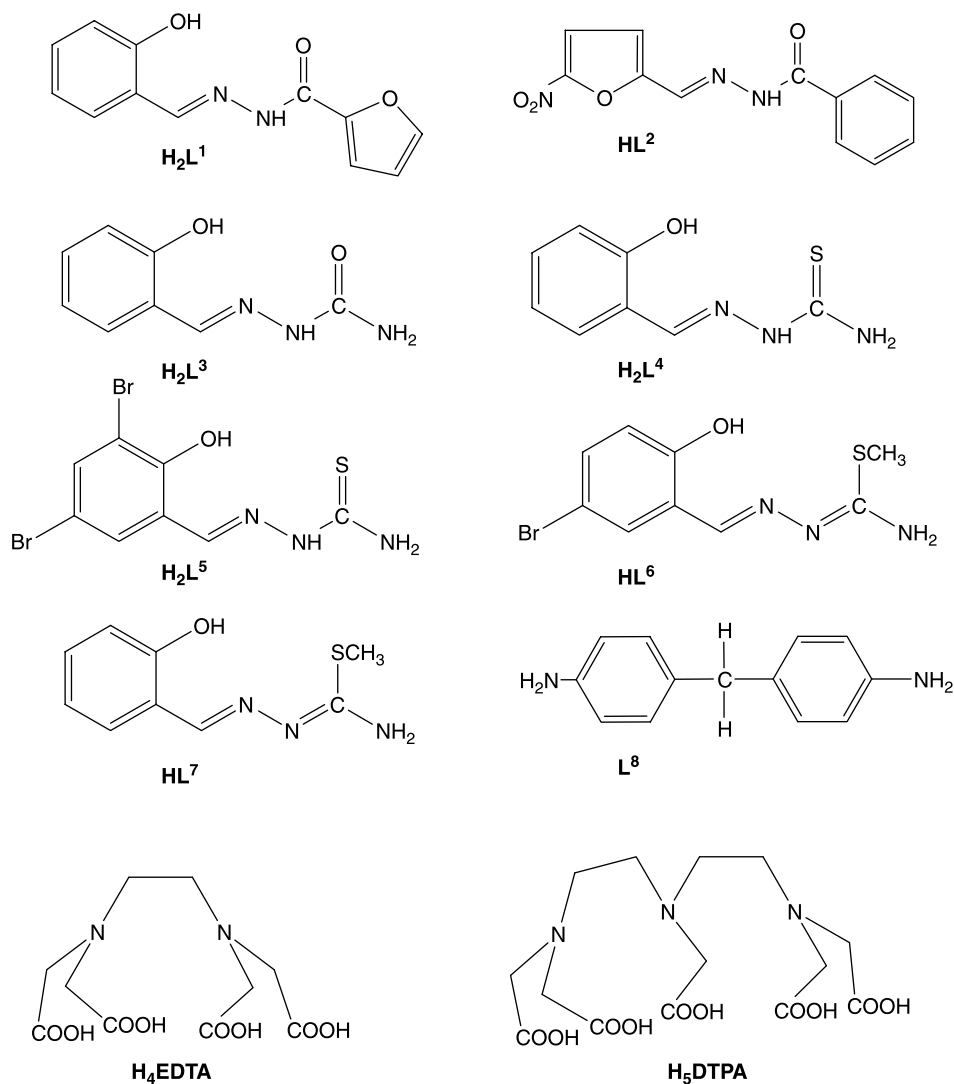


Figure 1. The chemical structure of organic ligands used in the preparation of metal complexes I-XXVII.

[13–15]. In continuation of this approach, the present paper describes the chemical synthesis, characterization and biological *in vitro* evaluation of different classes (ammoniacates, dioximates, carboxylates, semi- and thiosemicarbazidates) of Co(II), Co(III), Cu(II), Ni(II), Mn(II), Zn(II) and Fe(III) complexes. Schiff bases **H₂L¹** - **HL⁷** obtained from interaction of salicyl aldehydes and appropriate amine were used as tridentate ligands containing ONO, ONS or ONN as donor atoms (Figure 1). Diamine **L⁸**, **H₄EDTA** and **H₅DTPA** were also used as ligands. The composition and structure of synthesized complexes were analysed by elemental analysis, IR and NMR spectroscopies, magnetochemical, thermoanalytical and molar conductance measurements. All complexes were tested as inhibitors of human leukemia (HL-60) cells growth. The most potent, the Cu(II) complexes, have been also tested for their *in vitro* antibacterial activity against *Staphylococcus aureus* (Wood-46, Smith, 209-P), *Staphylococcus saprophyticus*,

Streptococcus faecalis, *Escherichia coli* (O-111), *Salmonella typhimurium*, *Salmonella enteritidis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Proteus mirabilis* and antifungal activity against laboratory stems *Aspergillus niger*, *Aspergillus fumigatus*, *Candida albicans* and *Penicillium*.

Materials and methods

General

All commercially available reagents and chemicals were of analytical- or reagent-grade purity and used as received. ¹H and ¹³C nuclear magnetic resonance (NMR) spectra were recorded at room temperature with a Bruker DRX 400 spectrometer. All chemical shifts (¹H, ¹³C) are given in ppm versus SiMe₄ using DMSO-d₆ as solvent. IR spectra were recorded on a Specord M80 and are reported in cm⁻¹. Classic methods were applied for C, H, N and Br elemental

analyses, which were performed at Academy of Sciences of Moldova (Institute of Chemistry). The complexes were analysed for their metal contents by EDTA titration [16]. TG/DT combined analyses were carried out using a SETARAM 92-1600 instrument. Each sample was deposited in a platinum crucible, which was heated ($60^{\circ}\text{C h}^{-1}$) in a current of air to allow evacuation of the products resulting from decomposition. Magnetic measurements were carried out on solid complexes using the Gouy's method [17].

Synthesis of Schiff's bases (H_2L^1 - HL^7)

General procedure with H_2L^1 . A hot solution (50°C) of salicylic aldehyde (10 mmol) in ethanol (25 mL) was added to a magnetically stirred solution of furoylhydrazine (10 mmol) in warm ethanol and the mixture was refluxed for 30 min. After completion of the reaction, the mixture was cooled and the solid residue was filtered, washed with cold ethanol, then with diethyl ether, and dried. Crystallization from ethanol gave H_2L^1 . The same method was applied for the synthesis of HL^2 - HL^7 by using corresponding benzoylhydrazine and 5-nitrofurfural (HL^2), salicylic aldehyde and semicarbazide (H_2L^3), salicylic aldehyde and thiosemicarbazide (H_2L^4), 3,5-dibromosalicylic aldehyde and thiosemicarbazide (H_2L^5), 5-bromo-salicylic aldehyde and S-methylthiosemicarbazide (HL^6), salicylic aldehyde and S-methylthio-semicarbazide (HL^7).

Salicylfuroylhydrazone (H_2L^1). Yield: 65%. IR (KBr): 3650 (m, OH), 3058 (m, NH), 1630 (m, C=O), 1586 (w, C=N), 1535 (m, NNH). ^1H NMR (DMSO- d_6): 12.28 (s, 1H, NNH), 8.74 (s, 1H, HC=N), 8.40 (s, 1H, OH), 7.88, 7.81, 6.75 and 6.74 (m, 4H, phenyl), 7.80, 7.27 and 6.74 (m, 3H, furan). ^{13}C NMR (DMSO- d_6): 152.4 (C=O), 150.5 (HC=N), 152.1 (C-OH), 135.9, 135.4, 121.2, 116.5 and 115.7 (phenyl), 151.9, 146.8, 114.5 and 114.5 (furan). Elemental analysis found for $\text{C}_{12}\text{H}_{10}\text{N}_2\text{O}_3$: C, 62.4; H, 4.3; N, 12.4; calculated: C, 62.6; H, 4.4; N, 12.1%.

5-Nitrofuroylidenbenzoylhydrazone (HL^2). Yield: 67%. IR (KBr): 3058 (m, NH), 1625 (m, C=O), 1585 (w, C=N), 1535 (m, NNH). ^1H NMR (DMSO- d_6): 12.26 (s, 1H, NNH), 8.41 (s, 1H, HC=N); 7.94, 7.92, 7.58, 7.56 and 7.64 (m, 5H, phenyl); 7.54 and 7.28 (d, 2H, $J=3$ Hz, furan). ^{13}C NMR (DMSO- d_6): 165.8 (C=O), 151.8 (HC=N), 135.5, 127.7, 127.3, 128.6, 128.3 and 132.2 (phenyl), 163.4, 151.9, 115.4 and 114.7 (furan). Elemental analysis found for $\text{C}_{12}\text{H}_9\text{N}_3\text{O}_4$: C, 55.9; H, 3.3; N, 16.2; calculated: C, 55.6; H, 3.5; N, 16.2%.

