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PRECLINICAL STUDIES

Cytotoxic activities of new iron(III) and nickel(II) chelates of some *S*-methyl-thiosemicarbazones on K562 and ECV304 cells

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Summary The S-methyl-thiosemicarbazones of the 2-hydroxy-R-benzaldehyde (R= H, 3-OH 3-OCH_3 or 4-OCH_3) reacted with the corresponding aldehydes in the presence of $FeCl_3$ and $NiCl_2$. New *ONNO* chelates of iron(III) and nickel (II) with hydroxy- or methoxy-substitued N^1,N^4-diarylidene-*S*-methyl-thiosemicarbazones were characterized by means of elemental analysis, conductivity and magnetic measurements, UV-Vis, IR and ^{1}H-NMR spectroscopies. Cytotoxic activities of the compounds were determined using K562 chronic myeloid leukemia and ECV304 human endothelial cell lines by MTT assay. It was determined that monochloro N^1-4-methoxysalicylidene-N^4-4-methoxysalicylidene-*S*-methyl-thiosemicarbazidato-iron(III) complex showed selective anti-leukemic effects in K562 cells while has no effect in ECV304 cells in the 0.53 μg/ml (IC_{50}) concentrations. Also, some methoxy-substitued nickel(II) chelates exhibit high cytotoxic activitiy against both of these cell lines in low concentrations. Cytotoxicity data were evaluated depending on cell lines origin and position of the substituents on aromatic rings.

Keywords Thiosemicarbazone · Iron complex · Nickel complex · Cytotoxicity · MTT

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Introduction

Treatment of refractory and relapsed leukemias remains a challenge. While intensive multi-chemotherapeutic approaches have had some limited success, overall, there is a substantial need for developing and testing novel therapeutics. The blast crisis of chronic myelogenous leukemia (CML) is refractory and resistant to most forms of cancer chemotherapy.

Thiosemicarbazones have a wide range of pharmacological activities. After the antibacterial effect of thiosemicarbazide derivatives were reported [1, 2] thiosemicarbazones have raised considerable interest, and so numerous articles on biologic potential of various thiosemicarbazones have been published. Metal complexes of thiosemicarbazones are a class of compounds presenting some biological applications as antiviral, antibacterial and antitumour depending on the parent aldehyde, ketone and metal ion [3–5]. In the last 20 years, several thiosemicarbazone complexes having biological activity were synthesized, and in particular, the copper(II) complexes have been studied with regard to their antitumour potentials [6, 7]. Some palladium(II) complexes of thiosemicarbazones have antitumour properties [8–10] and they are also known as antiviral agents [11–14]. However, the biological activity thematic studies are related to S-alkyl-thiosemicarbazones [15–17] and their metal complexes are especially limited [18, 19]. Research on cytotoxic properties of thiosemicarbazone-metal complexes have been centralized to platinum and palladium chelates. The object of the published papers is the *ONS* and *NNS* chelators such as benzaldehyde, 2-acetyl pyridine, phenanthrenequinone, and 2-benzoylpyridine derivatives which have no substituent on sulphur atom of thiosemicarbazone [9, 10, 20–23].

One of the most effective thiosemicarbazone is triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone). Triapine that is inhibited ribonucleotide reductase which is

played role in replication of cancer cells carry on with phase I and II clinical trials for the treatment of various metastatic, solid cancers and myeloid leukemia. [23, 24] Based on last data, it may be said that Triapine has given hope to in future investigations [25–28].

Chronic myelogenous leukemia (CML) is a myeloproliferative disorder of pluripotent hematopoietic stem cells that produces the *BCR-ABL* fusion gene (The Philadelphia chromosome) [29]. Adult lymphoblastic leukemias had more favorable 5-year survival rates than chronic myeloid leukemia (ALL, 63%; CML, 38%) Treatment decisions in patients with CML are based on the patient's age and phase of the disease [30, 31]. The blastic phase is more agressive than chronic phase and resistant to drugs. Response rates to chemotherapy combinations are reported to be 20% in patients with nonlymphoid blastic phase and the median survivals are 3–6 months [29].

In our previous paper, we were the first to demonstrate cyctotoxic activites of the iron(III) and nickel(II) complexes of S-methylthiosemicarbazones with *ONNO* type, and determined that the iron(III) complex of N^1-3-methoxysalicylidene-N^4-4-methoxysalicylidene-S-methyl-thiosemicarbazone has efficiently cytotoxic activity for K562 chronic myeloid leukemia cell in 3.5 μg /ml (IC_{50}) and mildly proliferative activity for ECV304 human endothelial cell at the same concentration [19].

By study, we synthesized, characterized fourteen of iron(III) and nickel(II) complexes with N^1,N^4-diarylidene-*S*-methyl-thiosemicarbazones having H, 3-OH, 3-OCH_3 or 4-OCH_3 substituents on phenol rings in order to analyze whether the different substituents have caused different cytotoxic effect (Fig. 1). Cytotoxic effects of the *ONNO* templates were determined by MTT test for K 562 chronic myeloid leukemia and ECV 304 human umbilical vein endothelial cell lines.

Materials, methods

Materials, methods and apparatus All chemicals were of reagent grade and used as commercially purchased without further purification. The elemental analyses were determined on a Thermo Finnigan Flash EA 1112 Series Elemantar Analyser. UV-Vis. Spectra were obtained from ATI-Unicam UV-Visible Spectrometer UV2 Series. Infrared spectra of the compounds were recorded on KBr pellets with a Mattson 1000 FT-IR spectrometer. The ^{1}H-NMR spectra were recorded on Bruker AVANCE- 500 model spectrometer. Magnetic measurements were carried out at room temperature by the Gouy technique with an MK I model device obtained from Sherwood Scientific. The molar conductivities of the compounds were measured in 10^{-3}M DMSO solution at 25± 1°C using a digital WPA CMD 750 conductivity meter. The ESI-MS analyses were carried out in positive and negative ion modes using a Thermo Finnigan LCQ Advantage MAX LC/MS/MS. The mobile phase consisted of MeOH. Hypersil Betabasic-8 (5 μ, 100 mm×4.6 mm) column was used at a flowrate of 0.3 ml/min at 25°C. ESI-MS inlet conditions; in the positive ion mode: heated capillary temp., 200°C; vaporizer temp., 1.60°C; sheath gas flow rate, 40 units; capillary voltage, (−20)-(−45) V and tube lens offset, 20 V; in the negative ion mode: heated capillary temp., 270–290°C; vaporizer temp., 1.60°C; sheath gas flow rate, 40 units; capillary voltage, 20 V and tube lens offset, 20 V.

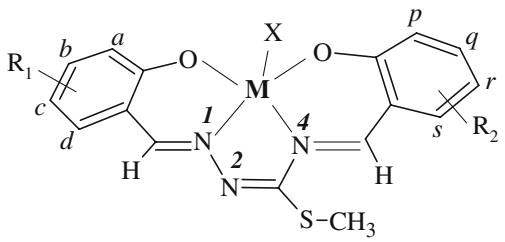

Fig. 1 The iron (**a,** where M/X = Fe/Cl) and nickel (**b,** where M/X = Ni/-) chelates R_1/R_2: H/3-OH **(Ia, Ib),** 3-OH/H **(IIa, IIb),** 3-OH/3-OH **(IIIa, IIIb)**, H/3-OCH_3 **(IVa, IVb),** 3-OCH_3/H **(Va, Vb),** 3-OCH_3/3-OCH_3 **(VIa, VIb),** 4-OCH_3/ 4-OCH_3 **(VIIa, VIIb)**

Cell cultures The K562 chronic myeloid leukemia cell line and ECV304 human umbilical vein endothelial cell line were purchased from ATTC. The cells were cultured in DMEM (for ECV304) and IMDM (for K562) medium (Sigma) supplemented with 10% fetal calf serum (GIBCO-BRL) and 1% penicillin-streptomycin. Experiments were conducted on cells seeded into 96-well culture plates at densities 10^5 cells/ml while maintaining the cells at 37°C in an atmosphere of 5% CO in air.

