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Journal of Enzyme Inhibition and Medicinal Chemistry

ISSN: 1475-6366 (Print) 1475-6374 (Online) Journal homepage: www.tandfonline.com/journals/ienz20

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To cite this article: R. Beklem Bostancıoğlu, Kenan Işık, Hatice Genç, Kadriye Benkli & Ayşe Tansu Koparal (2012) Studies on the cytotoxic, apoptotic and antitumoral effects of Au(III) and Pt(II) complexes of 1, 10-phenanthroline on V79 379A and A549 cell lines, Journal of Enzyme Inhibition and Medicinal Chemistry, 27:3, 458-466, DOI: 10.3109/14756366.2011.596835

To link to this article: https://doi.org/10.3109/14756366.2011.596835

Published online: 04 Aug 2011.

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Journal of Enzyme Inhibition and Medicinal Chemistry, 2012; 27(3): 458–466
© 2012 Informa UK, Ltd.
ISSN 1475-6366 print/ISSN 1475-6374 online
DOI: 10.3109/14756366.2011.596835

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RESEARCH ARTICLE

Studies on the cytotoxic, apoptotic and antitumoral effects of Au(III) and Pt(II) complexes of 1, 10-phenanthroline on V79 379A and A549 cell lines

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Abstract

In the present study, Au(III) and Pt(II) complexes of 1, 10-phenanthroline (phen) were synthesized and used as the test compounds. The structure elucidation of the synthesized compounds was performed by IR, ^{1}H-NMR and MASS spectroscopic data and the results of elemental analyses. The cytotoxic and apoptotic effects of test compounds were elucidated on V79 379A (Chinese hamster lung fibroblast like) and A549 (human lung carcinoma epithelial like) cell lines. Cytotoxicity was measured with MTT assay and antitumoral effect was determined by colony forming ability methods. In addition, nuclear fragmentation and activation of apoptotic enzyme (caspase-3) and DAPI staining were used to detect the apoptotic effect of the compounds. All the test compounds induced time and concentration-dependent cytotoxic and antitumoral effects. Significant increases in the levels of apoptosis were observed with increasing exposure concentration.

Keywords: Au(III), Pt(II), cytotoxicity, apoptosis, MTT assay, DNA fragmentation, colony forming ability assay (CFA), DAPI staining, active caspase-3

Introduction

The existence of a relationship between cancer and metals is widely accepted by researchers. Metals or metal containing compounds are known to be important agents for therapy in cancer and leukemia, dating back to the sixteenth century[1]. Since Rosenberg discovered the antitumor activity of cisplatin (*Cis*-diaminedichloroplatinum(II)) in 1967, it is the mainstay of treatment for testicular, ovarian, bladder, cervical, small-cell and non-small-cell lung cancers[2–5]. The success of cisplatin as an anticancer drug prompted medical researchers to use metals, including platinum derivatives such as carboplatin, oxoplatin, nedaplatin, lobaplatin, ruthenium and gold compounds in anticancer medicine[2,3,5,6]. Gold and platinum compounds are very significant therapeutic agents because of fewer side effects than existing metal anticancer drugs[7].

It is commonly believed that the cytotoxic effects of metal complexes are consequences of DNA binding. The goal is to cause DNA damage specifically in cancer cells, leading to cell death[5,7]. This cytotoxicity results from the formation of DNA monoadducts and crosslinks, which, in turn, promotes the development of apoptosis-inducing double-strand breaks during replication[8,9]. Scientist revealed that platinum(II) compounds were thought to interact with bio-molecules such as DNA and proteins. In contrast to cisplatin, DNA is not the primary target of gold(III) complexes. Rather, their cytotoxicity is mediated by their ability to alter mitochondrial function and inhibit protein synthesis[8,10]. Relevant antimitochondrial effects were demonstrated in some cases, eventually leading to cell apoptosis[7,10,11].