Salicylidensemicarbazone (H_2L^3). Yield: 87%. IR (KBr): 3600 (m OH), 3058 (m, NH), 1630 (m, C=O), 1586 (w, C=N), 1535 (m, NNH). ^1H NMR

(DMSO- d_6): 11.20 (s, 1H, NNH), 9.98 (s, 1H, OH), 8.35 (s, 1H, HC=N), 7.93 and 8.02 (1s 2H, NH_2), 8.20, 7.21, 6.85 and 6.80 (m, 4H, phenyl). ^{13}C NMR (DMSO- d_6): 197.6 (C=O), 154.4 (HC=N), 140.6 (C-OH), 116.2, 132.1, 121.4, 126.7 and 118.0 (phenyl). Elemental analysis found for $\text{C}_8\text{H}_9\text{N}_3\text{O}_2$: C, 59.3; H, 4.9; N, 23.4; calculated: C, 59.6; H, 5.1; N, 23.5%.

Salicylidenthiosemicarbazone (H_2L^4). Yield: 75%. IR (KBr): 3600 (m, OH), 3058 (m, NH), 1560 (s, C=S), 1586 (w, C=N), 1535 (m, NNH). ^1H NMR (DMSO- d_6): 11.39 (s, 1H, NNH), 9.88 (s, 1H, OH), 8.37 (s, 1H, HC=N), 7.93 and 7.91 (2s, 2H, NH_2), 8.20, 7.21, 6.85 and 6.80 (m, 4H, phenyl). ^{13}C NMR (DMSO- d_6): 177.6 (C=S), 156.4 (HC=N), 139.6 (C-OH); 116.0, 131.1, 120.4, 126.7 and 118.9 (phenyl). Elemental analysis found for $\text{C}_8\text{H}_9\text{N}_3\text{OS}$: C, 49.4; H, 4.9; N, 21.3; calculated: C, 49.2; H, 4.9; N, 21.5%.

3,5-Dibromosalicylidenthiosemicarbazone (H_2L^5). Yield: 72%. IR (KBr): 3650 (m, OH), 3058 (m, NH), 1560 (s, C=S), 1586 (w, C=N), 1535 (m, NNH), ^1H NMR (DMSO- d_6): 11.45 (s, 1H, NNH), 10.55 (s, 1H, OH), 8.29 (s, 1H, HC=N), 8.10 and 8.01 (2s, 2H, NH_2), 8.20 and 7.56, (2s, 2H, phenyl). ^{13}C NMR (DMSO- d_6): 178.5 (C=S), 155.4 (HC=N), 150.2 (C-OH), 118.1, 137.5, 111.2, 130.8 and 123.0 (phenyl). Elemental analysis found for $\text{C}_8\text{H}_7\text{Br}_2\text{N}_3\text{OS}$: C, 27.0; H, 2.1; N, 11.8; Br, 44.3; S, 9.3%; calculated: C, 27.2; H, 2.0; N, 11.9; Br, 45.3; S, 9.1%.

5-Bromosalicyliden-S-methylthiosemicarbazone (HL^6). Yield: 58%. IR (KBr): 3600 (m, OH), 3058 (m, NH), 1658 (s, C-S), 1586 (w, C=N). ^1H NMR (DMSO- d_6): 9.88 (s, 1H, OH), 8.34 (s, 1H, HC=N), 8.60 (s, 2H, NH_2), 8.20, 7.34 and 6.80 (m, 3H, phenyl); 2.10 (s, 3H, CH_3). ^{13}C NMR (DMSO- d_6): 160.6 (C-S), 154.2 (HC=N), 137.2 (C-OH), 118.0, 133.2, 111.4, 128.7 and 122.9 (phenyl), 7.9 (S- CH_3). Elemental analysis found for $\text{C}_9\text{H}_{10}\text{BrN}_3\text{OS}$: C, 37.4; H, 3.6; N, 14.5; calculated: C, 37.5; H, 3.5; N, 14.6%.

Salicyliden-S-methylthiosemicarbazone (HL^7). Yield: 65%. IR (KBr): 3600 (m, OH), 3058 (m, NH), 1586 (w, C=N). ^1H NMR (DMSO- d_6): 9.80 (s, 1H, OH), 8.30 (s, 1H, HC=N), 8.40 (s, 2H, NH_2), 8.11, 7.21, 6.90 and 6.80 (m, 4H, phenyl), 2.11 (s, 3H, CH_3). ^{13}C NMR (DMSO- d_6): 160.0 (C-S), 155.2 (HC=N), 137.0 (C-OH), 118.0, 130.2, 120.4, 131.7, 115.9 and 118.4 (phenyl), 7.9 (S- CH_3). Elemental analysis found for $\text{C}_9\text{H}_{11}\text{N}_3\text{OS}$: C, 51.7; H, 5.2; N, 20.3; calculated: C, 51.6; H, 5.3; N, 20.1%.

Bis(4-aminophenyl)methane (L^8). This ligand was commercially available.

Syntheses of metal complexes (Table I)

The reagents for the synthesis of **I** and **II** were Bi(HEDTA) · 2H₂O, obtained according to a published method [18], BaCO₃ and the two sulphates [Co(NxH)₂(An)₂]₂SO₄ · 5H₂O and [Co(NxH)₂(*p*-Tol)₂]₂SO₄ · 5H₂O. The dioximates were prepared by reaction of CoSO₄, NxH (1,2-cyclohexanedionedioxime) and the aromatic amine aniline (An) or *para*-toluidine (*p*-Tol) in a molar ratio of 1:2:3 in the presence of oxygen. The compounds **I** and **II** are crystalline substances stable in air, soluble in water, poorly soluble in alcohols, and insoluble in acetone and diethyl ether.

trans-[Co(NxH)₂(An)₂][Bi(EDTA)(H₂O)]₂ · 7H₂O (**I**). The complex Bi(HEDTA) · 2H₂O (1.068 g, 2 mmol) was dissolved in water (50 mL) upon heating. BaCO₃ (0.197 g, 1 mmol) was added and the mixture heated with stirring for 1 h to give Ba[Bi(EDTA)]₂ solution, then [Co(NxH)₂(An)₂]₂SO₄ · 5H₂O (0.577 g, 1 mmol) (NxH₂ = nioxime = 1,2-cyclohexanedione dioxime) in water (20 mL) was added. BaSO₄ was filtered off, and the clear solution was allowed to stand at room temperature. Transparent and brownish crystals of **I** were separated by filtration, washed with ethanol (2–5 mL) and diethyl ether (2–3 mL), and dried in air up to the constant weight. Yield: 65%. Water content: Exp. (TGA) 7.5%, Calc. 7.33%.

trans-[Co(NxH)₂(*p*-Tol)₂][Bi(EDTA)] · 4H₂O (**II**). Red-brown crystals of complex **II** were obtained as above by reaction of [Co(NxH)₂(*p*-Tol)₂]₂SO₄ · 5H₂O and Ba[Bi(EDTA)]₂. Yield: 52%. Water content: Exp. (TGA) 6.5%, Calc. 6.41%.

trans-[Co(NH₃)₄(NO₂)₂][Bi(EDTA)(H₂O)] · 2H₂O (**III**). A solution containing *trans*-[Co(NH₃)₄(NO₂)₂]₂SO₄ (0.534 g, 1 mmol) dissolved in a minimum of hot water was added with vigorous stirring to an aqueous solution of Ba[Bi(EDTA)]₂ (prepared from 1.068 g, 2 mmol, of Bi(HEDTA) · 2H₂O as described in literature [18]) and BaCO₃ (0.197 g, 1 mmol). After removal of precipitated BaSO₄, the solution was allowed to stand overnight. The resulting yellow crystalline product was collected by filtration, washed with ethanol and then dried in air. This complex is soluble in water but insoluble in alcohols, acetone, or diethyl ether. It can be recrystallized from water solution without change in composition. Yield: 80%.

[Co(NH₃)₅NCS][Bi(EDTA)]₂ · 4H₂O (**IV**). The complex **IV** was prepared by reaction of Ba[Bi(EDTA)]₂ and [Co(NH₃)₅NCS]SO₄ · 2H₂O in aqueous solution. Bi(HEDTA) · 2H₂O (1.068 g,

2 mmol) was dissolved in hot water (80 mL) and BaCO₃ (0.197 g, 1 mmol) was added. After complete dissolution, a solution of [Co(NH₃)₅NCS]SO₄ · 2H₂O (0.334 g, 1 mmol) of water (20 mL) was added with stirring. The precipitated BaSO₄ was removed by filtration. Ethanol was added and the solution was allowed to stand for 24 h. [Co(NH₃)₅NCS][Bi(EDTA)]₂ · 4H₂O crystallized as orange needles from a clear solution. The compound was recrystallized from hot water and dried in air. Yield: 72%.

