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Apoptotic mechanisms of nickel(II) complex with N1-acetylacetone-N4-4-methoxy-salicylidene-S-allyl-thiosemicarbazone on HL60 leukemia cells

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Abstract. Thiosemicarbozone complexes that have a broad spectrum of biological activity are formed as a result of condensation reaction between thiosemicarbazide [$H_2N(C=S)$-NH-NH_2] and carbonyl-containing compounds. A new nickel(II) complex with N1-acetylacetone-N4-4-methoxy-salicylidene-S-allyl-thiosemicarbazone ligand was synthesized and characterized. We studied the antileukemic activity of the Ni(II) thiosemicarbazone compound and assessed their potential for drug development. Specifically, the effects of this Ni(II) thiosemicarbazone compound on intracellular signal nodes and apoptotic pathways were investigated. According to our results, the Ni(II) thiosemicarbazone compound has apoptotic activity against HL60 cells. Moreover, while Ni(II) thiosemicarbazone compound significantly increased levels of p53 and cleaved caspase-3 proteins, it decreased level of Phospho-Akt1 protein in HL60 cells. The Ni(II) thiosemicarbazone compound could induce HL60 cell apoptosis through inhibiting of PI3K/Akt pathway. These results showed that Ni(II) thiosemicarbazone compound might be an antileukemic agent.

Key words: Thiosemicarbazone — Apoptosis — HL60 human promyelocytic leukemia cell — Signal transduction

Introduction

Thiosemicarbozone complexes that have a broad spectrum of biological activity are formed as a result of condensation reaction between thiosemicarbazide [$H_2N(C=S)$-NH-NH_2] and carbonyl-containing compounds. Thiosemicarbozones are polydentate ligands and their aromatic derivatives have chelating ability with various metals. Metal complexes of thiosemicarbazones is one of the popular research subjects because of their wide range pharmacological activity that provides diverse variety of compounds and metal ions with different biological activities (Casas et al. 2000; Tarasconi et al. 2000; Beraldo and Gambino 2004; Yanardag et al. 2009; Kesel 2011). Some of activities have antitumor (Afrasiabi et al. 2004; Vrdoljak et al. 2010), antimicrobial (De Logu et al. 2005; Rodriguez-Arguelles et al. 2005; Mendes et al. 2009), antiviral (Varadinova et al. 2001; Genova et al. 2004), and cytotoxic (Bal Demirci et al. 2007; Atasever et al. 2010) effects.

In various studies, it was found that metal complexes of some thiosemicarbozone compounds inhibit DNA synthesis and induce apoptosis. Especially, copper, palladium and platinum complexes of thiosemicarbazones are the most preferred compounds for drug potential studies (Otero et al. 2006; Matesanz and Souza 2007; Vieites et al. 2009; Raja et al. 2011). Furthermore, some significant findings regarding biological activities of vanadium (Noblia et al. 2005), manganese (Oliveira et al. 2014), iron and nickel (Bal Demirci et al. 2007; Atasever et al. 2010) chelates based on thiosemicarbazone have been shown.

Unfortunately, drug resistance still remains a major problem leukemias. Therefore developing new agents against

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leukemic cells is needed. Structures and biological activity make thiosemicarbazones strong therapeutics candidates.

In our previous studies, we demonstrated antileukemic activity of the iron (III) complexes of S-methylthiosemicarbazones with *ONNO* type. These previous results imply that their selective anticancer effects on various leukemic cells depends on not only metal ion in thiosemicarbazone complexes but also substituents and their location on aromatic rings. In this study, to analyse importance aromatic ring and OCH_3 group, we investigated effects of the new synthesized N1-acetylacetone-N4-4-methoxy-salicylidene-S-allyl-thiosemicarbazone chelating with nickel(II) against HL60 human promyelocytic leukemia cells (Figure 1). Specifically we studied its effects on intracellular signal nodes and apoptotic pathways in HL60 cells.

Materials and Methods

Synthesis of the compounds

Firstly, 2,4-pentanedione S-allyl-thiosemicarbazone was obtained as described in the literature (Yamazaki 1975). The cream colored compound melts at 144°C. Yield: 65%. Anal. Calc. for $C_9H_{15}N_3OS$ (213.29): C, 50.68; H, 7.09; N, 19.70; S, 15.03. Found: C, 50.65; H, 7.10; N, 19.71; S, 15.00 %. UV–Vis: 208 (5.57), 217 (5.58), 226 (5.58), 392 (3.71). IR: $\nu_{as}(NH_2)$ 3188, $\nu_s(NH_2)$ 3089, δ(NH), $\upsilon(C=N^1)$, $\upsilon(C=N^2)$ 1608-1555, ν(C-S) 725. ^{1}H NMR: 9.43, 8.83 (*cis*/*trans* ratio: 2/1, s, 2H, NH_2), 7.77, 7.56 (*cis*/*trans* ratio: 2/1, s, 1H, O*H*), 5.86 (m, 1H, C^2H), 5.36 (d, J=16.94, 2H, =C^3HH_a), 5.25 (d, J=9.76, 2H, =C^3HH_b) 3.95 (s, 2H, C-CH_2), 3.87 (dd, J= 5.85, J= 6.95, 2H, S-C^1H_2), 2.32 (s, 3H, C-CH_3), 2.28 (s, 3H, C-CH_3).