Over the recent 3 decades, various gold(III) and platinum(II) containing compounds have been synthesized and evaluated for *in vitro* anticancer properties[7,9]. Among new platinum complexes with potential therapeutic effects, satraplatin is currently undergoing phase III clinical trials in Europe for ovarian and prostate cancers[5]. Zhang and co-worker (2008) demonstrated that

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(Received 28 March 2011; revised 07 June 2011; accepted 08 June 2011)

tri-functional mononuclear platinum(II) complexes and new type binuclear platinum complexes have cytotoxic and antitumor effects against leukemia, colon carcinoma, breast carcinoma, bladder carcinoma and gastric carcinoma cell lines[4]. Otherwise, gold(I) phosphine complexes were also active against the growth of cultured tumour cells *in vitro* in the 1970s and 1980s. Afterwards, gold complexes with bis(diphenylphosphino)ethane ligands such as the cationic tetrahedral four coordinated $[Au(dppe)_2]Cl$ were developed and found cytotoxic to tumour cells, produced DNA-protein cross links and DNA strand breaks in cells and inhibited protein, DNA, and RNA synthesis[2]. Besides gold(I), gold(III) compounds have long been studied for anticancer treatment. Mirabelli et al. (1986) reported that gold(III) ions could induce DNA strand breaks. Studies showed that gold(III) compounds with tridentate terpy ligands could bind DNA via intercalation. This work was followed by the discovery of a variety of gold(III) compounds containing monodentate, bidentate or tridentate ligand(s) exhibiting favourable anticancer properties comparable to that of cisplatin[10]. Recently, Wang et al. (2007) demonstrated that eight new gold(III) complexes (1e8) of 5-aryl-3-(pyridin-2-yl)-4,5-dihydropyrazole-1-carbothioamide have cytotoxic effects against HeLa and A549 cell lines by MTT assay[7]. Moreover, a series of new gold(I) and gold(III) complexes were synthesized and characterized; in addition, antiproliferative profile was investigated on A2780S and its cisplatin resistant subline A2780R cells by Maiore and co-workers (2011)[12].

Among these gold(III) and platinum(II) containing compounds, Au(III) and Pt(II) complexes of 1,10-phenanthroline (phen) were reported to be cytotoxic to several tumour cells. Messori and co-workers (2002) demonstrated significant cytotoxic activity of Au(III) complexes with 1,10-phenanthroline (phen) against human ovarian cancer cells (A2780); on the other hand, Cusumano and co-workers (2006) showed that Pt(II) complexes of 1,10-phenanthroline (phen) have DNA binding properties[11,13,14]. It is also well known that 1,10-phenanthroline (phen) is the parent of an important class of chelating agents and has structural features such as a rigid planar, hydrophobic, and its electron-poor heteroaromatic system whose nitrogen atoms are beautifully placed to act cooperatively in cation binding. Some metal complexes containing 1,10-phenanthroline are also known to bind to DNA by an intercalative mode. At the same time, several metal complexes with 1,10-phenanthroline and natural products incorporating this heterocyclic nucleus possess interesting anticancer properties[15,16].

In summary, the aim of this study was to investigate the cytotoxic, apoptotic and antitumoral properties of gold(III) and platinum(II) complexes as potential anticancer drugs. For this purpose, after the synthesis of the Au(III) and Pt(II) complexes of 1.10-phenanthroline (phen), cytotoxic effects were demonstrated by MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoium bromide), and antitumoural effects by CFA (colony forming ability) assay. Besides, it was also assessed whether they cause apoptosis or not via DAPI staining and colorimetric caspase-3 assays on A549 (human lung carcinoma epithelial cell like) and V79 379A (Chinese hamster lung fibroblast like) cell lines. All the test compounds induced time and concentration-dependent cytotoxic effect. Significant increases in the levels of apoptosis were observed with increasing exposure concentration. The structure elucidation of the synthesized compounds was performed by IR, ^{1}H-NMR and MASS spectroscopic data and elemental analyses results.