[Co₂(-H₂O)(-CCl₃COO)₂(CCl₃COO)₂(H₂O)₄] · H₂O (**V**), [Mn₂(-H₂O)(-CCl₃COO)₂(CCl₃COO)₂(H₂O)₄] · H₂O (**VI**) and [Zn(CF₃COO)₂(H₂O)₂] · 2H₂O (**VII**). Non-symmetric Co(II), Mn(II) trichloroacetate complexes were prepared as described in the literature [19,20].

[Fe(L⁶)(H₂O)₃]₂SO₄ · or · 2H₂O (**VIII**). This complex was prepared according to the literature [21].

Compounds IX–XIII. The complexes of type M(HL¹)₂ · nH₂O (M = Mn²⁺, Co²⁺, Ni²⁺, Zn²⁺ and Cu²⁺; n=0–3) were synthesised from metal acetates and H₂L¹ in the presence of ammonium hydroxide solution (pH = 8) [22,23].

[Co(L⁷)(H₂O)₃]₂SO₄ · 2H₂O (**XIV**) and [Cu(L²)₂] · 2H₂O (**XV**). These complexes were obtained as described in the literature [21].

{[Cu(HL³)(H₂O)Bi(EDTA)(H₂O)] · 4H₂O}₂ (**XVI**). A solution of [Cu(HL³)(H₂O)]₂SO₄ · 2H₂O (0.521 g, 0.8 mmol), prepared as described in literature [24], in distilled water (50 mL) was added to a solution of Ba[Bi(EDTA)]₂ (0.8 mmol) obtained upon reacting Bi(EDTA) · 2H₂O (0.854 g, 1.6 mmol) with BaCO₃ (0.158 g, 0.8 mmol) in water (25 mL). BaSO₄ was filtered off and the resulting solution, after heating for half an hour on a water bath, was filtered hot and left for two days. The light-green crystals were collected by filtration, washed with water and ethanol prior to be dried. Yield: 75%.

[Cu(HL³)(H₂O)]₂[Bi(DTPA)] · 10H₂O (**XVII**). In a 100 mL container, [Cu(HL³)(H₂O)]₂SO₄ · 2H₂O (0.521 g, 0.8 mmol) was dissolved in deionized water (50 mL). Separately, BiH₂DTPA · 2H₂O (0.508 g, 8 mmol) [18,24] was dissolved in distilled water (25 mL) under stirring and heating, and BaCO₃ (0.158 g, 8 mmol) was gradually added to this clear solution. The second solution was then added

Table I. Physical and analytical data of the metal complexes I-XXVII.

No	Metal complex and molecular formula ^a	Mr ^b	μ eff ^c (B.M.)	C, H, N, calc (found) (%)	M (3d) ^d (%)	IR band (cm ⁻¹)	η ^e (%)	T dec. ^f (°C)
I	<i>trans</i> -[Co(NxH) ₂ (An) ₂] ₂ [Bi(EDTA)(H ₂ O)] ₂ · 7H ₂ O C ₃₄ H ₅₃ BiCoN ₈ O ₁₆	1106	dia	36.9(37.0); 4.8(4.7); 10.1(9.8)	5.3(5.5)	1750 $\nu_{as}(\text{C—O})_{\text{COO}}$; 1330 $\nu_s(\text{C—O})_{\text{COO}}$; 1080; 1055 ν (C—N, CN); 460 (M-O); 400 (M-N)	65	490
II	<i>trans</i> -[Co(NxH) ₂ (<i>p</i> -Tol) ₂] ₂ [Bi(EDTA)]·4H ₂ O C ₃₆ H ₅₆ BiCoN ₈ O ₁₆	1125	dia	38.4(38.1); 5.0(4.6); 9.9(9.5)	5.2(5.3)	1750 $\nu_{as}(\text{C—O})_{\text{COO}}$; 1330 $\nu_s(\text{C—O})_{\text{COO}}$; 1080; 1055 $\nu(\text{C—N, CN})$; 460 (M-O); 400 (M-N)	52	480
III	<i>trans</i> -[Co(NH ₃) ₄ (NO ₂) ₂] ₂ [Bi(EDTA)(H ₂ O)]·2H ₂ O C ₁₀ H ₃₀ [BiCoN ₈ O ₁₅	770	dia	15.6(15.8), 3.9(3.9), 14.5(14.8)	7.7(7.6)	1750 $\nu_{as}(\text{C—O})_{\text{COO}}$; 1330 $\nu_s(\text{C—O})_{\text{COO}}$; 1080; 1055 $\nu(\text{C—N, CN})$, 1355 ν (N—O) _{NO₂} ; 460 (M-O); 400 (M-N)	80	480
IV	[Co(NH ₃) ₅ NCS](Bi(EDTA)) ₂ ·4H ₂ O C ₂₁ H ₄₇ Bi ₂ CoN ₁₀ O ₂₀ S	1326	dia	19.9(20.1); 3.7(3.6); 11.0(11.2)	4.6(4.7)	1750 $\nu_{as}(\text{C—O})_{\text{COO}}$; 1330 $\nu_s(\text{C—O})_{\text{COO}}$; 1080; 1055 $\nu(\text{C—N, CN})$; 460 (M-O); 400 (M-N)	72	490
V	[Co ₂ (-H ₂ O)(μ -CCl ₃ COO) ₂ (CCl ₃ COO) ₂ (H ₂ O) ₄]·H ₂ O C ₈ H ₁₂ Co ₂ O ₁₄ Cl ₁₂	896	4.8	10.6(10.2); 1.3(1.4)	13.5(13.1)	1750 $\nu_{as}(\text{C—O})_{\text{COO}}$; 1330 $\nu_s(\text{C—O})_{\text{COO}}$; 460 (M-O)	85	350
VI	[Mn ₂ (-H ₂ O)(μ -CCl ₃ COO) ₂ (CCl ₃ COO) ₂ (H ₂ O) ₄]·H ₂ O C ₈ H ₁₂ Mn ₂ O ₁₄ Cl ₁₂	868	5.5	11.1(11.0); 1.4(1.3)	12.7(12.9)	1750 $\nu_{as}(\text{C—O})_{\text{COO}}$; 1330 $\nu_s(\text{C—O})_{\text{COO}}$; 460 (M-O)	70	360
VII	[Zn(CF ₃ COO) ₂ (H ₂ O) ₂]·2H ₂ O C ₄ H ₈ ZnO ₈ F ₆	361	dia	13.2(13.4); 2.2(2,1)	17.9(18.0)	1750 $\nu_{as}(\text{C—O})_{\text{COO}}$; 1330 $\nu_s(\text{C—O})_{\text{COO}}$; 460 (M-O)	92	320
VIII	[Fe(HL ⁶)(H ₂ O) ₃]SO ₄ ·2H ₂ O C ₉ H ₁₉ FeN ₃ O ₁₀ SBr	496	5.8	21.8(22.0); 3.8(3.7); 8.5(8.8)	11.3(11.0)	1600 (C = N); 1510 (NNH); 460 (M-O); 400 (M-N)	72	420
IX	[Mn(HL ¹) ₂]·2H ₂ O C ₂₄ H ₂₂ MnN ₄ O ₈	547	5.9	52.6(52.1); 4.0(3.9); 10.2(10.5)	10.0(10.1)	1560 (C = NN); 460 (M-O); 400 (M-N)	56	445
X	[Co(HL ¹) ₂]·3H ₂ O C ₂₄ H ₂₄ CoN ₄ O ₉	569	4.9	50.6(50.2); 3.9(3.7); 9.8(9.7)	10.4(10.0)	1560 (C = NN); 460 (M-O); 400 (M-N)	68	460
XI	[Ni(HL ¹) ₂]·2H ₂ O C ₂₄ H ₂₂ NiN ₄ O ₈	551	3.1	52.3(52.7); 4.0(3.8); 10.2(10.5)	10.7(10.2)	1560 (C = NN); 460 (M-O); 400 (M-N)	71	450
XII	[Zn(L ¹) ₂] C ₂₄ H ₁₈ ZnN ₄ O ₆	521	dia	55.3(55.6); 3.5(3.4); 10.7(10.9)	12.1(12.0)	1560 (C = NN); 460 (M-O); 400 (M-N)	85	475
XIII	[Cu ₂ (HL ¹) ₂]·2H ₂ O C ₂₄ H ₂₀ Cu ₂ N ₄ O ₈	619	1.6	46.5(46.4); 3.2(3,1); 9.0(8.8).	20.4(20.3)	1560 (C = NN); 460 (M-O); 400 (M-N)	65	400
XIV	[CoL ⁷ (H ₂ O) ₃] ₂ SO ₄ ·2H ₂ O C ₁₈ H ₃₆ Co ₂ N ₆ O ₁₄ S ₄	774	5.2	27.9(28.3); 4.7(4.5); 10.8(10.9)	15.2(15.0)	1600 (C = N); 1510 (NNH); 460 (M-O); 400 (M-N)	68	380
XV	[Cu(L ²) ₂]·2H ₂ O C ₂₄ H ₂₂ CuN ₆ O ₁₀	617	1.9	46.7(46.9); 3.6(3.9); 13.6(13.8)	10.2(10.0)	400 (M-N)	70	410
XVI	{[Cu(HL ³)(H ₂ O)Bi(EDTA)(H ₂ O)]·4H ₂ O} ₂ C ₃₆ H ₆₄ Bi ₂ Cu ₂ N ₁₀ O ₃₂	1689	1.9	25.6(25.4); 3.8(3.4); 8.3(8.2)	7.5(7.2)	1648 $\nu(\text{C} = \text{O})_{\text{SSA}}$; 1628 $\nu(\text{C} = \text{N})_{\text{SSA}}$; 1572 $\nu_{as}(\text{C—O})_{\text{COO}}$; 1391 $\nu_s(\text{C—O})_{\text{COO}}$; 1252, $\nu(\text{CO})$ phenolic; 1117, 1087 ν (C—N) _{EDTA}	75	380
XVII	[Cu(HL ³)(H ₂ O)] ₂ [Bi(DTPA)]·10H ₂ O C ₃₀ H ₅₈ BiCu ₂ N ₉ O ₂₆	1296	1.9	27.8(27.1); 4.5(4.6); 9.7(8.9)	9.8(9.3)	1661 $\nu(\text{C} = \text{O})_{\text{SSA}}$; 1581 $\nu(\text{C} = \text{N})_{\text{SSA}}$; 1557 $\nu_{as}(\text{C—O})_{\text{COO}}$; 1393 $\nu_s(\text{C—O})_{\text{COO}}$; 1301 $\nu(\text{CO})$ phenolic; 1124; 1088 $\nu(\text{C—N})_{\text{EDTA}}$	85	460
XVIII	[Cu(L ⁸) ₂]SO ₄ C ₂₆ H ₂₈ CuN ₄ O ₄ S	556	1.7	56.1(56.4); 5.0(5.2); 10.1(10.2)	11.3(11.1)	1600 (C = N); 1510 (NNH); 460 (M-O); 400 (M-N);	60	460