Cytotoxicity assay Cytotoxic effects of the compounds were evaluated by MTT (3-[4,5- dimethylthiazol -2-yl]-2,5 diphenyltetrazolium bromide) assay which is reduced by living cells to yield a soluble formazan product using the method of Mossman modified by our laboratory [32]. Stock solutions compounds were prepared at 5 mg/ml in DMSO. The six concentrations (50 μg/ml, 10 μg/ml, 5 μg/ml, 1 μg/ml, 0.1v, 0.01 μg/ml) were prepared from each compound and 10 μl was added to wells each one as triplicate. Then, K562 and ECV304 cells were plated at 10^4 cells/well and incubated for 3 days at 37°C and in 5% CO_2 atmosphere. Control wells were prepared no compound. At least 3 independent experiments were conducted. After incubation period, acidified medium was aspirated from wells and MTT was added to 10 μl at 5 mg/ml . Cells were incubated at 37°C for 3 h, after which time and they were subsequently lysed by addition of 100 μl of acidic (0.04 M HCl) isopropanol alcohol. Overnight, plates were stored protecting light for disolved formazan. The next day, optical density (OD) of formazan was measured with 560 nm test wavelength and a 620 nm reference wavelength by ELISA multiwell spectrophotometer

(Diagnostics Pasteur LP 400). The absorbance of the DMSO blank was subtracted from all values. Cytotoxicity index (CI) was calculated to following formula comparing to control: % CI (Cytotoxicity index) = 1- OD treated wells / OD control wells × 100.

Also, inhibitory concentration$_{50}$ (IC_{50} = the concentration of the compound that inhibited 50 % cells) was calculated from dose-response curves.

Statistical analysis was performed using the Statistical Package for Social Statistics (SPSS). Student's t test was used to compare K 562 cells to ECV 304 cells, $p<0.05$ considered significant differences.

Synthesis of N^1-arylidene-S-methyl-thiosemicarbazones (I–IV) The R-substitued N^1-arylidene-S-methyl-thiosemicarbazone derivatives [R: H (**I**), 3-OH (**II**), 3-OCH_3 (**III**), 4-OCH_3 (**IV**)] were synthesized from the corresponding salicylaldehyde, methyl iodide and thiosemicarbazide in equimolar ratios as the reported procedure [19, 33, 34]. The colour, yield (%), m.p. (°C), Rf value (stationary/mobile phase), elemental analysis, UV-visible (λ_{max} nm, in DMF), IR (KBr, cm^{-1}) and ^{1}H-NMR (DMSO-d_6, 25°C, δ ppm) data of **I–IV** were given as follows:

I: light yellow, 160-161, 97, 0.1279 ($CHCl_3/CH_2Cl_2$) ; Anal.Calc. for $C_9H_{11}N_3OS$ (209 g): C, 51.67; H, 5.26; N,20.09; S, 15.31, found: C, 51.75; H, 5.31; N, 20.07; S, 15.34%. UV-vis: 260, 307, 337. IR (cm^{-1}): ν_a(NH) 3457, ν_s(NH) 3280, ν(OH) 3052, δ(NH) 1635, ν(C=N^1), ν(N^2=C) 1616, 1605, ν(C-O) 1150. ^{1}H-NMR: δ 11.58, 10.83 (cis/trans ratio: 3/2, s, 1H, OH), 8.47, 8.34 (syn/anti ratio:1/2, s, 1H, CH=N^1), 6.94 (s, 2H, NH_2), 7.59, 7.39 (d-d, *J*= 7.44, 1H, *d*), 7.24 (t, *J*= 7.63, *J*= 7.84, 1H, *b*), 6.90 (t, *J*= 7.50, *J*= 7.61 1H, *c*), 6.87(s, 1H, *a*), 2.43, 2.40 (cis/trans ratio:3/2, s, 3H, S-CH_3).

II: beige, 175-176, 84, 0.2632 ($CHCl_3$/$CHCl_3$:MeOH, 20:1), Anal.Calc. for $C_9H_{11}N_3O_2S$ (225 g): C, 48.00; H, 4.89; N,18.66; S, 14.22, found: C, 48.25; H, 4.82; N, 18.59; S, 14.18%. UV-vis: 243, 315. IR (cm^{-1}): ν_a(NH) 3472, ν_s(NH) 3349, ν(OH) 3218, δ(NH) 1620, ν(C=N^1), ν(N^2=C) 1618, 1582, ν(C-O) 1162, 1139. ^{1}H-NMR: δ 11.59, 10.69 (cis/trans ratio: 5/2, s, 1H, OH), 9.05, 8.91 (cis/trans ratio: 3/7, s, 1H, R(OH)), 8.41, 8.28 (syn/anti ratio:3/7, s, 1H, CH=N^1), 6.88 (s, 2H, NH_2), 6.97-6.83 (d-d, *J*= 7.81, *J*= 1.46, 1H, *d*), 6.81-6.77 (d-d, *J*= 7.81, *J*= 1.46, 1H, *b*), 6.70 (t, 1H, *J*= 7.81, *c*), 2.44, 2.38 (cis/trans ratio:5/2, s, 3H, S-CH_3).

III: cream, 164-165, 93, 0.12 ($CHCl_3/CH_2Cl_2$), Anal.Calc. for $C_{10}H_{13}N_3O_2S$ (239 g): C, 50.21; H, 5.44; N,17.57; S, 13.39, found: C, 50.25; H, 5.42; N, 17.56; S, 13.40%. UV-vis: 248, 312. IR (cm^{-1}): ν_a(NH)3412, ν_s(NH) 3306, ν(OH) 3129, δ(NH) 1651, ν(C=N^1), ν(N^2=C) 1628, 1601, ν(C-O) 1154. ^{1}H-NMR: δ 11.58, 10.71(cis/trans ratio: 2/1, s, 1H, OH), 8.44, 8.30 (syn/anti ratio:2/3, s, 1H, CH=N^1), 6.84 (s, 2H, NH_2), 7.14-6.99 (d-d, *J*= 7.43, J= 1.25, 1H, *d*), 6.94 (d, *J*= 6.92, 1H, *b*), 6.80 (t, 1H, *J*= 8.04, *c*), 2.42, 2.37 (cis/trans ratio:3/2, s, 3H, S-CH_3), 3.77 (s, 3H, OCH_3).

IV: beige, 170-171, 91, 0.15 ($CHCl_3/CH_2Cl_2$), Anal.Calc. for $C_{10}H_{13}N_3O_2S$ (239 g): C, 50.21; H, 5.44; N,17.57; S, 13.39, found: C, 50.22; H, 5.46; N, 17.51; S, 13.32%. UV-vis.: 257, 336. IR (cm^{-1}): ν_a(NH) 3453, ν_s(NH) 3303, ν(OH) 3064, (NH) 1651, ν(C=N^1), ν(N^2=C) 1624, 1601, ν(C-O) 1150. ^{1}H-NMR: δ 11.78, 10.11(cis/trans ratio: 2/1, s, 1H, OH), 8.38, 8.26 (syn/anti ratio:1/2, s, 1H, CH=N^1), 6.68 (s, 2H, NH_2), 7.41-7.28 (d-d, *J*= 8.3, 1H, d), 6.48 (split.d, 1H, *J*= 8.25, c), 6.43 (d, J= 2.37, 1H, a), 2.42, 2.37 (cis/trans ratio:1/1, s, 3H, S-CH_3), 3.76 (s, 3H, OCH_3).

Synthesis of the N^1,N^4-diarylidene-S-methyl-thiosemicarbazidato chelates (I–VIIa,b) The iron(III) and nickel(II) chelates displayed in Fig. 1 were isolated as following procedure. R-Substitue-salicylaldehyde-S-methyl thiosemicarbazone (1 mmol) and the corresponding R_2-substitued 2-hydroxy-benzaldehyde(1 mmol) were dissolved in EtOH (25 ml) and added to the solution of metal chloride in the EtOH (25 ml) by stirring. The mixture was stirred 2 h. After 2 days, the precipitate was collected by filtration. The complexes in the powder crystal form were dried *in vacuo* over P_2O_5.