For synthesis of the nikel(II) complex, $NiCl_2.6H_2O$ (0.24 g, 1 mmol) was dissolved in methanol (5 ml) and then 1.5 ml of orthoformic ester was added to the solution. After standing for 24 h at room temperature, a solution of the thiosemicarbazone (0.21 g, 1 mmol) and 2-hydroxy-4-methoxybenzaldehyde (0.152 g, 1 mmol) in 5 ml methanol was added dropwise to the metal salt solution. After the additon of triethylamine (0.1 mmol), the mixture was left to stand at room temperature overnight. The red crystals was filtered, and recrystalized from ethanol-dichloromethane. Yield: 45%. m.p. 189.5 °C. Anal. Calc. for $C_{17}H_{19}N_3NiO_3S$ (404.109 g): C, 50.53; H, 4.47; N, 10.40; S, 7.93. Found: C, 50.55; H, 4.50; N, 10.38; S, 7.90 %. UV–Vis: 228 (5.64), 306 (4.91), 339 (4.64) 355 (4.66), 409 (4.73), 425 (4.75), 515 (3.42). IR: $\nu(C=N^1)$ 1612, $\nu(C=N^2)$ 1583, $\nu(N^4=C)$ 1542, ν(C-O) 1173, 1135. ^{1}H NMR: 7.71(s, 1H, N^4=C*H*), 7.15 (d, *J*=9.27, 1H, *c*), 6.54 (s,1H, *a*), 6.3 (d, *J* = 8.78, 1H, *b*), 5.87 (m, 1H, C^2H), 5.23 (d, J=17.08, 2H, =C^3HH_a), 5.11 (d, J=9.76, 2H, =C^3HH_b), 5.16 (s, 1H, =C*H*), 3.74 (d, J= 7.32 2H, S-C^1H_2), 3.72 (s, 3H, O-CH_3), 2.25 (s, 3H, C-CH_3), 2.05 (s, 3H, C-CH_3).

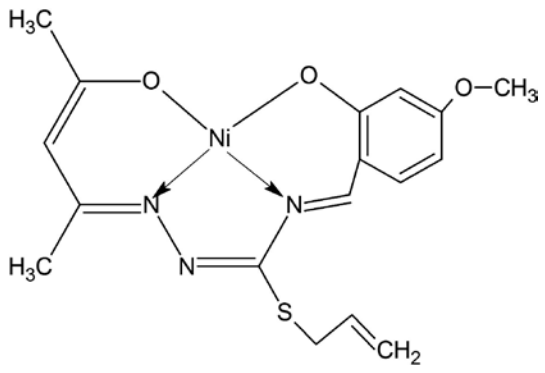

Figure 1. The N_2O_2 nickel complex.

Mammalian cell culture

Cytotoxic potential of Ni(II) thiosemicarbazone compound was assessed on HL60 cells. ECV304 (human umbilical vein endothelial cell line) was used as non-cancerous cellular control because ECV304 cell line presents many features of endothelial cells (Suda et al. 2001). The cell lines were purchased from ATCC.

RPMI medium for suspension cells (Panbiotech P04-16100) and DMEM medium for adhesive cells (HyClone, 16777-133) were supplemented with 10% fetal bovine serum (HyClone, SH3007003HI), 1% penicillin-streptomycin, 1% L-Glutamine and 0.1%, and MEM non-essential amino acids. Cell lines were incubated in a humidified atmosphere containing 5% CO_2 at 37°C. 75 ml polystyrene cell culture flasks and 96-well plates (Greiner) were used for cell culture and viability assays, respectively. Adhesive ECV304 cells, were detached by 0.5% Trypsin-EDTA solution (Sigma, T3924), washed once with 10% FBS containing medium and twice with PBS and resuspended in DMEM at density of 1×10^5 cells/ml.

Cytotoxicity assay

The cytotoxic effect of Ni(II) thiosemicarbazone compound on HL60 human promyelocytic leukemia and ECV304 human endothelial cells were measured with MTT (3-(4,5-dimethylthiazol-2yl)2,5-diphenyl tetrazolium bromide) assay (Sigma, M-5655) as previously described Pirildar et al. (2010), Svobodova et al. (2012) and Atasever-Arslan et al. (2015). HL60 cells were resuspended at 1×10^6 cells/ml in RPMI medium, ECV304 cells were resuspended at 1×10^6 cells/ml in DMEM medium at 37°C in a humidified atmosphere containing 5% CO_2.

Stock solutions of the thiosemicarbazone were prepared in DMSO at a concentration of 10 mg/ml. Serial dilutions of the stock solution were prepared in absolute methanol.

90 µl of cell cultures were dispensed into 96-well round-bottom plates containing 10 µl of Ni(II) thiosemicarbazone compound dilutions. Thus, the final concentrations of the compound were 124, 25, 12, 2.5, 0.25 µmol/l. As a negative control, only 10 µl of medium containing appropriately diluted methanol was used instead of Ni(II) thiosemicarbazone compound. After 48 hours of incubation, 10 µl freshly prepared MTT solution (5 mg/ml) in phosphate buffer saline (PBS) was added to each well and the plates were incubated 3 h at 37°C. Supernatants were removed from all wells and 100 ml of sodium dodecyl sulfate (SDS, pH 5.5) containing isopropyl alcohol was added to the wells and the microplates were stored at room temperature in the dark, in order to dissolve the formazan crystals formed by reduction of MTT in living cells. Optical density (OD) of each well was measured at 570 nm test wavelength and at 655 nm reference wavelength on a Bio-Rad Benchmark Microplate Reader. Cytotoxicity index was calculated with the formula:

Cytotoxicity index = 1– [OD (treated wells) / OD (control wells)] ×100). The cytotoxicity assay was repeated six times for each concentration of the compound.

According to MTT results, inhibitory concentration (IC_{50} = the concentration of the compound that inhibited 50% cells) was calculated from the data obtained using GraphPad Prism software.

Flow cytometry analysis

To determine the apoptotic and necrotic effects of the Ni(II) thiosemicarbazone compound on HL60 cells, we stained with Annexin V and Propodium iodide (PI) (Biolegend Inc.) and analyzed by flow cytometry (Atasever-Arslan et. al. 2016). Treated HL60 cells, were harvested from plates by centrifugation to remove the media and then washed with 1XFACS buffer (1X HBSS, 0.5% sodium azide and 0.5% BSA). Annexin V was diluted 1:100 with Annexin V binding buffer (0.1 M HEPES/NaOH (pH 7.4), 1.4 M NaCl, 0.05 M $CaCl_2$) and cells were resuspended in 500 µl of this mixture. Samples were incubated for 20 minutes at room temperature in the dark, stored on ice and PI (124 µmol/l) was added immediately before analysis. Flow cytometric measurement of the samples was performed on a FACS Canto flow cytometer (BD Biosciences). Flow cytometry was repeated three times for each concentration of the compound. Data were analyzed with FlowJo 9.4.3 software (Treestar).