Table I – continued

No	Metal complex and molecular formula ^a	Mr ^b	μ eff ^c (B.M.)	C, H, N, calc (found) (%)	M (3d) ^d (%)	IR band (cm ⁻¹)	η ^e (%)	T dec. ^f (°C)
XXIX	[Cu(HL ⁴)(H ₂ O)][Bi(EDTA)]·H ₂ O C ₁₈ H ₂₄ BiCuN ₅ O ₁₁ S	790	1.8	25.5(25.4); 3.8(3.9); 8.9(8.8)	8.0(7.6)	1606 ν (C = N) _{TSSA} ; 1575 ν_{as} (C-O) _{COO} ; 1359 ν_s (C-O) _{COO} ; 1283 ν (CO)phenolic; 1108; 1090 ν (C–N) _{EDTA}	52	480
XX	[Cu(HL ⁴ H ₂ O) ₂][BiDTPA]·6H ₂ O C ₃₀ H ₅₀ BiCu ₂ N ₉ O ₂₀ S ₁	1256	1.8	28.6(27.7); 4.0(4.3); 10.0(10.3)	10.1(9.7)	1602 ν (C = N) _{TSSA} ; 1570 ν_{as} (C-O) _{COO} ; 1369 ν_s (C-O) _{COO} ; 1294 ν (CO) phenolic; 1081 ν (C–N) _{EDTA}	46	475
XXI	[Cu(HL ⁵ Cl)] C ₈ H ₆ Br ₂ ClCuN ₃ OS	451	1.9	21.3(21.0); 1.3(1.1); 9.3(9.1) Br 35.4(35.6)	14.2(13.8)	1610 (C = N); 455 (Cu-O); 400 (Cu-N); 320 (Cl-O); 300 (Cu-S)	79	470
XXII	[Cu(HL ⁵ Br)] C ₈ H ₆ Br ₃ CuN ₃ O ₂ S	513	1.8	18.7(18.5); 1.6(1.3); 8.2(8.1); Br 46.7(46.5)	12.5(12.4)	1618 (C = N); 455 (Cu-O); 400 (Cu-N); 300 (Cu-S); 250 (Cu-Br)	85	460
XXIII	[Cu(HL ⁵ (NO ₃))] C ₈ H ₆ Br ₂ CuN ₄ O ₄ S	480	2.0	20.0(19.9); 1.2(1.0); 11.7(11.6); Br 33.5(33.2)	13.4(13.2)	1612 (C = N); 455 (Cu-O); 400 (Cu-N); 300 (Cu-S)	80	425
XXIV	[Cu(HL ⁵ (H ₂ O)) ₂ SO ₄] C ₁₆ H ₂₂ Br ₄ Cu ₂ N ₆ O ₁₁ S	1016	2.1	18.9(18.6); 2.2(2.0); 8.3(8.0); Br 31.4(31.1)	12.6(12.3)	1612 (C = N); 455 (Cu-O); 400 (Cu-N); 300 (Cu-S)	82	430
XXV	[Cu(L ⁶ (NO ₃))] C ₉ H ₁₀ CuN ₄ O ₄ S	333	1.8	32.4(32.0); 3.0(3.3); 16.8(16.5)	19.1(19.5)	1612 (C = N); 455 (Cu-O); 400 (Cu-N);	81	420
XXVI	[Cu(HL ⁴ (thio)) ₂ SO ₄]·H ₂ O C ₁₈ H ₂₆ Cu ₂ N ₁₀ O ₇ S ₅	780	1.6	27.7(27.5); 3.3(3.1); 17.9(18.1)	16.1(16.4)	1600 (C = N); 1510 (NNH); 460 (M-O); 400 (M-N); 300 (M-S)	72	450
XXVII	[Cu(HL ⁴ (H ₂ O)) ₂ SO ₄]·3H ₂ O C ₁₆ H ₂₆ Cu ₂ N ₆ O ₁₁ S ₃	700	1.7	27.4(27.0); 3.7(3.9); 12.0(12.3)	18.0(18.4)	1600 (C = N); 1510 (NNH); 460 (M-O); 400 (M-N); 300(M-S)	85	435

^aThe chemical structure of ligands **H₂L¹-L⁸**, H₄EDTA and H₅DTPA used in the preparation of complexes are reported in Figure 1.

^bMr: relative molecular masse; ^c μ_{eff} : magnetic moment; ^dM (3d): metal 3d; ^e η : yield; ^fT_{dec}: decomposition temperature

dropwise to the first one. BaSO₄ was filtered off and the resulting solution was left for crystallization at room temperature. The light-green crystalline powder was collected by filtration, washed with water and ethanol prior to be dried. Yield: 85%.

[Cu(L⁸)₂]SO₄ (**XVIII**). This complex was prepared as previously described [25].

[Cu(HL⁴)(H₂O)][Bi(EDTA)] · H₂O (**XIX**). A solution containing [Cu(HL⁴)(H₂O)]₂SO₄ · 3H₂O (0.137 g, 0.2 mmol), prepared by reacting equimolar quantities of CuSO₄ · 5H₂O and H₂L⁴ in ethanol, in distilled water (150 mL) was added to a solution of Ba[Bi(EDTA)]₂ (0.2 mmol) obtained upon reacting Bi(HEDTA) · 2H₂O [18] (0.214 g, 0.4 mmol) with BaCO₃ (0.0395 g, 0.2 mmol) in water (25 mL). BaSO₄ was filtered off and the resulting green-bluish substance was collected by filtration, washed with water and ethanol prior to be dried. Yield: 52%.

[Cu(HL⁴H₂O)₂][BiDTPA] · 6H₂O (**XX**). A solution containing [Cu(HL⁴)(H₂O)]₂SO₄ · 3H₂O (0.1366 g (0.2 mmol), prepared by reacting equimolar quantities of CuSO₄ · 5H₂O and salicylidenthiosemicarbazone (H₂L⁴) in ethanol, in distilled water (150 mL) was added to a solution of Ba[Bi(DTPA)] (0.2 mmol), which was obtained upon reacting BiH₂DTPA · 2H₂O [26] (0.127 g, 0.2 mmol) with BaCO₃ (0.039 g, 0.2 mmol) in water (25 mL). BaSO₄ was filtered off and the resulting green powder was collected by filtration, washed with water and ethanol prior to be dried. Yield: 46%.