The anaytical and spectral data of **I–VIIa,b** are given in following order: yield (%), m.p. (^{0}C), molar conductance ($ohm^{-1}cm^2mol^{-1}$, in 10^{-3} M DMSO, 25±1°C), μ_{eff} (BM), elemental analysis, UV-visible (λmax (nm), ε ($dm^3cm^{-1}mol^{-1}$), in $CHCl_3$), FT-IR (KBr, cm^{-1}), mass (ESI, APCI) spectra (for iron complexes) and ^{1}H-NMR (DMSO-d_6, 25°C, δ ppm) data (for nickel complexes).

Ia: 21, >390; 20.89; 5.86; Anal. Calc. for $C_{16}H_{13}N_3O_3SFeCl$ (418,3 g): C, 45.90; H, 3.13; N, 10.04; S, 7.66, found: C, 45.91; H, 3.11; N, 10.01; S, 7.65%. UV-Vis: 260 (22540), 346 (13145), 440*sh* (9850), 532 *sh* (2310). IR (cm^{-1}): ν(OH) 3453, ν(C=N) 1607, 1595, 1576, ν(C-O) 1161, 1130. m/z (+c ESI-MS, %relative abundance): 383 [M-Cl] (100.00), 384 [M-Cl+H] (14.56), 385 [M-Cl+2H] (4.42), 415 [M-3H] (9.26), 669 [2M-2Cl-2H-2SCH_3] (9.90), 766 [2M-2Cl] (10.41), 1152 [3M-3Cl+3H] (16.46), 1194 [3M-3Cl+3CH_3] (16.11), 1471 [4M-4Cl-4CH_3] (13.42); m/z (-c ESI-MS, %relative abundance): 417 [M-H] (100.00), 418 [M] (23.38), 419 [M+H] (36.49), 420 [M+2H] (9.12), 433 [M+CH_3](10.02).

IIa: 9; >390; 19.52; 5.88; Anal. Calc. for $C_{16}H_{13}N_3O_3S$-FeCl (418,3 g): C, 45.90; H, 3.13; N,10.04; S, 7.66, found: C, 45.88; H, 3.12; N, 10.07; S, 7.66%. UV-Vis: 261 (15000), 297 (18140), 354*sh* (13605), 459*sh*

(5258), 529*sh* (2390). IR (cm^{-1}): ν(OH) 3438, ν(C=N) 1607, 1593, 1576, ν(C-O) 1169, 1153, 1130. m/z (+c ESI-MS, %relative abundance): 383 [M-Cl] (100.00), 384 [M-Cl+H] (20.39), 385 [M-Cl+2H] (7.59), 386 [M-Cl+3H] (8.77), 399 [M-Cl+H+CH_3] (12.02), 415 [M-3H] (33.50), 766 [2M-2Cl] (8.61), 787 [2M-SCH_3] (24.42), 788 [2M-SCH_3+H] (11.59), 803 [2M-Cl+2H] (18,90), 1185 [3M-2Cl+2H] (9.52), 1203 [3M-3Cl+3H_2O] (7.63), 1550 [4M-4Cl+H_2O] (8.42); m/z (-c ESI-MS, %relative abundance): 416 [M-2H] (2.48), 434 [M+H+CH_3] (6.20), 449 [M-H+CH_3+OH] (100.00), 450 [M+CH_3+OH] (39.86), 465 [M+SCH_3] (60.75), 466 [M+SCH_3+H] (17.32), 467 [M+SCH_3+2H] (44.03), 468 [M+SCH_3+3H] (10.32), 469 [M+SCH_3+4H] (12.70), 483 [M+SCH_3+H_2O] (13.76), 860 [2M+Na] (3.52).

IIIa: 26; >390; 16.56; 5.88; Anal. Calc. for $C_{16}H_{13}N_3O_4SFeCl$ (434,65 g): C, 44.21; H, 3.01; N,9.67; S, 7.38, found: C, 44.22; H, 2.99; N, 9.65; S, 7.38%. UV-Vis: 252 (17720), 325(18660), 360*sh*(14980), 447*sh*(6020), 546*sh*(830). IR (cm^{-1}): ν(OH) 3430, ν(C=N) 1615, 1597, 1584, ν(C-O) 1161, 1130. m/z (+c ESI-MS, %relative abundance): 399 [M -Cl] (100.00), 400 [M-Cl+H] (3.92), 401 [M-Cl+2H] (8.15), 435 [M] (8.24), 436 [M+H] (7.12), 437 [M+2H] (15.42), 438 [M+3H] (11.10), 798 [2M-2Cl] (8.14), 1197 [3M-3Cl] (5.15); m/z (-c ESI-MS, %relative abundance): 433 [M-2H] (4.15), 434 [M-H] (6.71), 435 [M] (100.00), 436 [M+H] (24.15), 437 [M+2H] (20.41).

IVa: 18; >390; 18.51; 5.90; Anal. Calc. for $C_{17}H_{15}N_3O_3SFeCl$ (432,68 g): C, 47.19; H, 3.49; N,9.71; S, 7.41, found: C, 47.21; H, 3.48; N, 9.71; S, 7.42%. UV-Vis: 262 (22150), 325 (19054), 442*sh* (8645), 776(125). IR (cm^{-1}): ν(C=N) 1607, 1595, 1584, ν(C-O)1161, 1153. m/z (+c ESI-MS, %relative abundance): 236 [CH_3O-C_6H_3-(O)-CH=N-C(SCH_3)=N-N] (2.18), 382 [M -Cl,-CH_3] (4.19), 396 [M-Cl-H] (4.92), 397 [M-Cl] (100.00), 398 [M-Cl+H] (21.24), 399 [M-Cl+2H] (15.42), 400 [M-Cl+3H] (17.82), 401 [M-Cl+4H] (9.64), 402 [M-Cl+5H] (4.52), 427 [M-5H] (8.87), 455 [M+Na] (5.77), 823 [2M-Cl-6H] (5.84), 824 [2M-Cl-5H] (5.00), 825 [2M-Cl-4H] (18.02), 826 [2M-Cl-3H] (15.24), 827 [2M-Cl-2H] (6.84), 828 [2M-Cl-H] (7.02); m/z (-c ESI-MS, %relative abundance): 206 [C_6H_4-(O)-CH=N-N=C(SCH_3)-N] (2.65), 433 [M] (2.85), 459 [M+4H+Na] (2.02), 688 [2M+6H-2Cl-2Fe] (100.00), 1227 [3M+H-2Cl] (28.25), 1259 [3M-3Cl+3Na] (9.15), 1610 [4M-4Cl+Na] (13.12).

Va: 27; 222-223; 20.52; 5.89; Anal. Calc. for $C_{17}H_{15}N_3O_3$ SFeCl (432,68 g): C, 47.19; H, 3.49; N,9.71; S, 7.41, found: C, 47.18; H, 3.48; N, 9.69; S, 7.40%. UV-Vis: 273 (20920), 310 (18500), 357*sh* (4480), 425*sh* (1800), 522 *sh* (1040), 763 (160). IR (cm^{-1}): ν(C=N) 1600, 1593, 1576, ν(C-O)1169, 1,146. m/z (+c ESI-MS, %relative abundance): 238 [CH_3O-C_6H_3-(O)-CH=N-N=C(NH_2)-SCH_3] (19.91), 367 [M-Cl+H-OCH_3] (12.86), 382 [M-Cl-CH_3] (8.92), 397 [M-Cl] (100.00), 398 [M-Cl+H] (19.64), 399 [M-Cl+2H] (5.42), 427 [M-5H] (7.87), 428 [M-4H] (12.77), 429 [M-3H] (3.83), 455 [M+Na] (29.13), 457 [M+2H+Na] (12.09), 788 [2M-2Cl-6H] (3.59); m/z (-c ESI-MS, % relative abundance): 236 [CH_3O-C_6H_3-(O)-CH=N-N=C(SCH_3)N-] (100.00), 386 [M-SCH_3] (3.00), 553 [M+ C_6H_4-(O)-CH=NH] (4.53), 593 [M+ C_6H_4-(O)-CH=N-C=N-NH] (3.31).

