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Anticancer activities and mechanism of action of 2 novel metal complexes, $C_{16}H_{34}N_8O_5Ag_2Cd$ and $C_{11}H_{16}N_7O_2Ag_3Ni$

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Abstract: The discovery of anticancer activity in cisplatin triggered the development of novel drugs containing metals such as platinum or ruthenium. Extremely diverse structural chemistry and the interaction of metal complexes with biomolecules resulted in the exploration of novel metal complexes with drug potential. In the present study, the anticancer and cytotoxic activities and the mechanisms of action were investigated for $C_{16}H_{34}N_8O_5Ag_2Cd$ (**AN1**) and $C_{11}H_{16}N_7O_2Ag_3Ni$ (**AN7**), 2 newly synthesized dicyanidoargentate(I) complexes. The anticancer and cytotoxic activities of **AN1** and **AN7** on several cancer cell lines were tested by cell proliferation and cytotoxic activity assays, respectively. The apoptotic and replication inhibitory potentials of the compounds were investigated using terminal deoxynucleotidyl transferase dUTP nick and labeling (TUNEL) and DNA topoisomerase inhibition assays. **AN1** and **AN7** showed significant ($P < 0.05$) anticancer activity and lower cytotoxicity against all cell lines tested. The TUNEL assay results indicated that **AN1** and **AN7** may inhibit cell proliferation by inducing apoptosis. The compounds showed very significant DNA topoisomerase I inhibitory activity. Based on the results, it is suggested that compounds **AN1** and **AN7** are potential anticancer drug candidates.

Key words: Metal complexes, anticancer activity, cytotoxic activity, apoptosis

1. Introduction

Cancer is considered the second most common disease causing death (Tuncer, 2008). It is estimated that 19.3 million new cancer cases will be detected per year by 2025. The incidence, morbidity, and mortality of cancer are expected to be much higher in developing countries such as Turkey. Efforts to cure cancer or reduce its occurrence will be significantly important (Tuncer, 2008).

Although many anticancer drugs have been used to treat cancer, they have some limitations, such as side effects, tumor specificity, and tumor cell resistance (Roche, 2002). Therefore, new anticancer drug candidates with few or no side effects need to be developed as alternatives to current chemotherapeutic drugs. Metals and metal compounds have been used in medicine as antiprotozoal, antiulcer, antiarthritic, antimalarial, antimicrobial, and anticancer drugs (Avendańo, 2008). Nowadays, the application of metal complexes in medicine is being investigated very extensively. Thati et al. (2007) reported that silver complexes of coumarin derivatives possess anticancer activity against certain types of cancer. Zhu et al. (2003) reported that silver carboxylate dimers exert anticancer activity against human carcinoma cells. The phosphine complexes of silver have been shown to possess anticancer activity (Liu et al., 2008).

The discovery of the anticancer potential of cisplatin (Kelland et al., 2007) triggered the development of novel metallodrugs. Several platinum- or ruthenium-based metal coordination complexes such as carboplatin, oxaplatin, and NAMI-1 have been used or have been under trial as anticancer drugs (Gielen and Tiekink, 2005). The extremely diverse structural chemistry and interactions of metal complexes with biomolecules such as nucleic acids and proteins have resulted in the exploration of anticancer activities of other metal coordination complexes.

Various silver compounds with interesting antitumor activity have been reported previously. Zachariadis et al. (2004) determined the anticancer activity of silver(I) complexes of heterocyclic thioamide 2-mercapto-3,4,5,6-tetrahydropyrimidine derivatives against certain types of cancer. In addition, El-Din et al. (2011) reported the anticancer activity of $[SnMe_3(1,2\text{-bis}(4\text{-pyridyl})\text{ethane})][Ag(CN)_2].2H_2O$ against human carcinoma cells. In particular, silver complexes have been found to be promising alternatives to current anticancer drugs lacking activity against various cancer types. Serious

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limitations have prompted many researchers to develop alternative strategies to treat cancer based on different metal complexes.