DNA fragmentation assay

After HL60 cells was incubated with the Ni(II) thiosemicarbazone compound (124 µmol/l) for 24 hours, cells were collected by centrifugation at 13,200 rpm for 20 seconds at room temperature. The supernatant was discarded and the pellet was transferred to a fresh microcentrifuge tube containing 600 µl of ice-cold cell lysis buffer (10 mM Tris-Cl (ph8), 1 mM EDTA (ph8), %0.1 (w/v) SDS). Three µl of proteinase K solution (20 mg/ml) was added and incubated for 3 hours at 55°C followed by 1.5 µl of RNAse (4mg/ml) and an additional incubation for 15 min at 37°C. Then, samples were cooled down to room temperature and 200 µl potassium acetate solution was added and mixed. The samples were centrifuged (13,200 rpm) for 3 minutes at 4°C. Supernatants were transferred to fresh microcentrifuge tubes followed by the addition of 600 µl isopropanol. The solution was mixed well and centrifuged at 13,200 rpm for 1 min at room temperature. The supernatant was removed and 600 µl of 70% ethanol was added to the DNA pellet. The tube was inverted several times and centrifuged at 13,200 rpm for 1 min at room temperature. The supernatant was removed and the DNA pellet air dried for 15 min before being dissolved in 100 µl TE buffer (Takaki et al. 2014). Isolated genomic DNA was resolved by 2% agarose gel electrophoresis for 40 minutes at 100 V, stained in an Ethidium bromide solution (0.625 mg/ml) for x minutes and analyzed on a gel imaging system (Vilber Lourmat -02). The DNA fragmentation analyses were repeated twice.

Spectrophotometric analysis of intracellular signal nodes and apoptotic signal pathways

CST PathScan® Signaling Nodes and Apoptosis Multi-Target Sandwich ELISA kits (Cell Signaling Technologies, USA) were used to determine the effects of Ni(II) thiosemicarbazone compound on HL60 cells on signaling nodes and intracellular apoptotic signal pathways. Ni(II)-treated HL60 cells were processed according to the manufacturer's instructions. Briefly, 1 million cells were lysed in lysis buffer, lysates aliquoted onto 96 well plates coated with antibodies and a sandwich ELISA was performed using specific antibodies and HRP labelled secondary antibodies, incubated with substrate. Assays were repeated six times. HRP enzymatic activity detected on Multiskan™ GO Microplate Spectrophotometer (Spectrophotometric Determination) to quantify antigen concentration. Optical density (OD) of each well was measured at 450 nm test wavelength.

Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) software. Results were expressed as the mean ± standard deviation (SD). Statistical differences were assessed by Student's unpaired *t*-test, with $p < 0.05$ as a statistical significance cut-off.

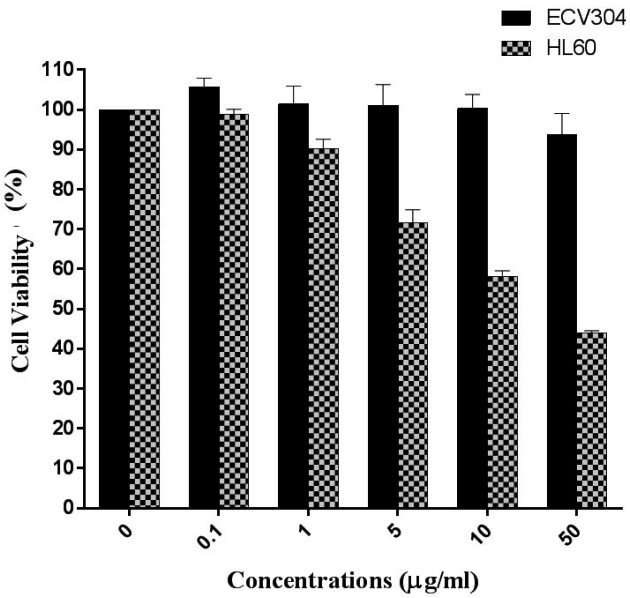

Figure 2. Cytotoxic effects of Ni(II) thiosemicarbazone compound against HL60 and ECV304 cell lines. The graphs show level of cell viability according to concentration of the compound. Cell viability of control group was accepted 100% and decrease in cell viability has been shown at 0.25, 2.5, 12, 25 and 124 µmol/l, respectively.

Results

A nickel(II) complex with N_2O_2 chelating N1-acetylacetone-N4-4-methoxy-salicylidene-S-allyl-thiosemicarbazone ligand was synthesized and confirmed expected structure by elemental analysis, infrared and ^{1}H NMR spectra.

We investigated cytotoxic effect of Ni(II) thiosemicarbazone compound on HL60 human promyelocytic leukemia cells by using MTT cytotoxicity assay. As a control group, we used ECV304 human endothelial cells. HL60 and ECV304 cells were treated with Ni(II) thiosemicarbazone compound at different concentration (124, 25, 12, 2.5, 0.25 µmol/l) and cell viability was measured *via* MTT assay. Figure 2 shows the cytotoxic activity of Ni(II) thiosemicarbazone compound against HL60 human promyelocytic leukemia cells and ECV304 human endothelial cells. Ni(II) thiosemicarbazone compound did not show a significant cytotoxicity on ECV304 non-cancer control group cells. In addition, this compound caused proliferation of ECV304 cells. Ni(II) thiosemicarbazone compound showed cytotoxic activity above 55% against HL60 cells in 124 µmol/l (Figure 2). Furthermore, it showed about 40% cytotoxicity on HL60 cells in 25 µmol/l. According to the cytotoxicity results, IC_{50} values of HL60 and ECV304 cells are 58.8 µmol/l and >124 µmol/l, respectively. Apoptotic effect of the Ni(II) thiosemicarbazone compound on HL60 cells was analysed by using flow cytometry analysis. In order to discriminate apoptotic effects from necrotic effects, Annexin V and PI staining were performed (Figure 3A). It was found that Ni(II) thiosemicarbazone compound had apoptotic effect on HL60 cells. Amount of Annexin V and PI double positive HL60 cells was 31.1% after 24 hours incubation with this compound in 124 µmol/l. On the other hand, only Annexin V positive HL60 cells constituted 12% of all cells. Our results showed total amount of apoptotic cells was 43.1% after 24 hours comparing with controls.