VIa: 32; 218(decomp); 18.65; 5.87; Anal. Calc. for $C_{18}H_{17}N_3O_4SFeCl$ (462,3 g): C, 46.72; H, 3.70; N,9.08; S, 6.93, found: C, 46.72; H, 3.68; N, 9.08; S, 6.92%. UV-Vis: 269 (29528), 310(18186), 458*sh* (8406), 793(143). IR (cm^{-1}): ν(C=N) 1607, 1593, 1584, ν(C-O) 1161, 1153. m/z (+c ESI-MS, %relative abundance): 238 [CH_3O-C_6H_3-(O)-CH=N-N=C(NH_2)-SCH_3] (12.26), 427 [M-Cl] (100.00), 428 [M+H-Cl] (21.19), 429 [M+2H-Cl] (18.12), 852 [2 M-2Cl-2H] (6.42); m/z (-c ESI-MS, %relative abundance): 236 [CH_3O-C_6H_3-(O)-CH=N-N=C(SCH_3)N-] (12.42), 489 [M+2H+Na] (100.00), [M+4H+Na] (18.25).

VIIa: 24; >390; 12.16; 5.89; Anal. Calc. for $C_{18}H_{17}N_3O_4$ SFeCl (462,3 g): C, 46.72; H, 3.70; N,9.08; S, 6.93, found: C, 46.68; H, 3.71; N, 9.04; S, 6.90%. UV-Vis: 269 (23350), 325(16371), 387*sh* (8940), 438*sh* (6970), 763(150). IR (cm^{-1}): ν(C=N) 1615, 1607, 1576, ν(C-O) 1161, 1153, 1130. m/z (+c ESI-MS, % relative abundance): 425 [M-Cl-2H] (5.14), 427 [M-Cl] (100.00), 428 [M+H-Cl] (19.34), 429 [M+2H-Cl] (5.08), 481 [MH+H_2O] (6.99), 852 [2M-2Cl-2H] (3.36), 860 [2M-Cl+6H] (3.67), 885 [2M-Cl-4H] (11.58), 886 [2M-Cl-3H] (9.25), 887 [2M-Cl-2H] (4.76); m/z (-c ESI-MS, %relative abundance): 489 [M+4H+Na] (100.00), 490 [M+5H+Na] (12.45), 491 [M+6H+Na] (6.12), 739 [2M-2Cl-2Fe-3H] (2.82), 740 [2M-2Cl-2Fe-2H] (4.19), 742 [2M-2Cl-2Fe] (7.70), 744 [2M-2Cl-2Fe+2H] (3.48).

Ib: 39; 272-273; 6.8; 0.23; Anal. Calc. for $C_{16}H_{13}N_3O_3SNi$ (386,05 g): C, 49.78; H, 3.39; N,10.88; S, 8.31, found: C, 49.62; H, 3.38; N, 10.89; S, 8.20%. UV-Vis: 263 (28410), 317 (17740), 399 (18120), 483*sh*(4690), 546*sh*(3210), 825(89). IR (cm^{-1}): ν(OH) 3418, ν(C=N) 1608, 1597, 1582 ν(C-O) 1168, 1150,1135. ^{1}H-NMR: δ 8.88, 8.42 (cis/trans ratio:6/1, s, 1H, $OH_{(R2)}$), 8.59 (s, 1H, CH=N^1), 8.31 (s, 1H, CH=N^4), 6.95 (d, *J*= 8.29, 1H, *a*), 7.33 (ddd, *J*= 6.83, *J*= 1.95 1H, *b*), 6.68 (t, *J*= 8.29, 1H, *c*), 7.57 (dd, *J*= 8.3, *J*= 1.46 1H, *d*), 6.89 (dd, *J*= 7.32, *J*= 1.46, 1H, *q*), 6.55 (t, *J*= 7.32, 1H, *r*), 7.21 (dd, *J*= 8.3, J= 1.46 1H, *s*), 2.72 (s, 3H, S-CH_3).

IIb: 52; 268-269; 5.2; 0.16; Anal. Calc. for $C_{16}H_{13}N_3O_3SNi$ (386,05 g): C, 49.78; H, 3.39; N,10.88; S, 8.31, found: C, 49.81; H, 3.36; N, 10.85; S, 8.33%. UV-Vis: 260 (16460), 300 (8270), 328 (7720), 401(10020), 483*sh* (2490), 549*sh* (1760), 816(62). IR (cm^{-1}): ν(OH) 3426, ν(C=N) 1612, 1593, 1582 ν(C-O) 1166, 1146, 1127. ^{1}H-NMR: δ 8.85, 8.43 (cis/trans ratio: 2/9, s, 1H, $OH_{(R1)}$), 8.59 (d, *J*= 4.4, 1H, CH=N^1), 8.31 (d, *J*= 6.34, 1H, CH=N^4), 7.01 (d, *J*= 8.78, 1H, *b*), 7.49 (ddd, *J*= 6.83, *J*= 1.95, 1H, *c*), 7.78 (dd, *J*= 8.3, *J*= 1.95 1H, *d*), 6.81 (dd, *J*= 7.32, *J*= 1.47 1H, *p*), 6.74 (t, *J*= 7.81, 1H, *q*), 6.51 (t, *J*= 7.81, 1H, *r*), 7.03 (dd, *J*= 8.3, J= 1.46 1H, *s*), 2.73 (s, 3H, S-CH_3).

IIIb: 68; 326(decomp); 8.3; 0.12; Anal. Calc. for $C_{16}H_{13}N_3O_4SNi$ (402,05 g): C, 47.80; H, 3.26; N,10.45; S, 7.98, found: C, 47.82; H, 3.24; N, 10.47; S, 7.98%. UV-Vis: 261 (15940), 315 (10090), 400 (10250), 524(1770), 549*sh* (1710), 819(67). IR (cm^{-1}): ν(OH) 3422, 3407, ν(C=N) 1612, 1597, 1582, ν(C-O) 1168, 1150. ^{1}H-NMR: δ 8.58, 8.38 (s,s, 2H, $OH_{(R1,R2)}$), 8.64 (s, 1H, CH=N^1), 8.37 (s, 1H, CH=N^4), 6.90 (dd, *J*= 7.32, *J*= 1.46, 1H, *b*), 6.59 (ddd, *J*= 8.3, 1H, *c*), 7.23 (dd, *J*= 8.78, *J*= 1.46 1H, *d*), 6.80 (dd, *J*= 7.32, *J*= 1.46, 1H, *q*), 6.53 (t, *J*= 7.81, 1H, *r*), 7.03 (dd, *J*= 8.3, *J*= 1.47 1H, *s*), 2.73 (s, 3H, S-C*H*$_3$).

IVb: 48; 196-197; 5.9; 0.08; Anal. Calc. for $C_{17}H_{15}N_3O_3SNi$ (400,08 g): C, 51.04; H, 3.78; N,10.50; S, 8.01, found: C, 51.08; H, 3.78; N, 10.42; S, 8.03%. UV-Vis: 262 (16510), 314 (7750), 396 (8230), 483*sh* (2280), 558 (1810), 825(70). IR (cm^{-1}): ν(C=N) 1608, 1593, 1582, ν(C-O) 1154, 1131, 1108. ^{1}H-NMR: δ 8.51 (syn/anti ratio: 4/1, s, 1H, CH=N^1), 8.28 (syn/anti ratio: 1/4, s, 1H, CH=N^4), 6.96 (d, *J*= 7.32, 1H, *a*), 7.32 (ddd, *J*= 6.83, *J*= 1.47, 1H, *b*), 6.62 (t, *J*= 8.3, 1H, *c*), 7.56 (dd, *J*= 8.3, *J*= 1.46, 1H, *d*), 6.92 (d, *J*= 8.78, 1H, *q*), 6.67 (ddd, *J*= 6.83, *J*= 0.98, 1H, *r*), 7.30 (dd, *J*= 7.81, *J*= 1.47, 1H, *s*), 3.76, 3.73 (isomer ratio: 7/2, s, 3H, -$OCH_{3(R2)}$), 2.71 (s, 3H, S-CH_3).