Methods

Chemical synthesis

Potassium tetrachloroplatinate K_2PtCl_4 and sodium tetrachloroaurate dihydrate $NaAuCl_4.2H_2O$ were purchased from Fluka. All other reagents were used as purchased without any further purification. Chemical analysis was performed for C, H, and N on a Perkin-Elmer elemental analyzer. Infrared spectra were recorded in the 4000–200 cm^{-1} range on Perkin-Elmer FT-IR. KBr pellets were used to record spectra. ^{1}H-NMR spectra were obtained in DMSO-d_6 solution on Bruker DX400 instrument. $[Au(phen)Cl_2]Cl$ and $Pt(phen)Cl_2$ complexes were synthesized as reported[13,14].

$[Au(phen)Cl_2]Cl$

The mixture of (phen) 1,10-phenanthroline solution in EtOH-H_2O and $NaAuCl_4$ in H_2O was stirred at 30°C for 2 h. The precipitate was filtered and washed several times with water and diethylether, and then dried in vacuum desiccator. The compound was characterized by IR, ^{1}H-NMR and MASS spectroscopic data.

Selected IR (KBr)ν_{maks}(cm^{-1})

3148,3110,3075(ArC-H),1645,1630,1450,1391(C=N,C=C), 325 (M-Cl), 287 (M-N). ^{1}H-NMR(300 MHz) (DMSO-d_6) δ(ppm): 7.52–9.25 (8H, m). MS (ES): *m/z*: 447 [M+1].

$Pt(phen)Cl_2$

The mixture of (phen)1,10-phenanthroline solution in EtOH-H_2O and K_2PtCl_4 in H_2O was stirred at 30°C for 8 h. The precipitate was filtered and washed several times with water and diethylether, and then dried in vacuum desiccator. The compound was characterized by IR, ^{1}H-NMR and MASS spectroscopic data. Selected IR (KBr)ν_{maks}(cm^{-1}): 3148, 3100, 3087 (Ar C-H), 1640, 1632, 1456, 1392 (C=N, C=C), 327 (M-Cl), 289 (M-N). ^{1}H-NMR(300 MHz) (DMSO-d_6) δ(ppm): 7.60–9.20 (8H, m). MS (ES): *m/z*: 445 [M+1].

Cell culture

V79 379A (Chinese hamster lung fibroblast like) and A549 (human lung carcinoma epithelial like) cell lines were obtained from the Institute for Fermentation, Osaka (IFO, Japan). V79 379A cells were maintained as a monolayer in Dulbecco's Modified Eagle Medium (DMEM)

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(Sigma) containing 10% (v/v) fetal bovine serum (Sigma), penicilin-streptomycin (Sigma) and sodium hydrogen carbonate. A549 cells were maintained as a monolayer in Nutrient Mixture F-12 HAM Medium (Sigma) containing 10% (v/v) fetal bovine serum (Sigma), penicillin-streptomycin (Sigma) and sodium hydrogen carbonate. V79 379A and A549 cells were incubated at 37°C in a humidified atmosphere of 5% (v/v) CO_2 in air.

MTT cell proliferation assay

The growth inhibitory effects of the test compounds against V79 379A (Chinese hamster lung fibroblast like) and A549 (human lung carcinoma epithelial like) cell lines were measured by using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazoium bromide) assay as previously described[17]. Compounds were dissolved in dimethyl sulfoxide (DMSO) and screened at a range of concentrations against cancer and normal cells. Just before the experiments, stock solutions were diluted with the supplemented medium to obtain final concentrations of 1.25, 2.5, 5, 10, 20, 40 and 80 μM. The cytotoxic activity was determined after 24, 48 and 72 h. All experiments were repeated at least three times.

Soft agar colony forming assay

The antineoplastic activities of [Au(phen)Cl_2]Cl and Pt(phen)Cl_2 were investigated through CFA assay as described in Li et al.[18] with modifications in A549 cell line. Colony inhibition was calculated with the following formula: Colony forming inhibition % = 100 × (1- colony number of drug treated group/colony number of control group)[19].