To confirm the apoptotic effects of this compound, we also used DNA fragmentation analysis. DNA fragmentation is one of the characteristics of apoptosis (McCarthy

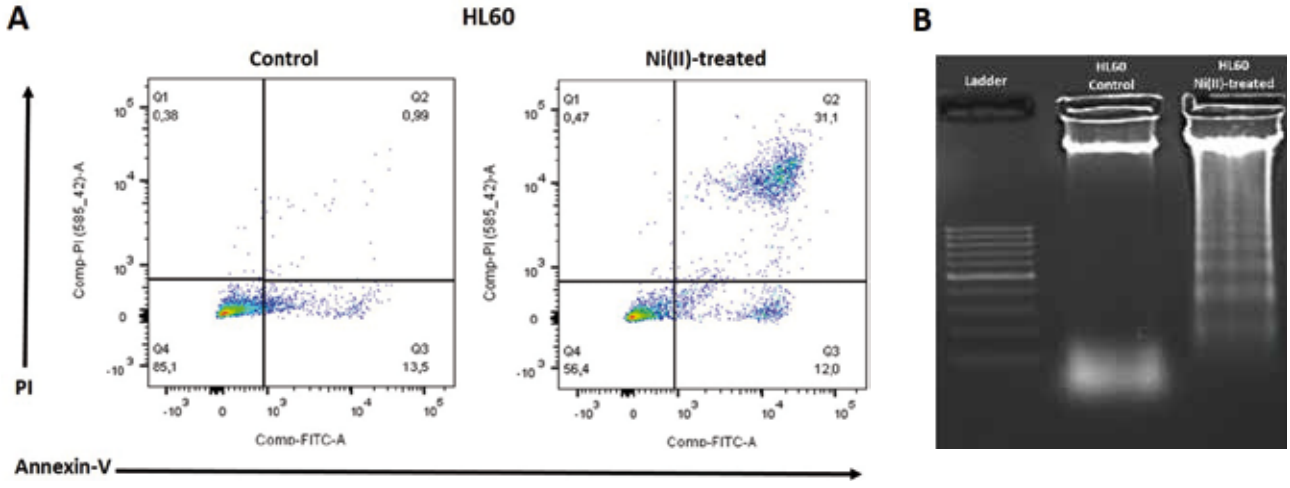

Figure 3. A. Flow cytometry analysis of apoptotic effects of Ni(II) thiosemicarbazone compound on HL60 cells. **B.** Screening apoptotic effects of Ni(II) thiosemicarbazone compound on HL60 with DNA fragmentation method.

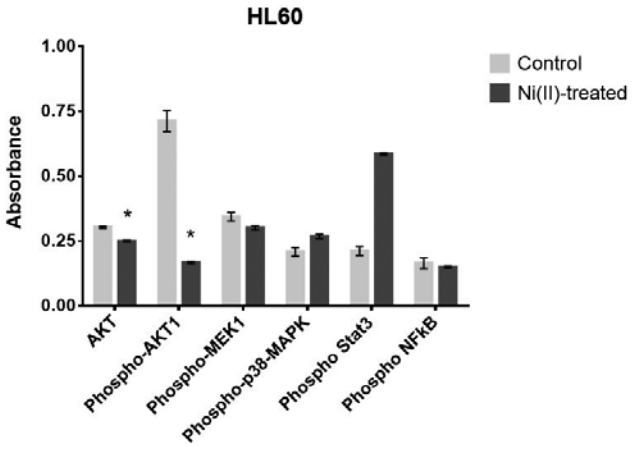

Figure 4. Effects of Ni(II) thiosemicarbazone compound on the levels of Phospho-Akt1, Phospho-MEK1, Phospho-p38-MAPK,Phospho-Stat3, Phospho-NFκB protein in HL60 cells. Optical density of each well was measured at 450 nm test wavelength. * $p < 0.05$.

and Evan 1998). Fragmented genomic DNA of HL60 cells that was treated with Ni(II) thiosemicarbazone compound was screened *via* DNA fragmentation assay (Figure 3B). Our flow cytometry analysis and DNA fragmentation assay results showed that the Ni(II) thiosemicarbazone compound induces apoptosis in HL60 cells (Figure 3). Because of this apoptotic properties, Ni(II) thiosemicarbazone compound may have anticancer drug potential for HL60 human promyelocytic leukemia cells.

In order to search the possible roles of Ni(II) thiosemicarbazone compound on the changes of the levels of intracellular signal nodes of HL60 cells, we measured levels of Phospho NFκB (Ser536), Phospho Stat3 (Tyr705), Phospho-p38 MAPK (Thr180/Tyr182), Phospho-MEK1 (Ser217/221) and Phospho-Akt1(Ser473) quantities in the cells treated with this Ni(II) thiosemicarbazone compound. We used Signaling Nodes Sandwich ELISA kits to determine the effects of the Ni(II) thiosemicarbazone compound on HL60 cells on intracellular signaling nodes. The results and their significances were shown in Figure 4. According to our results, Ni(II) thiosemicarbazone compound significantly decreased the level of Phospho-Akt1 protein. However, other protein levels did not change significantly after 24 hour incubation of HL60 cells with the Ni(II) thiosemicarbazone compound (Figure 4).