Vb: 48; 223; 6.1; 0.09; Anal. Calc. for $C_{17}H_{15}N_3O_3SNi$ (400,08 g): C, 51.04; H, 3.78; N,10.50; S, 8.01, found: C, 51.03; H, 3.75; N, 10.51; S, 8.00%. UV-Vis: 262 (12410), 301 (5860), 325 (5470), 351*sh* (4120), 399(7360), 483*sh* (1870), 546*sh* (1420), 835(80). IR (cm^{-1}): ν(C=N) 1608, 1597, 1582, ν(C-O) 1170, 1150, 1127. ^{1}H-NMR: δ 8.53 (syn/anti ratio: 1/1, s, 1H, CH=N^1), 8.29 (syn/anti ratio: 3/2, s, 1H, CH=N^4), 6.88 (dd, *J*= 7.31, *J*= 1.46, 1H, *b*), 7.48 (ddd, *J*= 8.78, *J*= 1.95, 1H, *c*), 7.77 (dd, *J*= 8.29, *J*= 1.95, 1H, *d*), 6.99 (d, *J*= 8.3, 1H, *p*), 6.73 (t, *J*= 7.8, 1H, *q*), 6.58 (t, *J*= 7.81, 1H, *r*), 7.13 (dd, *J*= 7.81, *J*= 1.46, 1H, *s*), 3.76, 3.74 (isomer ratio: 4/7, s, 3H, -$OCH_{3(R1)}$), 2.72 (s, 3H, S-CH_3).

VIb: 38; 326(decomp); 8.6; 0.23; Anal. Calc. for $C_{18}H_{17}N_3O_4SNi$ (430,10 g): C, 50.27; H, 3.98; N, 9.77; S, 7.46, found: C, 50.25; H, 3.99; N, 9.77; S, 7.48%. UV-Vis: 264 (23710), 315 (11700), 396 (12190), 511(2860), 565$_{sh}$ (2420), 821(60). IR (cm^{-1}): ν(C=N) 1612, 1597, 1582, ν(C-O) 1173, 1154, 1108. ^{1}H-NMR: δ 8.54 (s, 1H, CH=N^1), 8.29 (s, 1H, CH=N^4), 6.98 (dd, *J*= 7.81, *J*= 1.47, 1H, *b*), 6.64 (t, *J*= 8.3, 1H, *c*), 7.32 (dd, *J*= 8.78, *J*= 1.46 1H, *d*), 6.90 (dd, *J*= 7.80, *J*= 1.46, 1H, *q*), 6.59 (t, *J*= 8.3, 1H, *r*), 7.15 (dd, *J*= 8.3, *J*= 1.46, 1H, *s*), 3.77, 3.75 (s,s, 6H, -$OCH_{3(R1,R2)}$), 2.72 (s, 3H, S-CH_3).

VIIb: 36; 294-295; 6.1; 0.12; Anal. Calc. for $C_{18}H_{17}N_3O_4SNi$ (430,10 g): C, 50.27; H, 3.98; N, 9.77; S, 7.46, found: C, 50.28; H, 3.94; N, 9.71; S, 7.49%. UV-Vis: 264 (23710), 317 (28750), 417 (22450), 460$_{sh}$ (1880), 511 (2860), 956(20). IR (cm^{-1}): ν(C=N) 1612, 1597, 1585, ν(C-O) 1181, 1154, 1116. ^{1}H-NMR: δ 8.31 (d, *J*= 9.15, 1H, CH=N^1), 8.04 (d, *J*= 8.69, 1H, CH=N^4), 7.63 (d, *J*= 9.15, 1H, *d*), 7.42 (d, *J*= 8.69, 1H, *s*), 6.43 (d, *J*= 2.28, 1H, *p*), 6.40 (dd, *J*= 2.75, *J*= 8.69, 1H, *c*), 6.39 (d, *J*= 2.75, 1H, *a*), 6.32 (dd, *J*= 2.29, *J*= 8.7, 1H, *r*), 3.85, 3.80 (s,s, 6H, -$OCH_{3(R1,R2)}$), 2.68 (s, 3H, S-CH_3).

Results and discussion

Synthesis and some physical properties The substitued-N^1-arylidene-*S*-methyl-thiosemicarbazones, **I**–**IV**, were soluble in ethanol and chlorinated hydrocarbons. The template reactions of these thiosemicarbazones with the substitued salicyl aldehydes in the presence of iron(III) or nickel(II) chloride salts give the chelates which have the [Fe(L)Cl] and [Ni(L)] compositions (Fig. 1). The crystalline powder of the iron templates are bright black, the nickel templates are in claret colour and all metal complexes soluble in alcohol and chloroform, and very soluble in DMF and DMSO.

The μ_{eff} values of iron(III) chelates (**I–VIIa**), are in 5.86–5.90 BM, are equivalent to five unpaired electrons and so high-spin state of iron(III) indicates the [Fe(L)Cl] composition. Magnetic measurement results of nickel(II) chelates (**I–VIIb**) showed that they are diamagnetic and in squar-planar structure. The molar conductance values of nickel complexes are in the range 5.2–8.3 $\Omega^{-1}cm^2mol^{-1}$ indicating their non-electrolytic behavior. The iron complexes have the relative high conductance values between 16.56 and 20.89 causing to the chloro atom on the iron(III) centre.

Electronic spectra UV-Vis spectra of the starting thiosemicarbazones **(I–IV)** in the DMF and the chelates (**I–VIIa**,**b**) in $CHCl_3$ were obtained in the range 200–1,000 nm in 10^{-4} M solution. For all compounds, the spectra showed the π→π* transitions of the aromatic rings at 243–264 nm, and

the broad band in the range of 312–400 nm assignable to n→π* and π→π* transitions of the imin and thioamide region of thiosemicarbazone moiety [35, 36].

The spectra of the iron complexes, **I–VIIa**, show a main band in the 425–458 nm region which attributed to CT absorptions. The bands (for **I–IIIa** and **Va**) in the region 522–546 nm may be due to the axial coordination of the chloro atom to the metal center as seen in porphyrin complexes, [37, 38] and so these bands support the square-pyramidal structure of the penta coordinated iron(III). The iron templates, **IV–VIIa**, exhibit the spin forbidden d-d transitions bands between 763 nm and 793 nm in the low intensities, and **I–IIIa** are too weak to be observed.

The electronic spectra of the nickel templates (**I–VIIb**) display two bands in 483–511 and 546–565 nm ranges can be attributed to $^1A_{1g}\rightarrow{}^1B_{1g}$ and $^1A_{1g}\rightarrow{}^1A_{2g}$ transitions, respectively. The bands at 816–835 nm are due to the *Laporte* forbidden (spin-allowed) transitions which have very low ε values between 60 $dm^3cm^{-1}mol^{-1}$ and 89 $dm^3cm^{-1}mol^{-1}$ [39, 40]. The appearances of these bands and the diamagnetic nature of **I–VIIb** are consistent with the square-planar geometry around the nickel(II) ion.

Infrared spectra The infrared spectra of **I–IV** show strong band in the range 3,220–3,052 cm^{-1} corresponding to the presence of hydroxyl group of the thiosemicarbazones. The sharp bands in the 3,470–3,412, 3,349–3,280 and 1,650–1,620 cm^{-1} regions are assigned to $\nu_a(NH)$, $\nu_s(NH)$ and $\delta(NH_2)$ vibrations, respectively. The imine bands are observed in the range 1,628–1,582 cm^{-1}.