In the present study, 2 silver complexes, $[Cd_2(edbea)_2][Ag(CN)_2]_2.H_2O$ (**AN1**) and $[Ni(N\text{-bishydeten})Ag_3(CN)_5]$ (**AN7**), were successfully synthesized and tested for their anticancer and cytotoxic activities against C6 (rat brain tumor), HeLa (human cervical cancer), HT29 (human colon cancer), and Vero (African green monkey kidney) cell lines. The solutions were made in a tube with concentrations ranging from 5 to 100 µg/mL for each compound. The antiproliferative and apoptotic activities of these compounds and ligand $[Ag(CN)_2]^1$ on cancer cell lines were screened using BrdU cell proliferation assay (BCPA) and DNA laddering assay, respectively. 5-Fluorouracil (5FU) was used as a standard compound. According to the BCPA test results, these compounds and ligand exhibited very high antiproliferative effects on cancer cells, whereas 5FU had very low antiproliferative effects with the same administered dose. Another goal was testing for cytotoxic or cytostatic activity in vitro against cancer cell lines. An ideal anticancer drug would destroy cancer cells without harming normal cells. The cytotoxic activities of these compounds and ligand were evaluated by lactate dehydrogenase (LDH) assay. Any compound that has cytotoxic effects may induce necrosis, in which cells lose membrane integrity and die rapidly as a result of cell lysis, or the cells can activate apoptosis. In LDH tests, these compounds showed the same cytotoxic effects as 5FU, whereas the ligand revealed higher cytotoxic effects than 5FU on cancer cell lines in a dose-dependent manner ($P < 0.05$).

The antiproliferative activities of these compounds on cancer cell lines were also screened using terminal deoxynucleotidyl transferase dUTP nick and labeling (TUNEL) assay and topoisomerase I assay. The results of the TUNEL assay indicated that the novel complexes may inhibit cell proliferation through the induction of apoptosis. Based on initial experiments, these compounds were selected for further preclinical development due to a potent antitopoisomerase I feature. The results indicated that the ligand does not have cytostatic activity, but the compounds do have such activity. In conclusion, the results demonstrated that these compounds possess favorable qualities as anticancer agents. Nevertheless, further studies are required to show effectiveness in animal models.

2. Materials and methods

2.1. Synthesis of $[Cd_2(edbea)_2][Ag(CN)_2]_2.H_2O$ (AN1) and $[Ni(N\text{-bishydeten})Ag_3(CN)_5]$ (AN7)

Edbea (2,2′-(ethylenedioxy)bis(ethylamine)) and N-bishydeten (N,N-bis(2-hydroxyethyl) ethylenediamine) were used as ligands that can coordinate to a metal ion (Ni or Cd) through their donor atoms. Dicyanidoargentate $\{[Ag(CN)_2]^-\}$ is a linear anion of considerable stability and simplicity. The novel complexes $[Cd_2(edbea)_2][Ag(CN)_2]_2.H_2O$ (**AN1**) and $[Ni(N\text{-bishydeten})Ag_3(CN)_5]$ (**AN7**) were successfully synthesized as described by Korkmaz (2014). **AN1** was obtained at a yield of 62%. The elemental analysis calculated (%) for $C_{16}H_{34}N_8O_5Ag_2Cd$ indicated C 25.74, H 4.59, N 15.01, and the following were found: C 26.53, H 5.66, N 16.08. IR spectra (KBr disk cm^{-1}) 3631 [υ(O–H)]; 3378, 3336, 3282 [υ(N–H)]; 2923, 2877, 2897 [υ(C–H)]; 2154 [υ(C≡N)]; 1587 [δ(N–H)]; 1743 [$\delta(CH_2)$]; 1101 [υ(C-N)]; 1033 [υ(C-O)]. **AN7** was obtained at a yield of 35%. The elemental analysis calculated (%) for $C_{11}H_{16}N_7O_2Ag_3Ni$ indicated C 20.00, H 2.44, N 14.84, and the following were found: C 19.68, H 2.14, N 14.78. IR spectra (KBr disk cm^{-1}) 3596 [υ(O–H)]; 3336, 3280, 3191 [υ(N–H)]; 2979, 2904, 2861 [υ(C–H)]; 2163, 2129 [υ(C≡N)]; 1602 [δ(N–H)]; 1452 [$\delta(CH_2)$]; 1197 [υ(C-N)]; 1031 [υ(C-O)].

2.2. Cell culture

C6, HT29, HeLa, and Vero cell lines (American Type Culture Collection, USA) were maintained in Dulbecco's modified Eagle's medium (DMEM, Sigma, Germany) supplemented with 10% (v/v) fetal bovine serum (Sigma) and PenStrep solution (10,000 U/10 mg) (Sigma) (Murphy et al., 2009; Yoshimoto et al., 2012). At confluence, cells were detached from the flasks using 4 mL of trypsin-EDTA (Sigma) and centrifuged, and the cell pellet was resuspended with 4 mL of supplemented DMEM.