To understand apoptotic mechanisms of the Ni(II) thiosemicarbazone compound on HL60 cells, we investigated effects of Ni(II) thiosemicarbazone compound on the apoptotic signaling pathway by using Apoptosis Multi-Target Sandwich ELISA kit. HL60 cells were incubated with Ni(II) thiosemicarbazone compound and the changes levels of Phospho-p53(Ser15), p53, Cleaved caspase 3 (Asp175), Cleaved PARP(Asp214), Phospho-BAD(Ser112) and BAD proteins were determined. The results were shown in Figure 5. While the Ni(II) thiosemicarbazone compound decreased levels of cleaved PARP, phospho-Bad, Bad proteins, it significantly increased the levels of the p53 and cleaved caspase-3 proteins in HL60 cells.

Discussion

HL60 and ECV304 cells were treated with Ni(II) thiosemicarbazone compound at different concentration (124, 25, 12, 2.5, 0.25 μmol/l) and cell viability was measured *via* MTT (assay. Ni(II) compound showed cytotoxic activity above 55% against HL60 human promyelocytic leukemia cells in 124 μmol/l (Figure 2). Furthermore, it showed about 40% cytotoxicity on HL60 cells in 25 μmol/l. According to the cytotoxicity results, IC_{50} values of HL60 and ECV304 cells are 58.8 μmol/l and >124 μmol/l, respectively. Ni(II) thiosemicarbazone compound, of which IC_{50} value is not cytotoxic for ECV304 cells, have therapeutic potential as antitumor agents.

Apoptotic effect of the Ni(II) thiosemicarbazone compound on HL60 cells was analysed by using flow cytometry analysis. Results demonstrated that Ni(II) thiosemicarbazone compound had apoptotic effect on HL60 cells. Amount of both Annexin V and PI double positive HL60 cells was 31.1% after 24 hours incubation with this compound at 124 μmol/l concentration. Whereas, only Annexin V positive HL60 cells constituted 12% of all cells. Our results showed that total amount of apoptotic cells was 43.1% after 24 hours comparing with controls. Also DNA fragmentation assays demonstrated that the Ni(II) thiosemicarbazone compound induces apoptosis in HL60 cells (Figure 3). Their apoptotic properties imply that

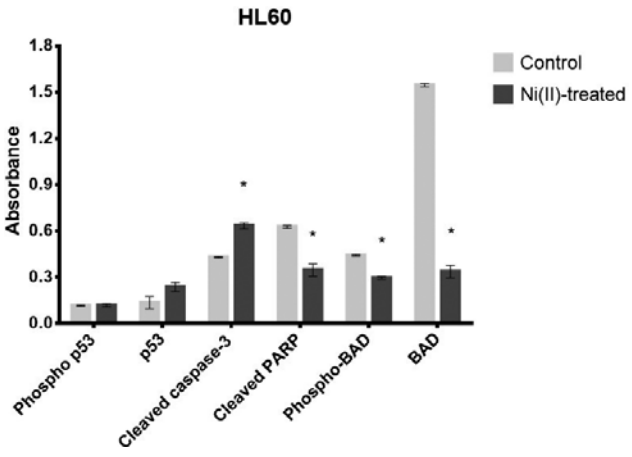

Figure 5. Effects of Ni(II) thiosemicarbazone compound on the levels of Phospho-p53,p53,Cleaved caspase 3,Cleaved PARP, Phospho-BAD and BAD protein in HL60 cells. Optical density of each well was measured at 450 nm test wavelength. * $p < 0.05$.

Ni(II) thiosemicarbazone compound may have anticancer drug potential for HL60 human promyelocytic leukemia cells.

In order to search whether a possible role of Ni(II) thiosemicarbazone compound on the changes of the levels of intracellular signal nodes of HL60 cells, we measured levels of Phospho NFκB (Ser536), Phospho Stat3 (Tyr705), Phospho-p38 MAPK (Thr180/Tyr182), Phospho-MEK1 (Ser217/221) and Phospho-Akt1(Ser473) quantities in the cells treated with this Ni(II) compound. According to our results, Ni(II) compound significantly decreased the level of Phospho-Akt1 protein (Figure 4).

Akt protein plays role in cell survival and apoptosis (Zhou et al. 2000). Activation of Akt has a fundamental role in cell survival signalling (Franke et al. 2003). Therefore, decreasing of Akt activation induces apoptosis by preventing cancer cell growth. Moreover, previous studies have showed that Akt inhibitors induce apoptosis in chronic lymphocytic leukemia cells (de Frias et al. 2009). In this study we showed Phospho-Akt1 protein level decreased with the Ni(II) thiosemicarbazone compound treatment on leukemic HL60 cell line compared with untreated HL60 cells. According to our data, the Ni(II) thiosemicarbazone compound decreased Akt1 level and induces apoptosis in HL60 cells.

To determine apoptotic mechanisms of the Ni(II) thiosemicarbazone compound, and the changes in levels of Phospho-p53(Ser15), p53, Cleaved caspase 3 (Asp175), Cleaved PARP(Asp214), Phospho-BAD(Ser112) and BAD proteins, HL60 cells were treated with Ni(II) thiosemicarbazone compound. While the Ni(II) thiosemicarbazone compound decreased levels of cleaved PARP, phospho-Bad, Bad proteins, it significantly increased the levels of the p53 and cleaved caspase-3 proteins in HL60 cells.

Protein p53 plays a crucial role in DNA repair, genomic stability, senescence, cell cycle control, and apoptosis (Harris 1996; Levine 1997; Baell and Huang 2002; Cory et al. 2003). Our results propose that increased level of p53 protein can stimulate apoptosis by inhibiting Bcl-2 anti-apoptotic protein and accelerate death process of cancer cells.