[Cu(HL⁵)Cl] (**XXI**). To a solution of Cu(II) chloride (10 mmol) in ethanol (30 mL), heated (50–55°C) and mixed continuously with a magnetic agitator, was added a solution of 3,5-dibromosalicyliden-thiosemicarbazone (H₂L⁵) (10 mmol) in ethanol (120 mL) and the mixture was heated for 30–40 min. After cooling, the small green crystals formed from the reaction mixture were filtered on glass filter, washed with ethanol and diethyl ether, and dried in air. Yield: 81%.

[Cu(HL⁵)Br] (**XXII**), [Cu(HL⁵)NO₃] (**XXIII**) and [Cu(HL⁵)(H₂O)]₂SO₄ (**XXIV**). According to the method reported above for **XXI** and using Cu(II) bromide, Cu(II) nitrate trihydrate or Cu(II) sulphate pentahydrate with 3,5-dibromosalicylic aldehyde thiosemicarbazone as initial substances in the 1:1 molar ratio, we synthesized **XXII**, **XXIII** and **XXIV**, respectively. Yields are 85% for **XXII**, 79% for **XXIII** and 80% for **XXIV**.

[Cu(HL⁴)NO₃] (**XXV**), [Cu(HL⁴)(thio)]₂SO₄ · H₂O (**XXVI**) and (Cu(HL⁴)(H₂O))₂SO₄ (**XXVII**). These complexes were prepared as previously described [25].

Antileukemia bioassay (Table II)

Cell culture. Human promyelocytic leukemia cells HL-60 (ATCC, Rockville, MD, USA) were routinely grown in suspension in 90% RPMI-1640 (Sigma, Saint Louis, USA) containing L-glutamine (2 nM), antibiotics (100 IU penicillin/mL, 100 µg streptomycin/mL) and supplemented with 10% (v/v) foetal bovine serum (FBS), in a 5% CO₂ humidified atmosphere at 37°C. Cells were currently maintained twice a week by diluting the cells in RPMI 1640 medium containing 10% FBS.

Cell proliferation assay. The cell proliferation assay for complexes **I–XXVII** and ligands 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 cells. In brief, triplicate cultures of 10,000 cells in a total of

Table II. Antiproliferative activity of complexes **I–XXVII** on human leukemia (HL-60) cells at three concentrations.

Complex ^a	Inhibition of cell proliferation (%) ^b		
	10 µM	1 µM	0.1 µM
I	–	17	5
II	–	18	4
III	35	30	15
IV	28	15	10
V	88	40	20
VI	–	14	16
VII	–	14	17
VIII	–	0	0
IX	–	20	10
X	–	20	7
XI	–	17	7
XII	–	4	0
XIII	–	12	0
XIV	–	5	0
XV	–	17	0
XVI	20	0	0
XVII	90	5	0
XVIII	–	15	12
XIX	97	92	10
XX	99	99	5
XXI	–	30	8
XXII	–	20	5
XXIII	–	22	6
XXIV	–	60	8
XXV	–	10	0
XXVI	87	88	10
XXVII	88	50	4
Doxorubicin	99	98	15

^aThe molecular formula of complexes are reported in Table I.

^bSEM < ± 4% of a single experiment in triplicate.

Table III. Minimum inhibitor concentration (MIC) and minimum bactericide concentration (MBC) in $\mu\text{g/mL}$ for complexes **XXI-XXIV** in comparison with Furaciline.

Microorganism strain		Complex ^a				Furaciline	
		XXI	XXII	XXIII	XXIV		
<i>Staphylococcus aureus</i>	Wood-46	MIC	0.145	0.141	0.145	0.145	9.35
		MBC	0.58	0.58	0.145	0.58	18.7
	Smith	MIC	0.145	0.018	0.036	0.072	9.35
		MBC	0.58	0.29	0.145	0.145	9.35
	209-P	MIC	0.29	0.29	0.072	0.29	18.7
		MBC	0.58	1.16	0.145	0.58	37.5
<i>Staphylococcus saprophyticus</i>	MIC	0.29	0.29	0.072	0.145	9.35	
	MBC	0.29	0.58	0.29	0.58	18.7	
<i>Streptococcus faecalis</i>	MIC	0.036	0.018	0.58	1.16	37.5	
	MBC	0.145	0.072	0.58	4.67	75	
<i>Escherichia coli</i> (O-111)	MIC	1.16	4.67	9.35	2.33	18.7	
	MBC	37.5	4.67	9.35	0.58	37.5	
<i>Salmonella typhimurium</i>	MIC	0.145	0.145	0.145	0.58	75	
	MBC	18.7	9.35	4.67	2000	150	
<i>Salmonella enteritidis</i>	MIC	0.145	0.145	0.145	1.16	9.35	
	MBC	37.5	75	9.35	2000	9.35	
<i>Klebsiella pneumoniae</i>	MIC	0.29	0.145	0.145	0.29	> 300	
	MBC	2.33	0.29	0.29	300	> 300	
<i>Pseudomonas aeruginosa</i>	MIC	2000	300	300	1000	> 300	
	MBC	2000	2000	2000	> 4000	> 300	
<i>Proteus vulgaris</i>	MIC	2.33	4.67	75	2.33	150	
	MBC	300	75	> 300	1000	300	
<i>Proteus mirabilis</i>	MIC	0.145	0.145	0.29	1.16	150	
	MBC	300	75	300	2000	300	

^aThe molecular formula of complexes are reported in Table I.

100 μL medium in 96-well microtiter plates (Becton Dickinson and Company, Lincoln Park, NJ, USA) were incubated at 37°C, 5% CO_2 . Compounds were dissolved in ethanol to prepare the stock solution of 1×10^{-2} M. These compounds and doxorubicin (Novapharm, Toronto, Canada), as a positive control, were diluted at multiple concentrations with culture media, added to each well and incubated for 3 days. Following each treatment, MTS (20 μL) was added to each well and the mixture incubated for 4 h. MTS is converted to water-soluble colored formazan by dehydrogenase enzymes present in metabolically active cells. Subsequently, the plates were read at 490 nm using a microplate reader (Molecular Devices, Sunnyvale, CA).

Antibacterial bioassay (Table III)

The antibacterial activity of complexes **XXI-XXIV** and also of their prototype Furaciline was determined under liquid nutritive environment [2% of peptonate bullion (pH 7.0)] using successive dilutions method [11,12]. *Staphylococcus aureus* (Wood-46, Smith, 209-P), *Staphylococcus saprophyticus*, *Streptococcus faecalis*, *Escherichia coli* (O-111), *Salmonella typhimurium*, *Salmonella enteritidis*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Proteus mirabilis* standard stems were used as reference culture for *in vitro* experiment. 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 method previously reported [27].

Antifungal bioassay (Table IV)

Antimycotic properties of the complexes **XXI-XXIV** were investigated *in vitro* on laboratory stems: *Aspergillus niger*, *Aspergillus fumigatus*, *Candida albicans* and *Penicillium*. The activity was determined in liquid Sabouroud nutritive environment (pH 6.8). The inoculates were prepared from fungi stems which were harvested during 3–7 days. Their concentration in suspension is $(2-4) \times 10^6$ colonies forming units/mh. Sowings for levures and micelles were incubated at 37°C during 7 and 14 days, respectively.

Results and discussion

Chemistry

The heterometallic bismuth(III) complexes containing cobalt(III) dioximates (**I** and **II**) or ammoniacates (**III** and **IV**) are of cation-anion structure (Figure 2). The complex **I** is an unique example, which differs structurally from other $\text{Bi}(\text{EDTA})^-$ complexes by

Table IV. Antimicrobial activity (MIC / MBC) in $\mu\text{g}/\text{mL}$ for complexes **XXI-XXIV** in comparison with Nistatine.