The condensation reactions of the thiosemicarbazones and aldehydes can be easily monitored by means of IR and ^1H NMR spectra. In the spectra of the complexes, the NH_2 and 2-OH bands of the *S*-methylthiosemicarbazones disappeared due to the condensation. Only the ν(OH) band of the 3-substituted hydroxyl groups on the **I–IIIa**,**b** structures were recorded in the range 3,453–3,422 cm^{-1}. After chelating, the sharp intensity band observed with splitting at *ca.* 1,595 cm^{-1} belongs to a new imin group, (N^4=C), that is due to condensation of the thioamide nitrogen (N^4) and second aldehyde. Thus, the new conjugated backbone of the complexes include three imin bonds which are C=N^1, N^2=C and N^4=C. It is difficult to distinguish these imin vibrations, but it can be said that the shifting of (CH=N^1) band to a lower wave number by 10–20 cm^{-1} in the metal complexes in the comparison to the free ligands [41].

^1H NMR spectra The protons of starting materials, **I–IV,** have showed the expected chemical shift values, and even the systematic signals of *syn-anti* and *cis-trans* isomers belonging to the imin, hydroxyl and *S*-methyl protons have displayed [42, 51]. The ratios of signal integrations of imine groups are 1:2, 3:7, 2:3, 1:2 because of *syn-anti* isomerism, and also the hydroxyl groups gave *cis* and *trans* peaks in 3:2, 5:2, 2:1 and 2:1 ratios for **I–IV**, respectively.

In the **I–VIIb** spectra, the proton signals of 2-OH and N^4H_2 groups of **I–IV** disappear by chelation. The absence of these N^4H_2 hydrogens indicates their deprotonation and arising the N^4=CH signal which is a singlet and equivalent to integral value of one proton confirms the template formation around nickel(II). The signals of the proton in HC=N^1 groups which associated with nickel centre through N^1 nitrogen were recorded in higher frequencies according to the starting thiosemicarbazones. The ^1H NMR data of the nickel templates show any isomer peak except CH=N^1 protons of **IVb** and **Vb**.

As results, the analytical and spectral data become evident that the chelating N^1,N^4-diarylidene-*S*-methyl-thiosemicarbazidato ligands are bonded to metal atom through *ONNO* donor set and so it can be proposed the template structures in Fig. 1.

ESI-mass spectra In the positive conditions, all of the complexes give the [M-Cl] ion peak (%100 relative abundance) due to the loss of chlorine. The spectrum show the protonated and deprotonated molecular ion peaks { [M-H], [M-2H], [M+H], [M+2H], [M] etc.}, dimer, trimer, tetramer ion peaks corresponding to lose of chlorine { [2M-Cl], [3M-3Cl], [4M-4Cl] }, in both conditions.

Cytotoxicity results The cytotoxic potencies of the starting thiosemicarbazone derivatives (**I–IV**) and fourteen metal chelates (**I–VIIa**,**b**) were investigated in K562 and ECV304 cells by means of the colorimetric MTT assay (Table 1). K562 cells were the first myelogenous leukemia line to be established from CML patient in blast crisis and are suitable in vitro model for new drug testing. We preferred to use different cells because to compare our thiosemicarbazone compounds cytotoxicity on normal and tumor cells similary to numerous studies by Richardson *et. al*. [21, 31]. In addition, we wanted to compare the selective antitumor activity to the iron(III) chelates I of thiosemicarbazone that was synthesized in previous studies [19].

The novel compounds, **I–IV**, have no useful cytotoxic effect on both cell lines in all concentrations, data of **III** and **IV** were given in Table 1 and Fig. 2 as examples. The iron (III) and nickel(II) chelates of the N^1,N^4-diarylidene-*S*-methyl-thiosemicarbazones (**I–VIIa**,**b**) display cytotoxic effects against the leukemia (K562) and endothelial (ECV 304) cell lines in different levels.

The hydroxy-substitued metal chelates exhibit notorious cytotoxic activity against both of cell lines in the values of IC_{50} >5 μg/ml, except the dihydroxy iron chelate (**IIIa**, R_1=R_2= 3-OH) which has a notable cytotoxicity on ECV304 (Tables 2 and 3).

The methoxy-substitued nickel(II) chelate which has OCH_3 group at C-3 position of the aromatic ring of N^4-arylidene

Table 1 Cytotoxicity index (CI%) of **III**, **IV** and **I–VII a**,**b** on K562 and ECV 304 cell lines

Cell line	Comp.	50μgr/ml	10μgr/ml	5μgr/ml	1μgr/ml	0.1μgr/ml	0.01μgr/ml
K562							
	III	−0,96±10,67	−4,17±2,62	−11,45±9,81	−5,20±4,61	−2,50±6,86	0,86±7,97
	IV	−0,29±4,36	−3,61±6,87	−3,47±5,29	4,46±5,00	−4,80±7,79	−0,82±15,80
	Ia	30,15±12,65	13,88±3,29	22,63±1,24	26,60±10,95	13,50±15,41	3,92±18,62
	IIa	−2,16±7,54	24,81±12,74	15,01±8,53	15,11±10,07	13,94±13,37	16,74±6,38
	IIIa	28,21±3,85	13,05±10,68	17,62±10,65	0,88±4,84	14,35±12,69	13,48±15,70
	IVa	**84,43±1,69****	−16,64±5,07	−2,08±10,92	−9,38±40,50	−42,13±6,98	−32,86±13,77
	Va	**84,32±8,97***	4,66±3,68	12,74±5,57	11,00±18,17	−19,92±13,51	−8,78±17,43
	VIa	79,01±7,80	−12,74±4.36	−19,68±23.07	−17,13±30.27	11.82±21.07	10,95±9.13
	VIIa	**98,58±0,49***	**99,20±1.24***	**94,83±4.44***	**96,41±1.77***	**9.07±3.90***	10,93±6.24
	Ib	26,93±10,38	12,18±9,24	−1,40±7,50	−0,19±21,20	11,31±9,50	6,91±3,99
	IIb	28,28±5,83	−13,04±17,35	−4,40±22,19	6,48±9,61	15,31±5,77	18,12±5,55
	IIIb	0,84±9,06	1,16±2,96	7,18±7,29	10,28±4,79	**−1,68±17,63***	**9,54±10,57***
	IVb	**85,11±3,01****	81,25±3,43	76,98±5,24	30,38±8,11	8,73±13,17	23,15±12,45
	Vb	83,14±1,28	36,86±11,53	31,70±2,50	12,98±11,52	**18,57±6,24***	15,88±4,62
	VIb	**78,48±1,92****	**72,36±2.53****	**64,35±2.29****	61,71±2.63	10.49±12.02	16,50±10.57
	VIIb	35,29±5,85	**29,44±2.22****	**25,37±1.87****	**17,77±6.59****	**11.72±5.16***	**11,07±5.59***
ECV 304	**III**	3,47±12,05	4,67±5,11	2,99±5,13	**8,32±5,61***	4,43±7,85	0,83±5,75
	IV	0,04±10,94	0,51±10,20	−3,48±6,58	−5,39±5,48	−3,17±4,06	−2,13±6,16
	Ia	72,29±5,45	38,88±15,33	36,98±18,24	40,57±22,00	21,62±16,05	38,66±15,80
	IIa	**35,94±14,95***	16,67±10,89	20,40±10,88	5,92±1,55	14,67±13,42	17,14±8,38
	IIIa	**81,68±7,90****	**78,88±6,65****	**74,06±6,66****	**42,99±3,91****	29,94±13,49	**28,56±4,02***
	IVa	55,81±6,96	**44,34±13,97****	**32,40±13,35****	33,56±25,61	18,82±21,13	27,72±14,12
	Va	56,19±16,78	43,04±26,08	30,93±16,14	27,91±11,70	13,03±2,09	11,83±24,09
	VIa	75,60±3,20	**37,23±7.46****	**37,43±4,16****	**24,67±13,91***	**12,43±6,23***	**14,26±5,75****
	VIIa	51,05±17,89	47,90±16,36	20,63±41,36	18,16±43,92	−22,44±16,87	−0,27±10,29
	Ib	**38,85±3,04***	**37,34±4,46****	**31,23±6,56****	13,58±6,25	18,60±2,30	**13,32±2,84***
	IIb	**52,09±2,27****	**42,04±7,91****	**46,77±2,29****	**36,18±8,44****	20,99±5,95	20,27±3,09
	IIIb	8,70±25,59	**18,87±14,71***	11,98±14,51	−0,80±23,47	−47,66±47,77	−21,43±29,04
	IVb	74,66±2,64	85,64±5,31	**86,16±7,64***	**56,14±7,98***	16,67±11,57	23,80±18,38
	Vb	83,43±2,65	**86,98±4,53***	**79,31±10,35***	19,40±17,37	−2,02±14,47	5,36±13,97
	VIb	40,13±4,05	55,96±7,60	56,97±4,50	55,87±8,54	6,68±7,37	3,11±12,69
	VIIb	29,51±7,70	13,94±5,87	12,82±3,51	2,37±3,66	5,28±3,88	4,82±2,74