Moreover, caspases are important mediators of apoptosis. Especially, caspase-3 that is responsible for the proteolytic cleavage of many key proteins is a critical executioner of apoptosis (Porter and Jänicke 1999; Mcllwain et al. 2013). According to our result, increasing levels of cleaved caspase-3 protein with the Ni(II) thiosemicarbazone compound incubation can induce apoptosis in HL60 cells.

PARP protein plays role in repair of DNA damage and cell survival and is also a coactivator of NF-kB and NF-kB proteins linked each other in cell survival mechanism (Veuger et al. 2009; Nowsheen et al. 2012). We found that the Ni(II) thiosemicarbazone compound decreased levels of cleaved PARP protein and this result suggests that inhibition of PARP protein can inhibit cell survival and induce apoptosis.

Emirdağ-Öztürk et al. showed that Co(II) complexes of gypsogenin thiosemicarbazone glyoxime has apoptotic effect on HL60 cells. However, intracellular signalling pathways of this molecule in HL60 cells and its effects on non-cancerous cells are not known (Emirdag-Ozturk et al. 2014). Bioinformatic meta-analysis of interactions all new synthesized thiosemicarbazones in literature with intracellular signalling nodes in cancer cells can be helpful to understand importance of metal ions and structural properties of thiosemicarbazones against various cancer cells and non-cancerous cells.

In this study, we detected decreased Akt1 and phospho-BAD levels. BAD protein is a pro-apoptotic member of Bcl-2 gene family and pro-apoptotic activity of BAD can be inhibited by phosphorylation (Hsu et al. 1997). Several previous studies has suggested that BAD mediate the anti-apoptotic effects of PI3K/Akt pathway (Datta et al. 1997; del Peso et al. 1997). Akt protein phosphorylates BAD protein and blocks the dimerization of BAD with Bcl-2 or Bcl-xl (del Peso et al. 1997). Inhibitory effect of Ni(II) thiosemicarbazone compound on Akt prevents inhibition of BAD by decreasing phospho-BAD protein level. Moreover, the inhibition effect of Akt on phosphorylation of Bad might decrease level of antiapoptoic Bcl-2 and Bcl-xl protein and induce apoptosis (Fu et al. 2014).

Based on our result we suggest that decreasing level of phospho-BAD protein with the Ni(II) thiosemicarbazone compound can trigger apoptosis on HL60 leukemia cells. We analyzed and compared our results with the literature and predicted the possible apoptotic signaling pathways of the Ni(II) thiosemicarbazone compound on HL60 cells. Our results suggested that the Nickel(II) thiosemicarbazone compound, as a potential anticancer drug, could induce apoptosis through the inhibition of PI3K/Akt pathway or p53 pathway in HL60 cells. On the other hand, inhibition of PARP or BAD and increase caspase-3 levels are other possible apoptotic pathways for this new compound. Understanding of its apoptotic mechanism can be helpful to identity therapeutic index of this compound targeting various cancer cells. All these results imply that its structural properties are very important for selective cytotoxicity and apoptotic effect against HL60 cells.

Conflict of interest: The authors have no conflict of interest.

References

Afrasiabi Z., Sinn E., Chen J., Ma Y., Rheingold A. L., Zakharov L. N., Rath N., Padhye S. (2004): Appended **1,** 2-naphthoquinones as anticancer agents 1: synthesis, structural, spectral and antitumor activities of ortho-naphthaquinone thiosemicarbazone and its transition metal complexes. Inorg. Chim. Acta **357,** 271–278
http://dx.doi.org/10.1016/S0020-1693(03)00484-5

Atasever-Arslan B., Yilancioglu K., Bekaroglu M. G., Taskin E., Altinoz E., Cetiner S. (2015): Cytotoxic effect of extract from Dunaliella salina against SH-SY5Y neuroblastoma cells. Gen. Physiol. Biophys. **34,** 201–207
http://dx.doi.org/10.4149/gpb_2014034

Atasever-Arslan B., Yilancioglu K., Timucin A. C., Kalkan Z., Gur H., Deniz E., Erman B., Cetiner S. (2016): Screening of new antileukemic agents from essential oils of algae extracts and computational modeling of their interactions with intracellular signaling nodes. Eur. J. Pharm. Sci. **83,** 120–131
http://dx.doi.org/10.1016/j.ejps.2015.12.001

Atasever B., Ülküseven B., Bal-Demirci T., Erdem-Kuruca S., Solakoğlu Z. (2010): Cytotoxic activities of new iron(III) and nickel(II) chelates of some S-methyl-thiosemicarbazones on K562 and ECV304 cells. Invest. New Drugs **28,** 421–432
http://dx.doi.org/10.1007/s10637-009-9272-2

Baell J. B., Huang D. C. S. (2002): Prospects for targeting the Bcl-2 family of proteins to develop novel cytotoxic drugs. Biochem. Pharmacol. **64,** 851–863
http://dx.doi.org/10.1016/S0006-2952(02)01148-6

Bal Demirci T., Atasever B., Solakoğlu Z., Erdem-Kuruca S., Ülküseven B. (2007): Synthesis, characterisation and cytotoxic properties of the N1,N4-diarylidene-S-methyl-thiosemicarbazone chelates with Fe(III) and Ni(II). Eur. J. Med. Chem. **42,** 161–167
http://dx.doi.org/10.1016/j.ejmech.2006.09.004

Beraldo H., Gambino D. (2004): The wide pharmacological versatility of semicarbazones, thiosemicarba-zones and their metal complexes. Mini Rev. Med. Chem. **4,** 31–40
http://dx.doi.org/10.2174/1389557043487484

Casas J. S., Garcia-Tasende M. S., Sordo J. (2000): Main group metal complexes of semicarbazones and thiosemicarbazones. A structural review. Coord. Chem. Rev. **209,** 197–261
http://dx.doi.org/10.1016/S0010-8545(00)00363-5

Cory S., Huang D. C. S., Adams J. M. (2003): The Bcl-2 family: roles in cell survival and oncogenesis. Oncogene **22,** 8590–8607
http://dx.doi.org/10.1038/sj.onc.1207102