2.3. Cell proliferation assay

A cell suspension containing 3×10^3 cells in 100 µL was pipetted into the wells of 96-well cell culture plates (COSTAR, Corning, USA). The test compounds (**AN1**, **AN7**, and $[Ag(CN)_2]^{1-}$) and a positive control compound (5FU) were dissolved in sterile dimethyl sulfoxide (DMSO). The amount of DMSO was adjusted to 0.5% maximum. The cells were treated with **AN1**, **AN7**, $[Ag(CN)_2]^{1-}$, and 5FU at final concentrations of 5, 10, 20, 30, 40, 50, 75, and 100 µg/mL. Cell controls and solvent controls were treated with supplemented DMEM and sterile DMSO, respectively. The final volume of the wells was adjusted to 200 µL by supplemented DMEM.

The cells were then incubated at 37 °C with 5% CO_2 overnight. The antiproliferative activity of the compounds was determined using a BrdU cell proliferation enzyme-linked immunosorbent assay (ELISA) kit according to the manufacturer's protocol (Roche, USA) for a calorimetric immunoassay based on BrdU incorporation into the cellular DNA. Briefly, cells were exposed to BrdU labeling reagent for 4 h, followed by fixation in FixDenat solution for 30 min at room temperature. Cells were then cultured with a 1:100 dilution of anti-BrdU-POD for 90 min at room temperature. Substrate solution was added to each

well, and BrdU incorporation was measured at 450–650 nm using a microplate reader (Rayto, China). Each experiment was repeated at least 3 times for each cell line.

2.4. Calculation of IC_{50} and % inhibition

IC_{50} represents the concentration of an agent that is required for 50% inhibition in vitro. The half maximal inhibitory concentration (IC_{50}) of the test and control compounds was calculated using XLfit5 software (IDBS) and expressed in μg/mL at 95% confidence intervals. The cell proliferation assay results were reported as the percent inhibition of the test and control substances. The percent inhibition was calculated according to the following formula: % inhibition = [1 - (absorbance of treatments / absorbance of DMSO) × 100].

2.5. Cytotoxic activity assay

The cytotoxicity of **AN1**, **AN7**, $[Ag(CN)_2]^{1-}$, and 5FU on C6, HT29, HeLa, and Vero cells was determined with a LDH cytotoxicity detection kit (Roche) based on the measurement of LDH activity released from the cytosol of damaged cells into the supernatant according to manufacturer's instructions. Briefly, 3×10^4 cells in 100 μL were seeded into 96-well microtiter plates as triplicates and treated with IC_{50} concentrations of **AN1**, **AN7**, and the ligand as described above at 37 °C with 5% CO_2 overnight. LDH activity was determined by measuring absorbance at 492–630 nm using a microplate reader.

2.6. TUNEL assay

In vitro detection of apoptosis was assessed using a TUNEL assay kit (Roche) according to the manufacturer's protocol. HT29 cell lines (30,000 cells/well) were placed in a poly-L-lysine covered chamber slide. The cells were treated with the IC_{50} concentrations of **AN1** and **AN7** and left for 24 h of incubation. There were 2 controls for this assay: a positive control that had DNase I treatment, and a negative control that had no terminal deoxynucleotidyl transferase (TdT).

When the incubation time was over, the chamber was removed from the slide and washed with Dulbecco's phosphate buffered saline (DPBS) to remove the medium and unattached cells. All of the incubation and washing steps were done in a plastic jar. Slides were gently washed with DPBS, and, for fixation, 4% paraformaldehyde in DPBS at pH 7.4 was freshly prepared and added to the slides for 60 min at room temperature. Following incubation, the slides were washed twice with DPBS. The cells were blocked with freshly prepared 3% H_2O_2 in methanol for 10 min at room temperature. Following incubation, the slides were washed twice with DPBS.

The cells were permeabilized by prechilled 0.1% Triton X-100 and freshly prepared 0.1% sodium citrate in water and then incubated for 2 min on ice. All the slides were washed with DPBS twice for 5 min each. At this point, in order to prepare a DNase I enzyme-treated positive control, 100 μL of DNase I buffer was added to the slide and incubated at room temperature for 10 min. Fixative cells were transferred into a TUNEL reaction mixture (50 μL/section) containing TdT and fluorescein-dUTP. Intracellular DNA fragments were then labeled by exposing the cells to the TUNEL reaction mixture for 1 h at 37 °C in a humidified atmosphere, protected from light. After washing with DPBS twice, cells positive for apoptosis showed a green fluorescent signal and were visualized by a Leica fluorescent microscope (Leica DMIL LED Fluo, Germany).