Complex ^a	Fungi type			
	<i>Aspergillus niger</i>	<i>Aspergillus fumigatus</i>	<i>Candida albicans</i>	<i>Penicillium</i>
Nistatine	240/240	240/240	80/80	80/80
XXI	300/300	37.5/37.5	18.7/18.7	150/150
XXII	300/300	37.5/37.5	37.5/37.5	37.5/37.5
XXIII	300/300	37.5/37.5	75/75	37.5/37.5
XXIV	150/150	37.5/37.5	300/300	18.7/18.7

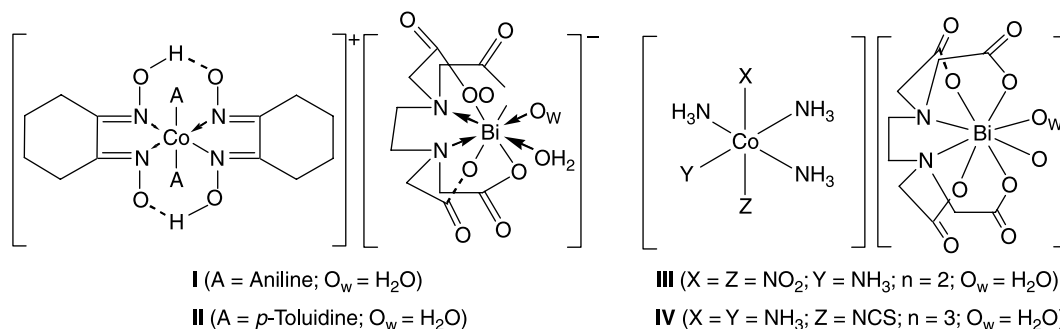
^aThe molecular formula of complexes are reported in Table I.

formation of a bridge between the cationic and anionic moieties through an oxime oxygen atom. The influence of the minor changes in the cationic moiety on the anionic sub-lattice is the subject of a special interest in this class of compounds. The heterometallic complexes **I-IV** have been obtained by an exchange reaction of barium EDTA bismuthate(III) with corresponding cobalt(III) dioximate/ ammoniacate sulphates. After removal of barium sulphate the resulting solution contains cobalt(III) nioximate/ammoniacate complex cation and bismuth(III) EDTA complex anion in molar ratio 1:1. Initial complex dioximates were prepared by passing an intense flow of air through a mixture of cobalt(II) sulphate heptahydrate, 1,2-cyclohexanedionedioxime, and corresponding amine in methanol–water solution. Complexes **I-IV** are brown crystalline substances readily soluble in water or dimethylsulfoxide and insoluble in acetone or diethyl ether. The complexes are stable to storage in air under normal conditions and can be recrystallized from water without change of their composition.

The ¹H NMR spectra of diamagnetic complexes **I** and **II** are similar. The following signals are characteristic for the coordinated *p*-toluidine: 2.24 ppm for the CH₃ group, 5.48 ppm for the NH₂ group and two doublets at 6.49 and 7.02 ppm ($\mathcal{J}_{\text{HH}} = 7.7$ Hz) for the aromatic hydrogen atoms. A confirmation of the *trans*-configuration of complex cations in **I** and **II** in solution is provided by the observation of a large singlet ($\Delta H/2 \approx 20$ Hz) at

17.7 ppm corresponding for two symmetric hydrogen bonds [28]. These data are in concordance with X-ray analysis in solid state [29]. The signals from CH₂-groups of nioxime ligands were observed at 2.54 and 1.51 ppm (1.50 ppm for complex **II**) as strong singlets. The singlet signal at 3.26 ppm was identified as that of ethylene protons of EDTA ligand. The NCH₂CH₂N component of EDTA in the domain of fast exchange and the signal from this group is a singlet. The four acetate methylene protons give rise to an AB quartet system $\mathcal{J}_{\text{AB}} = 15.9$ Hz for **I**, and 15.6 Hz for **II** in the range 3.68–3.73 ppm.

The thermogravimetric investigation of **I-IV** in air (5 °C/min) revealed four major steps corresponding to dehydration, deamination, ligand pyrolysis and formation of inorganic residue. The dehydration step occurs in a wide range of temperature, from 50 °C up to 160–170 °C. Deamination (removal of aniline or *p*-toluidine) begins at 180–195 °C, but it is overlapped by the strong exothermic effect that begins near 220 °C and finishes around 480–490 °C. The investigations of pyrolysis products were carried out using X-ray powder diffraction. X-ray powder diffraction patterns of all four residues are identical. The final products were identified as the sellinite-type phase with a minor mixture of the cobalt (II, III) oxide Co₃O₄ (~5%). X-ray diffraction data of the sellinite-type phase were indexed on the basis of a body-centred cubic structure with $a = 10.190(2)$ Å. In accordance with literature [30] this is the Bi_{26-x}Co_xO_{40-δ}-phase with the metal (Bi:Co) ratio $\approx 1:1$.

Figure 2. Representation of complexes **I-IV**.

The complexes **V** and **VI**, (μ -aqua)di(μ -trichloroacetato)-tri(aqua)cobalt(II)-aqua-bis(trichloroacetato)cobalt(II) and (μ -aqua)di(μ -trichloroacetato)-tri(aqua)-manganese(II)-aqua-bis(trichloroacetato)manganese(II), have an asymmetric structure including two nonequivalent metal atoms (Co or Mn) up bridged by one water molecule and two μ -trichloroacetate anions [19,20]. Main IR features are in good agreement with the proposed structure. The effective magnetic moments of **V** and **VI**, 4.8 B.M. for cobalt(II) and 5.5 B.M. for manganese(II), are characteristic for an octahedral environment of central atoms, but the values are a little lower than those for a theoretical high spin state. This fact confirms a weak antiferromagnetic interaction in dimeric bimetallic complexes **V** and **VI**. The zinc trifluoroacetate complex **VII** is diamagnetic in $3d^{10}$ ground electronic state.

The complexes **IX–XIII** of type $[M(HL^1)_2] \cdot nH_2O$ ($M = Mn^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}$ and Cu^{2+} ; $n = 0–3$) were synthesised from metal acetates and H_2L^1 in the presence of ammonia (pH = 8). Physico-chemical properties and composition of these metal complexes are shown in Table I and their structures are represented in Figure 3. The magnetic moment of 4.9 B.M. is indicative of three unpaired electrons for cobalt (II) compound **X** with pronounced spin orbital interaction in an octahedral environment. The nickel(II) complex **XI** showed a μ_{eff} of 3.1 B.M., which corresponds to two unpaired electrons per nickel(II) ion for a six-coordinated configuration.

The manganese(II) compound **IX** with an effective magnetic moment of 5.9 B.M. is an example of 5 electrons in a high state of octahedral coordination. The zinc(II) complex **XII** is diamagnetic. The thermal stability and total decomposition temperature (t) of the metal complexes **IX–XII** is influenced by the nature of the central atom according to the following relation: $t (Co) \geq t (Ni) \geq t (Zn) > t (Mn)$. For complex **XIII**, a comparison of the IR spectra of the Schiff's base H_2L^1 [31–32] to their metal chelates indicated that H_2L^1 is coordinated to the metal atom mainly in a deprotonated way acting

in a tridentate ONO manner including a phenolic oxygen, an azomethine nitrogen and an amidic oxygen, then forming five and six atom rings with the central metal atom. In IR, a band that appears at 1560 cm^{-1} due to the azomethine group was shifted to lower frequency by 28 cm^{-1} indicating the participation of azomethine nitrogen in the complexation. A new band appearing at 460 cm^{-1} was assigned to $\nu(M-O)$ [33] whereas the absence of the band at 1635 cm^{-1} demonstrated that the oxygen has formed a coordinative bond with metal ions in an enolic form. A weak band at 400 cm^{-1} was assigned to $\nu(M-N)$. The room temperature magnetic moment of the solid copper(II) complex **XIII**, 1.6 B.M., demonstrates the anti-ferromagnetic spin-spin interaction through a dimeric complex association.

The heterobimetallic (Bi,Cu) complexes containing the salicylic aldehyde semicarbazone (**XVI** or **XVII**) or salicylic aldehyde thiosemicarbazone (**XIX** or **XX**) were prepared by reacting $[Cu(HL^3)(H_2O)]_2SO_4 \cdot 2H_2O$ or $[Cu(HL^4)(H_2O)]_2SO_4 \cdot 3H_2O$ with solutions of $Ba[Bi(EDTA)]_2$ or $Ba[Bi(DTPA)]$.