Datta S. R., Dudek H., Tao X., Masters S., Fu H., Gotoh Y., Greenberg M. E. (1997): Akt phosphorylation of BAD couples survival signals to the cell-intrinsic death machinery. Cell **91,** 231–241
http://dx.doi.org/10.1016/S0092-8674(00)80405-5

de Frias M., Iglesias-Serret D., Cosialls A. M., Coll-Mulet L., Santidrián A. F., González-Gironès D. M., de la Banda E., Pons G., Gil J. (2009): Akt inhibitors induce apoptosis in chronic lymphocytic leukemia cells. Haematologica **94,** 1698–1707
http://dx.doi.org/10.3324/haematol.2008.004028

De Logu A., Saddi M., Onnis V., Sanna C., Congiu C., Borgna R., Cocco M. T. (2005): In vitro antimycobacterial activity of newly synthesised S-alkylisothiosemicarbazone derivatives and synergistic interactions in combination with rifamycins against Mycobacterium avium. Int. J. Antimicrob. Agents **26,** 28–32
http://dx.doi.org/10.1016/j.ijantimicag.2005.03.005

del Peso L., González-García M., Page C., Herrera R., Nu-ez G. (1997): Interleukin-3-induced phosphorylation of BAD through the protein kinase Akt. Science **278,** 687–689
http://dx.doi.org/10.1126/science.278.5338.687

Emirdağ-Öztürk S., Babahan İ., Özmen A. (2014): Synthesis, characterization and in vitro anti-neoplastic activity of gypsogenin derivatives. Bioorg. Chem. **53,** 15–23
http://dx.doi.org/10.1016/j.bioorg.2013.12.001

Franke T. F., Hornik C. P., Segev L., Shostak G. A., Sugimoto C. (2003): PI3K/Akt and apoptosis: size matters. Oncogene **22,** 8983–8998
http://dx.doi.org/10.1038/sj.onc.1207115

Fu Z., Ren L., Wei H., Lv J., Che X., Zhu Z., Jia J., Wang L., Lin G., Lu R., Yao Z. (2014): Effects of Tyroserleutide on phosphatidylinositol 3'-kinase/AKT pathway in human hepatocellular carcinoma cell. J. Drug Target **22,** 146–155
http://dx.doi.org/10.3109/1061186X.2013.844820

Genova P., Varadinova T., Matesanz A. I., Marinova D., Souza P. (2004): Toxic effects of bis(thiosemicarbazone) compounds and its palladium(II) complexes on herpes simplex virus growth. Toxicol. Appl. Pharmacol. **197,** 107–112
http://dx.doi.org/10.1016/j.taap.2004.02.006

Harris C. C. (1996): Structure and function of the p53 tumor suppressor gene: clues for rational cancer therapeutic strategies. J. Natl. Cancer Inst. **88,** 1442–1455
http://dx.doi.org/10.1093/jnci/88.20.1442

Hsu S. Y., Kaipia A., Zhu L., Hsueh A. J. (1997): Interference of BAD (Bcl-xL/Bcl-2-associated death promoter)-induced apoptosis in mammalian cells by 14-3-3 isoforms and P11. Mol. Endocrinol. **11,** 1858–1867

Kesel A. J. (2011): Broad-spectrum antiviral activity including human immunodeficiency and hepatitis C viruses mediated by a novel retinoid thiosemicarbazone derivative. Eur. J. Med. Chem. **46,** 1656–1664
http://dx.doi.org/10.1016/j.ejmech.2011.02.014

Levine A. J. (1997): p53, the cellular gatekeeper for growth and division. Cell **88,** 323–331
http://dx.doi.org/10.1016/S0092-8674(00)81871-1

Matesanz A. I., Souza P. (2007): Palladium and platinum **3,**5-diacetyl-1,2,4-triazol bis(thiosemicarbazones): chemistry, cytotoxic activity and structure-activity relationships. J. Inorg. Biochem. **101,** 245–253
http://dx.doi.org/10.1016/j.jinorgbio.2006.09.024

McCarthy N. J., Evan G. I. (1998): Methods for detecting and quantifying apoptosis. Curr. Top. Dev. Biol. **36,** 259–278
http://dx.doi.org/10.1016/S0070-2153(08)60507-4

Mcllwain D. R., Berger T., Mak T. W. (2013): Caspase functions in cell death and disease. Cold Spring Harb. Perspect. Biol. **5,** a008656

Mendes I. C., Costa F. B., de Lima G. M., Ardisson J. D., Garcia-Santos I., Castineiras A., Beraldo H. (2009): Tin(IV) complexes with 2-pyridineformamide-derived thiosemicarbazones: Antimicrobial and potential antineoplasic activities. Polyhedron. **28,** 1179–1185
http://dx.doi.org/10.1016/j.poly.2009.01.028

Noblia P., Vieites M., Parajón-Costa B. S., Baran E. J., Cerecetto H., Draper P., González M., Piro O. E., Castellano E. E., Azqueta A., López de Ceráin A., Monge-Vega A., Gambino D. (2005): Vanadium(V) complexes with salicylaldehyde semicarbazone derivatives bearing in vitro anti-tumor activity toward kidney tumor cells (TK-10): crystal structure of [VVO2(5-bromosalicylaldehyde semicarbazone)]. J. Inorg. Biochem. **99,** 443–451
http://dx.doi.org/10.1016/j.jinorgbio.2004.10.019

Nowsheen S., Cooper T., Bonner J. A., LoBuglio A. F., Yang E. S. (2012): HER2 overexpression renders human breast cancers sensitive

to PARP inhibition independently of any defect in homologous recombination DNA repair. Cancer Res. **72,** 4796–4806 http://dx.doi.org/10.1158/0008-5472.CAN-12-1287