2.7. DNA topoisomerase I inhibition assay

The DNA topoisomerase I inhibitory activities of **AN1** and **AN7** were evaluated using a cell-free topoisomerase I assay kit (TopoGen, USA). The principle of the assay is to measure the conversion of supercoiled pHOT1 plasmid DNA to its relaxed form in the presence of DNA topoisomerase I alone and with test compounds. The supercoiled substrate (pHOT1 plasmid DNA) and its relaxed product can easily be distinguished in agarose gel, because the relaxed isomers migrate more slowly than the supercoiled isomer. In brief, 20 μL of reaction mixture containing 1 μL of plasmid pHOT1 DNA in relaxation buffer was incubated with 2 U of recombinant human topoisomerase I enzyme in the presence of IC_{50} concentrations of C1, C2, or camptothecin as a positive control. The reactions were carried out at 37 °C for 30 min and then terminated by the addition of stop solution. After the termination, the sample was analyzed using 1% agarose gel at 4 V/cm for 60 min. After electrophoresis, DNA bands were stained with ethidium bromide (1 mg/mL) solution and photographed with a gel imaging system (UVP BioSpectrum, Germany).

2.8. Statistical analysis

The statistical significance of differences was determined by one-way analysis of variance (ANOVA) tests. Post hoc analyses of group differences were performed using the Tukey test, and the levels of probability were noted. SPSS for Windows was used for statistical analyses. The results are reported as the mean values ± standard error of the mean (SEM) of 3 independent assays, and differences between groups were considered to be significant at $P < 0.05$.

3. Results

3.1. Anticancer activity of AN1, AN7, and $[Ag(CN)_2]^{1-}$ against HT-29, HeLa, C6, and Vero cell lines

The anticancer activities of **AN1**, **AN7**, $[Ag(CN)_2]^{1-}$, and 5FU against HT-29, HeLa, C6, and Vero cell lines were determined using a BrdU cell proliferation ELISA kit (Figure 1). $[Ag(CN)_2]^{1-}$ was the most antiproliferative compound