Mean differences are significant between K562 and ECV 304 cells. Cytotoxicity index (CI%) was calculated to following formula comparing to control: CI %=1- OD (optical density) treated wells / OD control wells × 100

*p<0,050; **p<0,001

moiety (**IVb**, R_1/R_2= H/ 3-OCH_3) is more cytotoxic for ECV304 (IC_{50}=0.87 μg/ml) than K562 cells (IC_{50}= 2.27 μg/ml), but **Vb (**R_1/R_2= 3-OCH_3/ H**)** has cytotoxicity only in ECV304 cells at more high IC_{50} levels (3 μg/ml) (Fig. 3). However, the nickel(II) chelate, **VIb**, which has OCH_3 groups at the C-3 positions of N(1) and also N(4)-arylidene moities shows cytotoxic activity in ECV304 and K562 cells at same levels 0.9 μg/ml and 0.8 μg/ml, respectively.

Among the methoxy substitued iron(III) chelates, **VIIa** (R_1=R_2= 4-OCH_3) has selectively cytotoxic for K562 cells in very low concentrations (0.53 μg/ml=1 μM). This value is 6.6 times lower than the IC_{50} of the iron chelate with the substituents, 3-OCH_3 (R_1) and 4-OCH_3 (R_2), which was previously published [19]. As a superiority from the viewpoint of therapeutic potential, compound **VIIa** which is not cytotoxic for ECV304 cells at 0.53 μg/ml dose should have better therapeutic potential as antitumor agents.

Ferrari *et. al.* studies have been shown various thiosemicarbazone derivatives which have not inhibit cell growth in three leukemic human cell line (CEM, K562, U937) but copper complexes with 5-formyluracil thiosemicarbazone were only able to induce apoptosis at 40 μg/ml on CEM and K562 cell lines [43]. This concentration is too much high compared to our IC_{50} value. However they reported that three new 5-formyluracil thiosemicarbazone complexes were not able to induce apoptosis on all

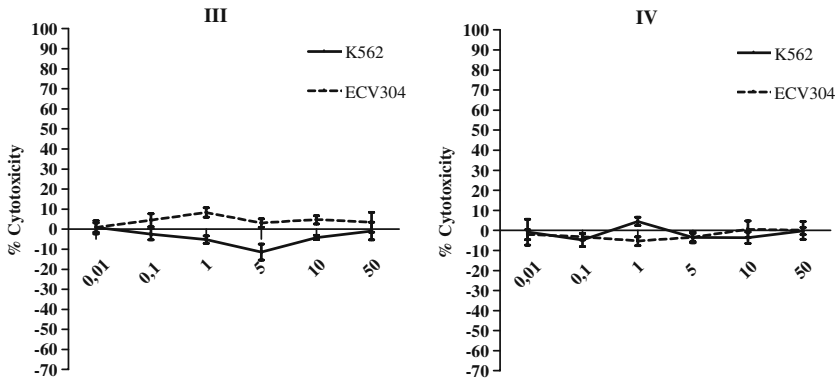

Fig. 2 Cytotoxic effects of the **III** and **IV** on K562 and ECV304 cell lines

leukemia cell lines in next study antogonistic previous study [44]. In latest study of same group, it was observed to induce an antiproliferative effect of new nickel complex on U937 cells at low concentrations (IC_{50}=14.4 μM) [45]. The effective HCTs [α-(N)-heterocyclic carboxaldehyde thiosemicarbazones, e.g., 3-AP and 3-AMP were also assessed in L1210 leukemia cells and L1210 leukemia bearing mice in vivo by Li et.al. They found IC_{50} values which were in 4.2–1.3 μM. These values are higher from our IC_{50} values but these differences may be possible as leukemic cell lines from different origin were used in studies [46]. Kovala-Demertzi et.al. have shown antitumor activity of platinum (II) complexes with thiosemicarbazones derived from 2-formyl and 2-acetyl pyridine in different cell lines and in leukemia P388-bearing mice [47].

The thiosemicarbazones effectiveness were seen to be in different doses when were evaluated studies performed

Table 2 Inhibitory concentration (IC_{50}) of **I–VII a**,**b** for K562 and ECV 304 cell lines

	IC_{50} (microgram/ml)	
	K562	**ECV304**
Ia	>5	>5
IIa	>5	>5
IIIa	>5	2
IVa	>5	>5
Va	>5	>5
VIa	>5	>5
VIIa	0,53	>5
Ib	>5	>5
IIb	>5	>5
IIIb	>5	>5
IVb	2,27	0,87
Vb	>5	3
VIb	0,8	0,9
VIIb	>5	>5

IC_{50} corresponds to the concentration required to inhibit a 50% of the cell growth when the cells are exposed to the compounds during 3 days

other cancer cell lines. Afrasiabi et.al. were reported that the nickel complexes of naphthaquinone thiosemicarbazone (Ni-NQTS) was exhibited the lowest IC_{50} value (2.25 μM) on MCF7 human breast cancer cells and Ni-NQTS was more effective than etoposide [33, 42, 48, 49]. In other study, The effective concentrations of palladium(II) complexes of 2-benzoylpyridine- thiosemicarbazones which were synthesized by Rebolledo et.al. are between 13.8 μM and 12.9 μM for MCF-7, TK-10 and UACC-62 cell lines [10]. Yuan et. al. have been shown marked and selective antitumor activity of Dp44mT that di-2-pyridyl thiosemicarbazones was the most active chelator, for example, an IC_{50} of 0.03 μM in SK-N-MC neuroepithelioma cells compared with more than 25 μM in MRC-5 fibroblasts [31]. Recently Richardson et.al. have reported the di-2-pyridyl ketone thiosemicarbazone (HDpT) chelators in particular the ligand, di-2-pyridyl ketone 4,4-dimethyl-3-thiosemicarbazone (HDp44mT) showing the highest antiproliferative activity of all chelators examined so far. These ligands demonstrated selective antitumor activity, having far less effect on the growth of normal cells. In addition, HDp44mT showed marked activity in vivo, reducing the growth of a murine M109 lung cancer by approximately 50% within 5 days of treatment, while having little effect on normal hematological indices [50]. In other studies, Richardson et.al. designed alternative some 2-Benzoylpyridine-thiosemicarbazones are effective on fibroblasts and neuroepithelioma in fairly low IC_{50} values (2.39–0.004 μM) [21]. Progressive studies were concentrated in triapine (3-aminopyridine-2-carboxaldehyde thiosemicarbazone) that is one of the most effective thiosemicarbazone. Triapine, an iron chelator and a potent inhibitor of ribonucleotide reductase, has significant anti-leukemia activity [23, 24, 27, 28]. Its antitumor effects against several tumor cell lines were dependent on achieving both a threshold concentration and a minimum duration of exposure. Triapine is also 100–1000-fold more potent than hydroxyurea and is active in some hydroxyurea-resistant leukemia cell lines [27, 28]. Preclinical experiments have shown that Triapine can increase the antitumor effects of standard cancer agents such