Oliveira C. G., Ivo da S., Maia P., Souza P. C., Pavan F. R., Leitec C. Q. F., Viana R. B., Batista A. A., Nascimento O. R., Deflon V. M. (2014): Manganese(II) complexes with thiosemicarbazones as potential anti-Mycobacterium tuberculosis agents. J. Inorg. Biochem. **132,** 21–29 http://dx.doi.org/10.1016/j.jinorgbio.2013.10.011

Otero L., Vieites M., Boiani L., Denicola A., Rigol C., Opazo L., Olea-Azar C., Maya J. D., Morello A., Krauth-Siegel R. L., Piro O. E., Castellano E., González M., Gambino D., Cerecetto H. (2006): Novel antitrypanosomal agents based on palladium nitrofurylthiosemicarbazone complexes: DNA and redox metabolism as potential therapeutic targets. J. Med. Chem. **49,** 3322–3331 http://dx.doi.org/10.1021/jm0512241

Pırıldar S., Sütlüpınar N., Atasever B., Erdem-Kuruca S., Papouskova B., Šimánek V. (2010): Chemical constituents of the different parts of Colchicum baytopiorum and their cytotoxic activities on K562 and HL60 cell lines. Pharm. Biol. **48,** 32–39 http://dx.doi.org/10.3109/13880200903029373

Porter A. G., Jänicke R. U. (1999): Emerging roles of caspase-3 in apoptosis. Cell Death Differ. **6,** 99–104 http://dx.doi.org/10.1038/sj.cdd.4400476

Raja D. S., Bhuvanesh N. S. P., Natarajan K. (2011): Biological evaluation of a novel water soluble sulphur bridged binuclear copper(II) thiosemicarbazone complex. Eur. J. Med. Chem. **46,** 4584–4594 http://dx.doi.org/10.1016/j.ejmech.2011.07.038

Rodriguez-Arguelles M. C., Lopez-Silva E. C., Sanmartin J., Pelagatti P., Zani F. (2005): Copper complexes of imidazole-2-, pyrrole-2- and indol-3-carbaldehyde thiosemicarbazones: inhibitory activity against fungi and bacteria. J. Inorg. Biochem. **99,** 2231–2239 http://dx.doi.org/10.1016/j.jinorgbio.2005.07.018

Suda K., Rothen-Rutishauser B., Günthert M., Wunderli-Allenspach H. (2001): Phenotypic characterization of human umbilical vein endothelial (ECV304) and urinary carcinoma (T24) cells: endothelial versus epithelial features. In Vitro Cell Dev. Biol. Anim. **37,** 505–514 http://dx.doi.org/10.1290/1071-2690(2001)037<0505:PCOHUV>2.0.CO;2

Svobodova H., Jost P., Stetina R. (2012): Cytotoxicity and genotoxicity evaluation of antidote oxime HI-6 tested on eight cell lines of human and rodent origin. Gen. Physiol. Biophys. **31,** 77–84 http://dx.doi.org/10.4149/gpb_2012_010

Takaki K., Higuchi Y., Hashii M., Ogino C., Shimizu N. (2014): Induction of apoptosis associated with chromosomal DNA fragmentation and caspase-3 activation in leukemia L1210 cells by TiO2 nanoparticles. J. Biosci. Bioeng. **117,** 129–133 http://dx.doi.org/10.1016/j.jbiosc.2013.06.003

Tarasconi P., Capacchi S., Pelosi G., Cornia M., Albertini R., Bonati A., Dall'Aglio P. P., Lunghi P., Pinelli S. (2000): Synthesis, spectroscopic characterization and biological properties of new natural aldehydes thiosemicarbazones. Bioorg. Med. Chem. **8,** 157–162 http://dx.doi.org/10.1016/S0968-0896(99)00260-6

Varadinova T., Kovala-Demertzi D., Rupelieva M., Demertzis M., Genova P. (2001): Antiviral activity of platinum (II) and palladium (II) complexes of pyridine-2-carbaldehyde thiosemicarbazone. Acta Virol. **45,** 87–94

Veuger S. J., Hunter J. E., Durkacz B. W. (2009): Ionizing radiation-induced NF-kappaB activation requires PARP-1 function to confer radioresistance. Oncogene **28,** 832–842 http://dx.doi.org/10.1038/onc.2008.439

Vieites M., Otero L., Santos D., Olea-Azar C., Norambuena E., Aguirre G., Cerecetto H., González M., Kemmerling U., Morello A., Diego Maya J., Gambino D. (2009): Platinum-based complexes of bioactive 3-(5-nitrofuryl)acroleine thiosemicarbazones showing anti-Trypanosoma cruzi activity. J. Inorg. Biochem. **103,** 411–418 http://dx.doi.org/10.1016/j.jinorgbio.2008.12.004

Vrdoljak V., DilovićI I., Rubcić M., Kraljević Pavelić S., Kralj M., Matković-Calogović D., Piantanida I., Novak P., Rozman A., Cindrić M.(2010): Synthesis and characterisation of thiosemicarbazonato molybdenum(VI) complexes and their in vitro antitumor activity. Eur. J. Med. Chem. **45,** 38–48 http://dx.doi.org/10.1016/j.ejmech.2009.09.021

Yamazaki C. (1975): The structure of isothiosemicarbazones. Can. J. Chem. **53,** 610–615 http://dx.doi.org/10.1139/v75-085

Yanardag R., Bal Demirci T., Ülküseven B., Bolkent S., Tunali S., Bolkent S. (2009): Synthesis, characterization and antidiabetic properties of N(1)-2,4-dihydroxybenzylidene-N(4)-2-hydroxybenzylidene-S-methyl-thiosemicarbazidato-oxovanadium(IV). Eur. J. Med. Chem. **44,** 818–826 http://dx.doi.org/10.1016/j.ejmech.2008.04.023

Zhou H., Li X. M., Meinkoth J., Pittman R. N. (2000): Akt regulates cell survival and apoptosis at a postmitochondrial level. J. Cell Biol. **151,** 483–494 http://dx.doi.org/10.1083/jcb.151.3.483

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