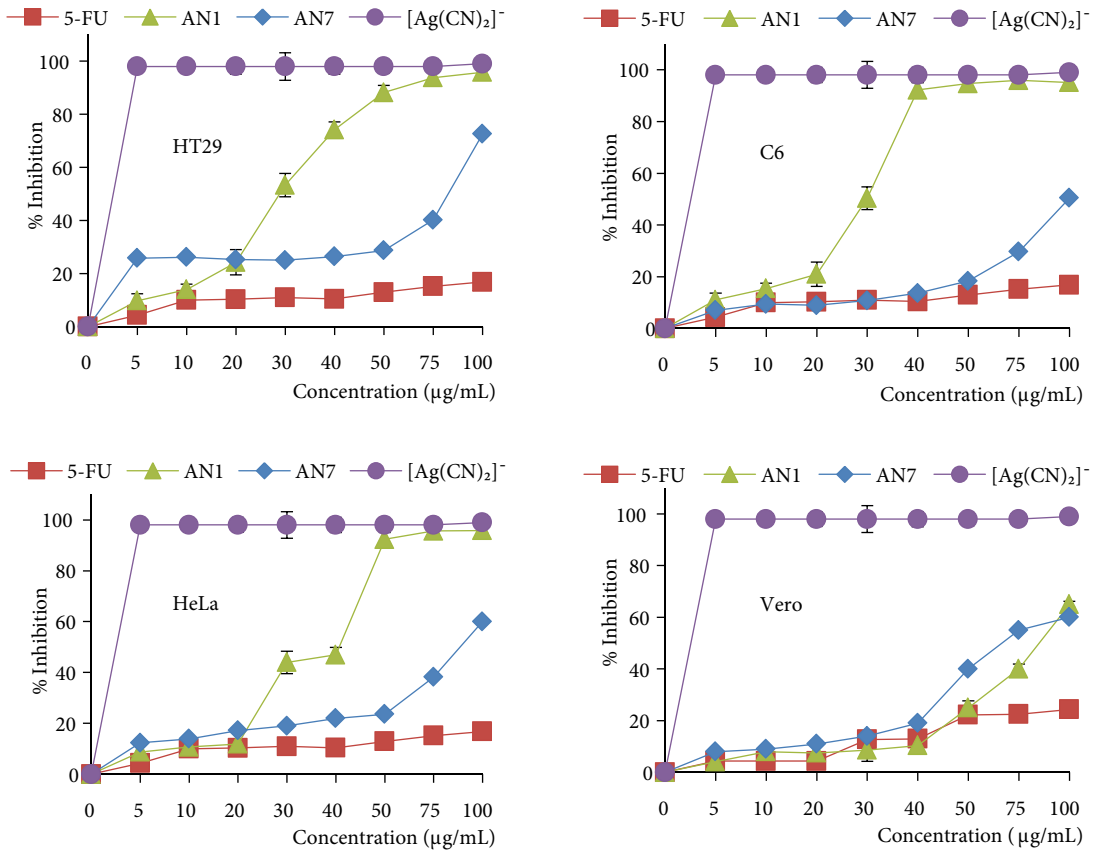

Figure 1. Antiproliferative activity of **AN1**, **AN7**, $[Ag(CN)_2]^{1-}$, and positive control compound (5FU) on HeLa, HT-29, C6, and Vero cell lines. Cell proliferation was measured using a BrdU cell ELISA assay kit. Percent inhibition was reported as mean value ± SEM of 3 independent assays ($P < 0.05$).

tested on all cell lines ($P < 0.01$), but it was found to be significantly toxic to the cells at concentrations of 5 µg/mL and higher (Figure 1). $[Ag(CN)_2]^{1-}$ was necrotic to cells, which may lead to rapid cell-membrane disintegration. **AN1** and **AN7** showed significantly ($P < 0.05$) higher antiproliferative activity than 5FU against the cancer cell lines tested, especially at high concentrations. Moreover, **AN1** and **AN7** were not necrotic to cells. In contrast to the cancer cell lines, the antiproliferative activities of the compounds were lower on nontumorigenic Vero cells (Figure 1). The data from the experiments suggest that the IC_{50} concentrations of **AN1**, **AN7**, the Ag ligand, and 5FU were 29.89, 65.15, 1.13, and 20.76 µg/mL for HeLa; 24.15, 70.39, 1.05, and 22.55 µg/mL for C6; 25.56, 56.09, 1.02, and 21.52 µg/mL for HT29; and 27.62, 58.19, 1.20, and 23.12 µg/mL for Vero, respectively.

3.2. Cytotoxic activity of AN1, AN7, $[Ag(CN)_2]^{1-}$, and 5FU on HT-29, HeLa, C6, and Vero cell lines

The cytotoxic activities of **AN1**, **AN7**, $[Ag(CN)_2]^{1-}$, and 5FU on HeLa, HT29, C6, and Vero cell lines were tested using an LDH cytotoxicity assay kit. In contrast to the high cytotoxicity of the ligand on the cell lines, the cytotoxicities of **AN1** and **AN7** were close to the cytotoxicity of 5FU at their IC_{50} concentrations (Figure 2). The percent cytotoxicity of **AN1**, **AN7**, and 5FU ranged from 15% to 25%, whereas $[Ag(CN)_2]^{1-}$ showed about 60% cytotoxicity ($P < 0.05$) against all cell lines (Figure 2). The results from the LDH assay were consistent with cell viability regarding the IC_{50} concentrations of **AN1** and **AN7**. Therefore, it is suggested that these compounds may have cytostatic potential rather than cytotoxic potential. The significantly lower cytotoxicity of **AN1** and **AN7** than $[Ag(CN)_2]^{1}$ alone may indicate that the cytotoxicity of $[Ag(CN)_2]^{1-}$ decreases to safe levels when complexed with (2,2′-(ethylenedioxy)bis(ethylamine)) and (N,N-bis(2-hydroxyethyl) ethylenediamine) in **AN1** and **AN7**, without reducing their antiproliferative potential.

3.3. Determination of apoptotic potential of AN1 and AN7

The apoptotic potential of **AN1** and **AN7** on HT29 cell lines was tested by TUNEL assay. As shown in Figure 3, **AN1**, **AN7**, and DNase I (PC, positive control) treated cells showed green fluorescence, indicating the fragmented DNA in apoptotic cells (A-AN1, B-AN7, and D-PC),