The coordinative compounds **XXI–XXIV** (figure 4) have been prepared in 79–85% yields by a reaction between hydrate of copper(II) chloride, bromide, nitrate or sulphate with 3,5-dibromosalicylic aldehyde thiosemicarbazone (H_2L^5) in the 1:1 molar ratio. The mechanism of the given reaction is connected with the addition of this ligand, which has the role of tridentate ONS ligand, to copper(II) ion. Chloride, bromide, nitrate or water occupies the fourth place in the inner coordination sphere. At the same time, the deprotonation of the phenol takes place in the reaction mixture. Complexes **XXI–XXIV** are stable in contact with air, poorly soluble in water and alcohol, soluble in dimethylformamide and dimethylsulfoxide, practically insoluble in diethyl ether. The composition and each structure have been determined from the elemental analysis, IR spectroscopy, magnetochemistry and thermogravimetry methods. By determining the molar electric conductivity in dimethylformamide, it was established that **XXI–XXIII** are

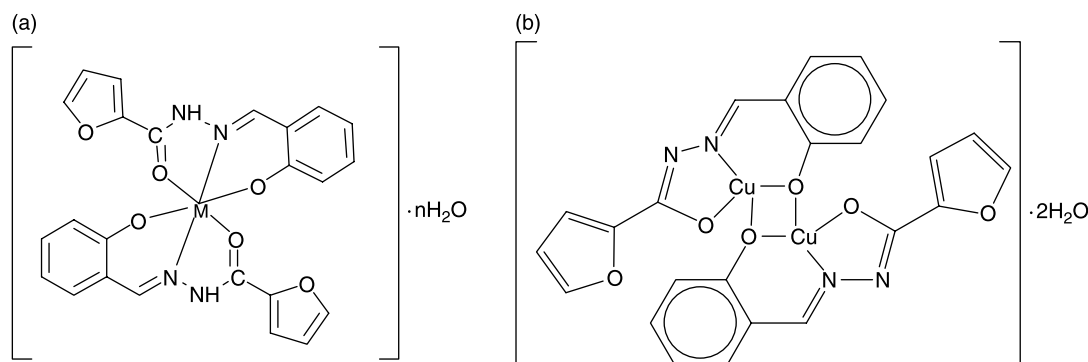


Figure 3. General structure of complexes **IX–XII** (a) and **XIII** (b).

non-electrolytes ($\mu_{20}^{1000} = 4-9 \text{ Ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$) whereas **XXIV** was a triple electrolyte ($\mu_{20}^{1000} = 149 \text{ Ohm}^{-1} \cdot \text{cm}^2 \cdot \text{mol}^{-1}$). According to the magnetochemical research of complexes at room temperature (293 K), the calculated values of their effective magnetic moment are close to the spin value for an uncoupled electron and represent 1.93 (**XXI**), 1.85 (**XXII**), 1.99 (**XXIII**) and 2.09 (**XXIV**) B.M. This fact allows us to suppose that the studied substances have a monomeric structure [17,34,35].

The comparative analysis of the IR spectra of the synthesized compounds and the ligand (3,5-dibromosalicylic aldehyde thiosemicarbazone) was made in order to determine the coordination mode of azomethine with copper(II) ion. It was established that the thiosemicarbazone in **XXI-XXIV** behaves as a monodeprotonated tridentate ONS ligand, connected to the central ion by a deprotonated phenolic oxygen atom, azomethine nitrogen and sulphur, forming metalocycles of five and six members. This fact finds the explanation in the disappearing of the $\delta(\text{OH})$ absorption band in the IR spectra, which can be observed in the free thiosemicarbazone in the range $1245-1240 \text{ cm}^{-1}$. In compounds **XXI-XXIV** and their structural analogues, $\nu(\text{C}=\text{N})$ absorption band is shifted by $35-30 \text{ cm}^{-1}$ to a smaller frequency [in starting thiosemicarbazone, $\nu(\text{C}=\text{N})$ is observed in the range of $1620-1610 \text{ cm}^{-1}$]. The mentioned coordination mode of 3,5-dibromosalicylic aldehyde thiosemicarbazone is supported by the appearance of a series of new absorption bands in the range $630-300 \text{ cm}^{-1}$, bands that according to the published data are detected as $\nu(\text{Cu}-\text{O})$, $\nu(\text{Cu}-\text{N})$ and $\nu(\text{Cu}-\text{S})$. Besides this, the confrontation of the absorption bands maxima, determined according to those described in [36], proves that the nitrate group in compound **XXIII** is coordinated to a central ion and behaves as a monodentate ligand in the interior coordination sphere [the intervals of the main oscillation frequencies coincide ($\nu_1(\text{A}_1) = 1295-1250$; $\nu_2(\text{A}_1) = 1035-970$; $\nu_4(\text{B}_1) = 1530-1480$; $\nu_6(\text{B}_2) = 800-780 \text{ cm}^{-1}$) and two weak absorption bands appear at 1780 and 1720 cm^{-1}]. However, the sulphate-ion in **XXIV** is placed in the exterior sphere. In fact, a single absorption band characteristic for this non-coordinated anion is observed in the range $1110-1120 \text{ cm}^{-1}$.

Thermal analysis of complexes **XXI-XXIV** showed that their thermolysis occurs in steps. An endothermic effect, which corresponds to the breaking of the crystallization water molecules (dehydration) of compounds **XXII** and **XXIV** in the temperature range of $75-96^\circ\text{C}$. In the case of **XXIV**, the process of deaquation occurs with endothermic effect at 155°C , but at 470 (**XXI**), 460 (**XXII**), 425 (**XXIII**) and 430 (**XXIV**) $^\circ\text{C}$, the complete thermooxidative destruction of the coordinated thiosemicarbazone with exothermic effect takes place.

Antileukemia activity

All 27 compounds were tested as inhibitors of HL-60 cells proliferation. These human promyelocytic leukemia cells were incubated for three days in the presence of synthetic compounds (ligands 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 ligands have insignificant inhibitor activity (data not shown), but some metal complexes selectively act in this biological process (Table II). 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. Among all the compounds tested, the most indicative are copper(II) complexes **XIX**, **XX**, **XXIV**, **XXVI** and **XXVII** and cobalt(II) complex **V**, which efficiently inhibited the HL-60 cell proliferation at $1 \mu\text{M}$.

The quite essential activity of copper complexes may be a consequence of Jahn-Teller distortion effect, which takes place along the dz^2 of copper(II) [37]. The activity of copper complexes is influenced also by the nature of the ligand donor atoms that are present in coordination polyhedra. In fact, complexes with coordinated ligand containing a sulphur donor atom (ONS) are essentially more active than complexes including inner sphere oxygen (ONO) or nitrogen (ONN) (figure 5).

In the case where a sulphur donor atom is blocked by a CH_3 group (as for ONN ligands **HL**⁶ and **HL**⁷), the biological activity of copper complex became insignificant as illustrated by a comparison of **XX** and **XXV**. If copper is capsulated in a dimeric complex as for **XIII** (Figure 3b) or polynuclear as for **XVI**, the cell growth inhibition dramatically change and became minimal. In the series of complexes $[\text{Cu}(\text{HL}^5)\text{Y}]$ (**XXI-XXIV**) having the same metal (copper) and tridentate ONS ligand **H₂L**⁵, the second inner sphere ligand Y (Figure 4), influence the biological activity in

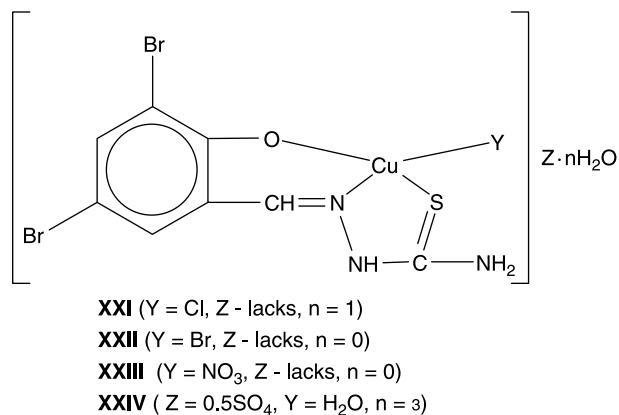


Figure 4. Representation of complexes **XXI-XXIV**.

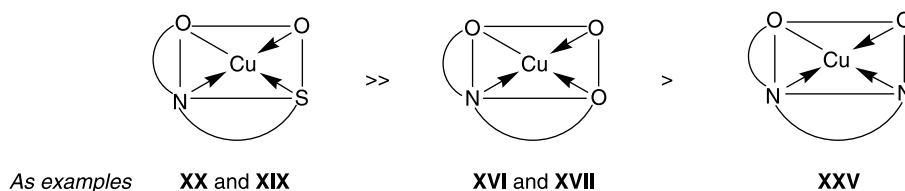


Figure 5. Copper(II) complexed by different ligand donor atoms (ONS, ONO and ONN).

the following order: H_2O (**XXIV**) > Cl^- (**XXI**) > Br^- (**XXII**) \cong NO_3^- (**XXIII**). In this case, the presence of an inner coordination sphere labile ligand, as a molecule of water, raises considerably (till 60%) the antiproliferative activity. Only one complex with a metal atom other than copper gave a significant inhibition of cell proliferation. Thus, the cobalt complex **V** inhibited 40% of cell growth at $1\ \mu\text{M}$. For the cobalt(II) and cobalt(III) octahedral complexes **V** and **X** with coordination number 6, the thermodynamic stability is also important as well as in the case of copper(II) complexes **XXI-XXIV**. In solutions, the most labile cobalt (II) complex **V** (Co^{2+} has the electronic configuration $3d^7$ with an inner coordination sphere lifetime of the ligand 10^{-4} to 10^{-5} s) is more active in comparison with the inert cobalt(III) complex **X** (Co^{3+} has the electronic configuration $3d^6$ with a lifetime 10^4 – 10^5 s) [38,39].