Table 3 Physicochemical data of **(Ia, Ib)–(VIIa, VIIb)**

Comp.	R_1	R_2	Formula	M.W.	M.p. (°C)
I	H	-	$C_9H_{11}N_3OS$	209 g	160–161
II	3-OH	-	$C_9H_{11}N_3O_2S$	225 g	175–176
III	3-OCH_3	-	$C_{10}H_{13}N_3O_2S$	239 g	164–165
IV	4-OCH_3	-	$C_{10}H_{13}N_3O_2S$	239 g	170–171
Ia	H	3-OH	$C_{16}H_{13}N_3O_3SFeCl$	418,66 g	>390
IIa	3-OH	H	$C_{16}H_{13}N_3O_3SFeCl$	418,66 g	>390
IIIa	3-OH	3-OH	$C_{16}H_{13}N_3O_4SFeCl$	434,65 g	>390
IVa	H	3-OCH_3	$C_{17}H_{15}N_3O_3SFeCl$	432,68 g	>390
Va	3-OCH_3	H	$C_{17}H_{15}N_3O_3SFeCl$	432,68 g	222–223
VIa	3-OCH_3	3-OCH_3	$C_{18}H_{17}N_3O_4SFeCl$	462,71 g	218(decomp)
VIIa	4-OCH_3	4-OCH_3	$C_{18}H_{17}N_3O_4SFeCl$	462,71 g	>390
Ib	H	3-OH	$C_{16}H_{13}N_3O_3SNi$	386,05 g	272–273
IIb	3-OH	H	$C_{16}H_{13}N_3O_3SNi$	386,05 g	268–269
IIIb	3-OH	3-OH	$C_{16}H_{13}N_3O_4SNi$	402,05 g	326(decomp)
IVb	H	3-OCH_3	$C_{17}H_{15}N_3O_3SNi$	400,08 g	196–197
Vb	3-OCH_3	H	$C_{17}H_{15}N_3O_3SNi$	400,08 g	223
VIb	3-OCH_3	3-OCH_3	$C_{18}H_{17}N_3O_4SNi$	430,10 g	326(decomp)
VIIb	4-OCH_3	4-OCH_3	$C_{18}H_{17}N_3O_4SNi$	430,10 g	294–295

as cisplatin, cyclophosphamide, and etoposide in mouse tumor models. The researchers have found effective plasma concentration of Triapine (2–7 μM) which was required to achieve in vitro/in vivo leukemia growth inhibition supported 50% reduction in white blood cell counts in patients [24, 28]. Effective serum concentrations and duration of Triapine exposure required for modulating [28]. Triapine is being evaluated in phase I and II clinical trials sponsored by the

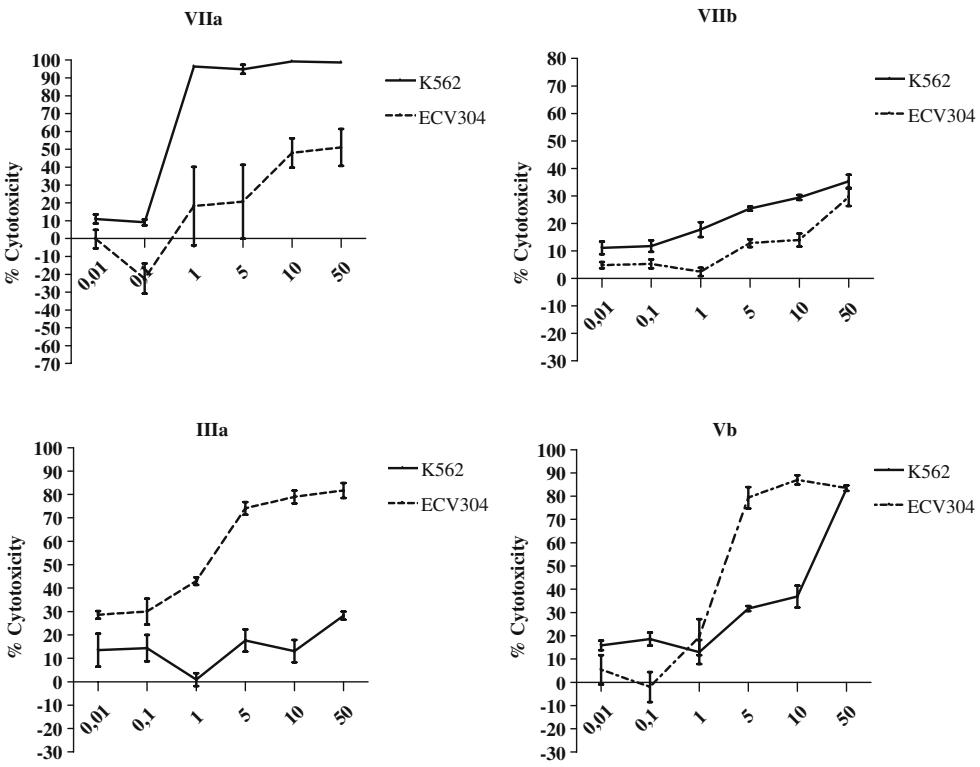

Fig. 3 Effect of iron and nickel chelates on K562 and ECV 304 cells in 0.01 μg/ml, 0.1 μg/ml, 1 μg/ml, 5 μg/ml, 10 μg/ml, 50 μg/ml. **VIIa** had selective cytotoxicity for K562 cells; **IIIa** and **Vb** were selectively cytotoxic for ECV304 cells. On the other hand, **VIIb** was not cytotoxic both cell line

National Cancer Institute for the treatment of various metastatic and solid cancers [23, 25, 26]. While further evaluations as first salvage therapy in phase II trials in patients with primary refractory and/or relapsed acute leukemias are warranted, it will take a randomized study to quantify the true contribution of Triapine to the combination regimen [23, 27, 28].

Considering the cytotoxicity results of these compounds with our findings for the *ONNO* chelates, it can be said that the iron chelate in previous paper [19], **IVb**, **VIb** and **VIIa** may be remarkable therapeutic drug potential due to their cytotoxicities at 1–5 μM against K562 cells. These results suggest an appreciable therapeutic index of our compounds, targeting cancer cells over normal cells. We can say anything about cytotoxic mechanisms probable by triggering apoptosis. Therefore we think to concentrate on important molecules of apoptotic mechanisms e.g. caspase 3, 8, 9 and cytochrome C pointed death pathway of active thiosemicarbazones. Subsequently, we will attempt testing in vitro combination our effective compounds with other chemotherapy drugs helping improvement therapy protocols.

Conclusion

The effective concentrations of palladium(II) complexes of 2-benzoylpyridine- thiosemicarbazones which were synthesized by Rebolledo *et.al.* are between 13.8 μM and 12.9 μM for MCF-7, TK-10 and UACC-62 cell lines [10]. Richardson et.al. had found that some 2-Benzoylpyridine-thiosemicarbazones are effective on MRC-5 fibroblasts and SK-N-MC neuroepithelioma in fairly low IC_{50} values (2.39–0.004 μM) [21]. In other study, one of the nickel compounds exhibits the lowest IC_{50} value (2.25 μM) on MCF7 human breast cancer cells [49]. Considering the cytotoxicity results of these compounds with our findings for the *ONNO* chelates, it can be said that the iron chelate in previous paper [19], **IVb**, **VIb**, **VIIa** and **VIIb** may be remarkable therapeutic drug potential due to their cytotoxicities at 1–7 μM against K562 cells accordingly in CML patients.

Results of our studies are tought that the 4-methoxy substitued iron chelates have a considerable cytotoxic activity against K562 while the 3-methoxy substitued nickel chelates are mostly cytotoxic against ECV304 and K562 cells. Taking into consideration the 4-methoxy substitued iron chelate (**VIIa**) which has best cytotoxic effect on K562, it should be pointed out that the OCH_3 groups at C-4 position of the aromatic ring are increased selectivity of the cytotoxicity.

All these results imply that selective cytotoxic potential depends on not only metal ion in complex structure but also substituents and their location on aromatic rings.

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