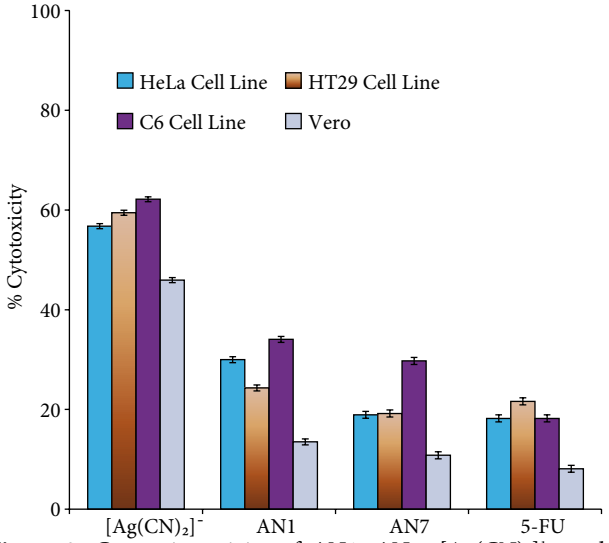

Figure 2. Cytotoxic activity of **AN1**, **AN7**, $[Ag(CN)_2]^{1-}$, and positive control compound (5FU) on HeLa, HT29, C6, and Vero cell lines. Cell lines were incubated with IC_{50} concentrations of test and control compounds for 24 h, and cytotoxicity was determined by LDH cytotoxicity assay kit. IC_{50} concentrations of **AN1**, **AN7**, and ligands Ag and 5FU were 29.89, 65.15, 1.13, and 20.76 µg/mL for HeLa; 24.15, 70.39, 1.05, and 22.55 µg/mL for C6; 25.56, 56.09, 1.02, and 21.52 µg/mL for HT29; and 27.62, 58.19, 1.20, and 23.12 µg/mL for Vero, respectively. Percent cytotoxicity was reported as mean value ± standard deviation of 3 independent assays ($P < 0.05$).

whereas the DMSO control was TUNEL-negative (C-NC). Phase-contrast images of the HT29 cancer cells (A′-AN1, B′-AN7, C′-NC (negative control), and D′-PC (positive control)) also overlapped the fluorescence images of the HT29 cancer cells. The TUNEL method indicated the presence of apoptosis in the HT29 cell line with treatments of 25.56 µg/mL for **AN1** and 56.09 µg/mL for **AN7** for 24 h. As in the case of image analysis, **AN1** and **AN7** caused more apoptosis in HT29 cells than the positive control.

3.4. Detection of DNA topoisomerase I inhibitory activity

The DNA topoisomerase I inhibitory activities of **AN1** and **AN7** were determined by DNA topoisomerase I inhibition assay. As shown in Figure 4, both compounds significantly inhibited the DNA relaxation activity of DNA topoisomerase I, similarly to the positive control drug, camptothecin. The results may indicate that the compounds inhibit cell proliferation by the suppression of DNA topoisomerase I action during replication. DNA topoisomerase I is a nuclear enzyme that plays essential roles in controlling the topological state of DNA to facilitate and remove barriers for vital cellular functions, including DNA replication and repair. Therefore, DNA topoisomerase I is an important target for anticancer agents.

4. Discussion

Although there has been promising progress in cancer research and the development of various anticancer drugs for chemotherapy, about 8.2 million cancer deaths were recorded in 2012 alone (http://globocan.iarc.fr/Pages/fact_sheets_cancer.aspx). Many cancer drugs have been withdrawn because of serious side effects, a loss of sensitivity to these drugs, and limited use for various tumor types. Therefore, the development of novel drugs with universal anticancer activity and no side effects is still important. The discovery of cisplatin (Kelland et al., 2007) resulted in the development and use of novel metallodrugs such as carboplatin, oxaplatin, and NAMI-1 in medicine (Gielen and Tiekink, 2005).

In the present study, the anticancer potential, cytotoxic activity, and mechanisms of action of newly synthesized metal complexes **AN1** and **AN7** were investigated on several tumor cell lines, such as HeLa, HT29, and C6. The reason we chose these tumor cell lines was the higher incidence, prevalence, and mortality of cervical, colon, and brain cancers in the world. The results of cell proliferation assay showed that these 2 metal compounds were significantly more antiproliferative than 5FU (Figure 1) against tumor cell lines but not nontumorigenic Vero cell lines, indicating their anticancer potential, as in previous studies (Iqbal et al., 2013; Medvetz et al., 2008; Wang et al., 2010). It is known that cisplatin-like metallodrugs inhibit cell proliferation by binding to cell DNA; however, it is not clear yet whether **AN1** and **AN7** bind cell DNA. In addition, **AN1** and **AN7** complexes were as cytotoxic as 5FU against all tumor cell lines tested at their IC_{50} concentrations (Figure 2). Therefore, we suggest that these compounds may have cytostatic potential rather than cytotoxic potential. It is very clear that the cytotoxicity of **AN1** and **AN7** against nontumorigenic Vero cells was significantly low.