Antibacterial activity

Four copper(II) complexes have been screened for their *in vitro* antibacterial and antifungal activity. Experimental results, obtained from the study of antimicrobial activity of compounds **XXI-XXIV**, are given in Table III. As can be seen, they display bacteriostatic activity towards gram-positive and gram-negative bacteria in 0.018–2000 $\mu\text{g}/\text{mL}$ concentration. *Pseudomonas aeruginosa* is an exception, for which MIC is 300–4000 $\mu\text{g}/\text{mL}$. For comparison, we also presented the antimicrobial data characteristic for Furaciline, a bactericide used in medical practice. The experimental data prove that complexes **XXI-XXIV** display an antimicrobial activity of 16–1052 times higher towards *staphylococci* and *streptococci* than Furaciline and outruns by 16–517 times its bacteriostatic activity towards majority of gram-negative microorganisms. At the same time the mentioned compounds are of 4–9 times more active towards gram-positive bacteria and not less of 130 times – towards gram-negative microorganisms than their structural analogue (after MIC).

Antifungal activity

The experimental data obtained from the study of antimycotic properties of selected compounds **XXI-XXIV** are given in Table IV. They also display selective

activity towards investigated fungi stems in the concentration range of 18.7–300 $\mu\text{g}/\text{mL}$. In order to make a comparison, data regarding the activity of Nistatine, an antifungal agent used in medicine for mycose treatment, are also given in Table IV. The data show that the synthesized complexes display an antimycotic activity of 6.4–1.1 times higher towards majority fungi than Nistatine. The properties found for synthesized and studied coordinative compounds are of interest from the view point of the growth of the arsenal of antimicrobial and antimycotic remedies.

Conclusion

Metal complexes have been efficiently elaborated by reacting a series of 3d metal with different organic ligands. They have various geometrical and electronic structure, thermodynamic and thermal stabilities, magnetic and conductance properties. All complexes are octahedral except those of Cu which are of square planar or pyramidal geometry. Some copper, cobalt and manganese compounds have dimeric or polymeric structure. From our investigations we have deduced that there are three most indicative criteria for future synthesis of biological active coordination compounds from the view point of the inhibitors of HL-60 cells proliferation:

- Use of copper (II) planar or pyramidal complexes;
- Presence of sulphur donor atom in the ligand composition;
- Use of ONS - tridentate ligands.

Our future investigations will be directed on the synthesis of copper(II) complexes with tridentate ONS containing ligands.

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References

- [1] Galanski M, Arion VB, Jakupec MA, Keppler BK. *Current Pharm Design* 2003;9:2078.
- [2] Chohan ZH, Pervez H, Rauf A, Khan KM, Supuran CT. *J Enz Inhib Med Chem* 2006;21–193 (and articles cited herein).
- [3] Chohan ZH, Arif M, Shafiq Z, Yaqub M, Supuran CT. *J Enz Inhib Med Chem* 2006;21:95.
- [4] Chohan ZH, Hassan MU, Khan KM, Supuran CT. *J Enz Inhib Med Chem* 2005;20:183.
- [5] Chohan ZH, Supuran CT, Scozafava A. *J Enz Inhib Med Chem* 2004;19:79.
- [6] Chohan ZH, Scozafava A, Supuran CT. *J Enz Inhib Med Chem* 2003;18:259.
- [7] Panchal PK, Parekh HM, Pansuriya PB, Patel MN. *J Enz Inhib Med Chem* 2006;21–203 (and articles cited herein).
- [8] Stavila V, Davidovich RL, Gulea A, Whitmire KH. *Coord Chem Rev* 2006;250:2782.
- [9] Gulea A, Poirier D, Roy J, Țapcov V, Stavila V. Patent of invention MD 2786. BOPI 2005;6–28.
- [10] Gulea A, Poirier D, Roy J, Popovschi L, Țapcov V. Patent of invention MD 285. BOPI 2005;9–24.
- [11] Samusi NS, Prisacari VI, Țapcov VI, Buraciov SA, Gulya AP. *Pharm Chem J* 2004;38:373.
- [12] Gulya AP, Samusi NS, Prisacari VI, Țapcov VI, Buraciov SA, Spinu SN, Begenari NP, Poirier D, Roy J. *Pharm Chem J* 2007;41:114.
- [13] Hall IH, Lackey CB, Kistler TD, Durham RW, Jr, Jouad EM, Khan M, Thanh XD, Djebbar-Sid S, Benali-Baitich O, Bouet GM. *Pharmazie* 2000;55:937.
- [14] Baldini M, Belicchi-Ferrari M, Bisceglie F, Dall'aglio PP, Pelosi G, Pinelli S, Tarasconi P. *Inorg Chem* 2004;43:7170.
- [15] Belicchi FM, Bisceglie F, Pelosi G, Tarasconi P, Albertini R, Dall'aglio PP, Pinelli S, Bergamo A, Sava G. *J Inorg Biochem* 2004;8:30.
- [16] Schwarenbach G, Flaschka H. *Die komplexometrische titration*. Ferdinand Euke Verlag Stuttgart; 1965.
- [17] Russell S, Drago. *Physical methods in chemistry*. 2 W.B. Saunders Company 1977.
- [18] Summers SP, Abboud KA, Farrah SR, Palenik GJ. *Inorg Chem* 1994;3:88.
- [19] Shova SG, Gulya AP, Novitski GV, Mazus MD. *Proceeding of The XI-th International Conference "Physical Methods in Coordination Chemistry"*. Kishinev 1993;162.
- [20] Shova SG, Turyatke LG, Novitskii GV, Mazus MD, Gulya AP. *Russian J Coord Chem* 1996;22:485.
- [21] Ямпольская МА, Гэрбэлэу НВ, Зеленцов ВВ, Ревенко МД, Туртэ КИ, Индричан КМ, Куявская БЯ, Быркэ МС. Координационные соединения железа(III) с четырехдентатными изоселеносемикарбазидами // *Журн неорг химии* 1988;3(10):2559.
- [22] Țapcov V, Prisacari V, Birca M, Gulea A. *Anale Științifice ale Universității de Stat din Moldova. Seria, Științe chimico-biologice, Chișinău*. 2002;358.
- [23] Gulea A, Poirier D, Roy J, Țapcov V. Patent of invention MD submitted 2006.
- [24] Gerasimenko AV, Davidovich RL, Bulimestru IG, Gulea AP, Ng SW. *Acta Cryst* 2005;E61:m1816.
- [25] Birca M, Țapcov V, Gulea A. XXVIII-a Conferința Națională de Chimie. Rezumate P. Călimănești-Căciulata. Vâlcea. România; 2004. p 133.
- [26] Brechbiel MW, Gansow OA, Pippin CG, Rogers RD, Planalp RP. *Inorg Chem* 1996;35:6343.
- [27] Pershin GN. *Meditina (Moscow)* 1971;357.
- [28] Gulya AP, Simonov YA, Bologa OA. *Koordinatsionnaya Khimiya* 1988;14–201.
- [29] Stavila V, Gulea A, Shova S, Simonov YA, Petrenko P. *Inorg Chim Acta* 2004;357:2060.
- [30] Rangavitta NI, Guru Row TN, Rao TN. *Eur J Solid State Inorg Chem* 1994;31–409.
- [31] Mohan M, Gupta NS, Kumar A, Kumar M. *Inorg Chim Acta* 1987;135:167.
- [32] Mohan M, Kumar A, Kumar MK. *Inorg Chim Acta* 1987;136:65.
- [33] Bellamy LJ. *The infrared spectra of complex molecules*. New York: John Wiley; 1971.
- [34] Samusi N, Prisacari V, Țapcov V, Buraciov S. Patent of invention MD 678. BOPI 1997;2–47.
- [35] Samusi NS, Prisacari VI, Țapcov VI, Buraciov SA, Gulya AP. *Pharm Chem J* 2004;38:373.
- [36] Nakamoto K. *Infrared spectra of inorganic and coordination compounds*. New York: Willey Interscience: 1986.
- [37] Bersuker I. *The Jahn – Teller effect*. Cambridge University Press: 2006.
- [38] Riblet F, Novitchi G, Helm L, Gulea A, Merbach A. *Chimia* 2006;60:224.
- [39] Novitchi G, Gulea A, Riblet F, Helm L, Scopelliti R, Merbach AE. *Magnetic Res in Chemistry* 2004;42:801.