It is known that **AN1** and **AN7** complexes contain the ligand $[Ag(CN)_2]^{1-}$, a very toxic compound against both tumorigenic and normal cells. Therefore, we compared the antiproliferative and cytotoxic activities of **AN1**, **AN7**, and the ligand to reveal the contribution of the ligand to the activities of **AN1** and **AN7**. As shown Figures 1 and 2, the antiproliferative and cytotoxic activities of **AN1** and **AN7** were significantly lower than that of the ligand, indicating that the extreme cytotoxicity of $[Ag(CN)_2]^{1-}$ decreased to safe levels in **AN1** and **AN7**, as reported by Batarseh (2013).

We tested the apoptotic potential of the compounds on the HT29 cell line using TUNEL assay to clarify their mechanism of action for their antiproliferative activity. As illustrated in Figure 3, the TUNEL results clearly show that the **AN1** and **AN7** significantly induced apoptosis in

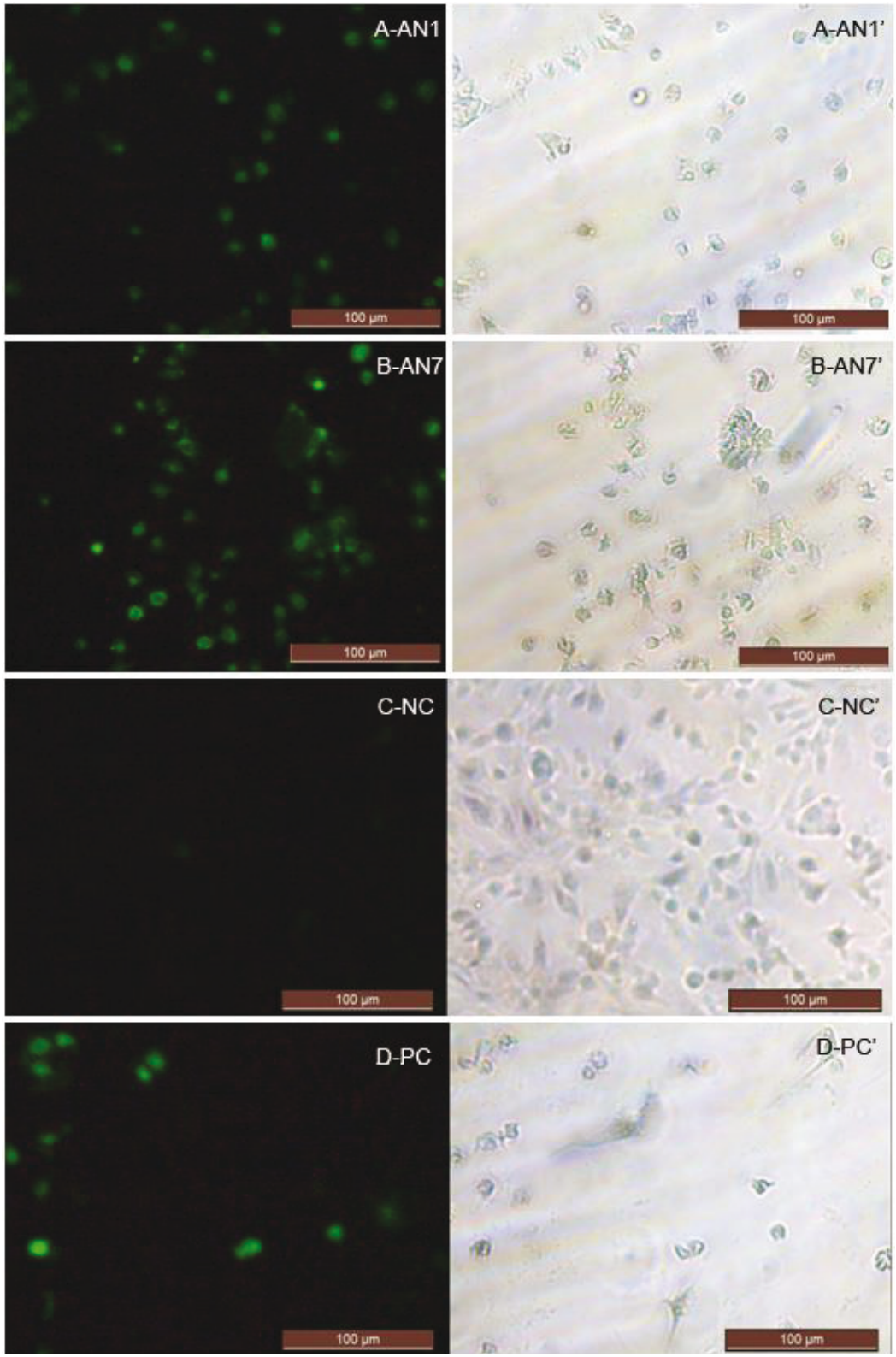

Figure 3. Fluorescence and phase-contrast images of the HT29 cancer cells treated with test and control compounds after TUNEL assay. TUNEL-positive cell nuclei were observed in brilliant green under fluorescence (A-AN1, B-AN7, C-NC (negative control), and D-PC (positive control)) and phase contrast (A′-AN1, B′-AN7, C′-NC (negative control), and D′-PC (positive control)).

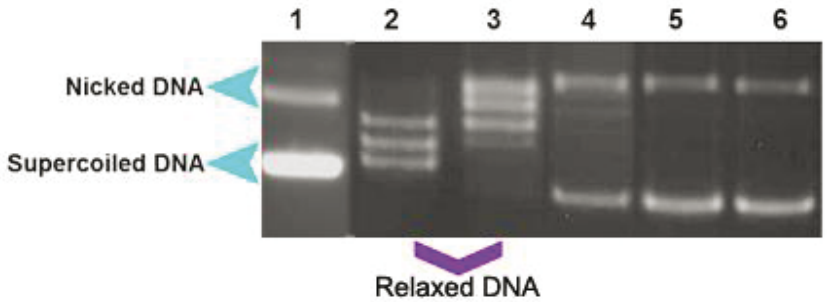

Figure 4. DNA topoisomerase I inhibitory activity of **AN1** and **AN7**. A significant increase in linear DNA and reduction of supercoiled DNA was observed with IC_{50} concentrations of **AN1** and **AN7**. Lane 1: Supercoiled marker DNA; Lane 2: Relaxed marker DNA; Lane 3: Negative control (supercoiled DNA + Topo I); Lane 4: Positive control (supercoiled DNA + Topo I + camptothecin); Lane 5: Supercoiled DNA + Topo I + **AN1**; Lane 6: Supercoiled DNA + Topo I + **AN7**.

HT29 cells. It is known that enhanced antitumor efficacy is associated with increased induction of apoptosis. These findings indicated that **AN1** and **AN7** inhibit cell proliferation by inducing apoptosis, as in previous studies by Govender et al. (2013) and Gandin et al. (2013). In addition, these complexes resulted in the DNA degradation of cancer cell DNA (data not shown), indicating their apoptotic potential. These results were somewhat similar to those of previous studies by Zachariadis et al. (2004), El-Din et al. (2011), Korany et al. (2013), Pettinari et al. (2011), and Banti and Hadjikakou (2013).

DNA topoisomerases are very effective antitumor targets because of their essential function of regulating DNA topology during DNA replication and recombination (Champoux, 2001). Several important topoisomerase inhibitors such as camptosar, irinotecan, and topotecan have been used in routine clinical practice. In order to understand whether the antiproliferative activity of these compounds involves the inhibition of DNA topoisomerases, we investigated the effects of **AN1** and **AN7** on the topoisomerase I-mediated relaxation of supercoiled plasmid (pHOT1) DNA. The results revealed that the IC_{50} concentrations of **AN1** (containing Cd) and **AN7** (containing Ni) inhibited almost all the DNA relaxation activity of topoisomerase I, similarly to camptothecin (Figure 4), indicating their antitopoisomerase potential for use as novel topoisomerase inhibitors. Similarly to Wu's (2011) results, these findings may also indicate the binding of these complexes not only to topoisomerase I, but also to other proteins. It was reported by Hall et al. (1997) that nickel(II) complexes of thiosemicarbazones significantly inhibited topoisomerase II. Similarly, Prabhakaran et al. (2011) showed that Ni(II) complexes containing N-substituted thiosemicarbazones considerably inhibited topoisomerase II.

In conclusion, our results have demonstrated that metal complexes **AN1** and **AN7** are potent anticancer drug candidates with higher antiproliferative, low cytotoxic, strong apoptosis-inducing, and effective DNA topoisomerase inhibitory characteristics. However, further in vitro and in vivo studies need to be performed to verify their anticancer drug potential.

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