Detection of apoptosis by DAPI staining

Apoptotic cells were detected via DAPI staining. DAPI is a DNA-staining agent and binds to grooves on the surface of the DNA helix. And apoptotic nuclei can be identified by the reduced nuclear size, condensed chromatin gathering at the periphery of the nuclear membrane or a total fragmented morphology of nuclear bodies[20,21]. After 24 h of seeding, cells were treated with various doses of [Au(phen)Cl_2]Cl and Pt(phen)Cl_2 for 12 h. Untreated control, solvent control (DMSO), [Au(phen)Cl_2]Cl and Pt(phen)Cl_2 treated cell monolayers grown on glass coverslips (Marienfield, Germany) were fixed with 3.7% (w/v) paraformaldehyde-phosphate buffer saline (PBS: 137 mM NaCl, 2.7 mM KCl, 15 mM KH_2PO_4, 8 mM $NaHPO_4$; pH 7.3) for 15 min at 37°C in the dark. Cells were rinsed with PBS. Fluorescent images were viewed using Olympus fluorescence microscope (Olympus IX70).

Determination of caspase-3 activity

The enzymatic activity of caspase-3 induced by compounds was investigated using a colorimetric assay kit (R&D Systems, Minneapolis, MN) according to the manufacturer's protocols. The cells were incubated in the absence and presence of agent for the indicated times. The cells were harvested and lysed in a lysis buffer for 15 min on an ice bath. The lysed cells were centrifuged at 10,000 rpm for 1 min, and equal amounts of protein (100 mg per 50 mL) were incubated for 50 mL reaction buffer 5 mL of colorimetric tetrapeptides. Asp-Glu-Val-Asp (DEVD)-p-nitroaniline (pDNA) for caspase-3 was at 37°C for 2 h. The optical density of the reaction mixture was quantified spectrophotometrically at a wavelength of 405 nm by using ELISA reader.

Statistical data analysis

The SPSS software was used for the statistical analyses of assessment of the MTT, CFA and caspase-3 assays. Data were evaluated using one-way analysis of variance followed by Tukey test. A value of $p < 0.05$ was considered significant.

Results

Chemistry

The Au(III) and Pt(II) complexes of 1,10-phenanthroline (phen), ([Au(phen)Cl_2]Cl and Pt(phen)Cl_2 were synthesized by using literature methods[13,14]. The compounds gave satisfactory elemental analysis. The ^{1}H-NMR spectral data were also consistent with the assigned structures, and all the protons were observed at expected regions. In the IR spectra, some significant stretching bands due N-H, C=N and C=C were at 3375–3105 cm^{-1}, 1580–1486 cm^{-1}, respectively. All spectral data conformed to literature[13,14].

Cytotoxicity assay

MTT assay is a well-documented cell viability assay as an index of intrinsic cytotoxicity and has been modified by several investigators since it was first developed by Mosmann (1983)[22]. The cytotoxic properties of ([Au(phen)Cl_2]Cl and Pt(phen)Cl_2) were evaluated against human lung carcinoma epithelial like-A549 and Chinese hamster lung fibroblast like cells-V79 379A *in vitro* using the MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoium bromide) assay. The inhibitory effect of compounds on the proliferation of A549 and V79 379A cells was observed to occur in a time and dose-dependent manner (Figure 1).

Results revealed that [Au(phen)Cl_2]Cl conferred substantially greater cytotoxicity than Pt(phen)Cl_2 against A549 cells even after 72 h of exposure time. But, both test compounds caused the same toxicity on V79 379A cells at the end of the 24 h. Under the same experimental conditions, [Au(phen)Cl_2]Cl demonstrated cytotoxic activity in the 2.5–80 μM both A549 and V79 379A cells. Moreover, Pt(phen)Cl_2 inhibited the cell proliferation at 40–80 μM range on A549 cells and 10–80 μM range on V79 379A cells. The results of MTT demonstrated that the IC_{50} of [Au(phen)Cl_2]Cl was about 20, 5 and 5 μM after 24, 48 and 72 h exposure on A549 cells. The IC_{50} of [Au(phen)Cl_2]Cl was 80, 2.5 and 1.25 μM after 24, 48 and 72 h exposure on V79 379A cells, respectively. Compounds of [Au(phen)Cl_2]Cl had low antiproliferative activity on healthy cells compared to cancer cell

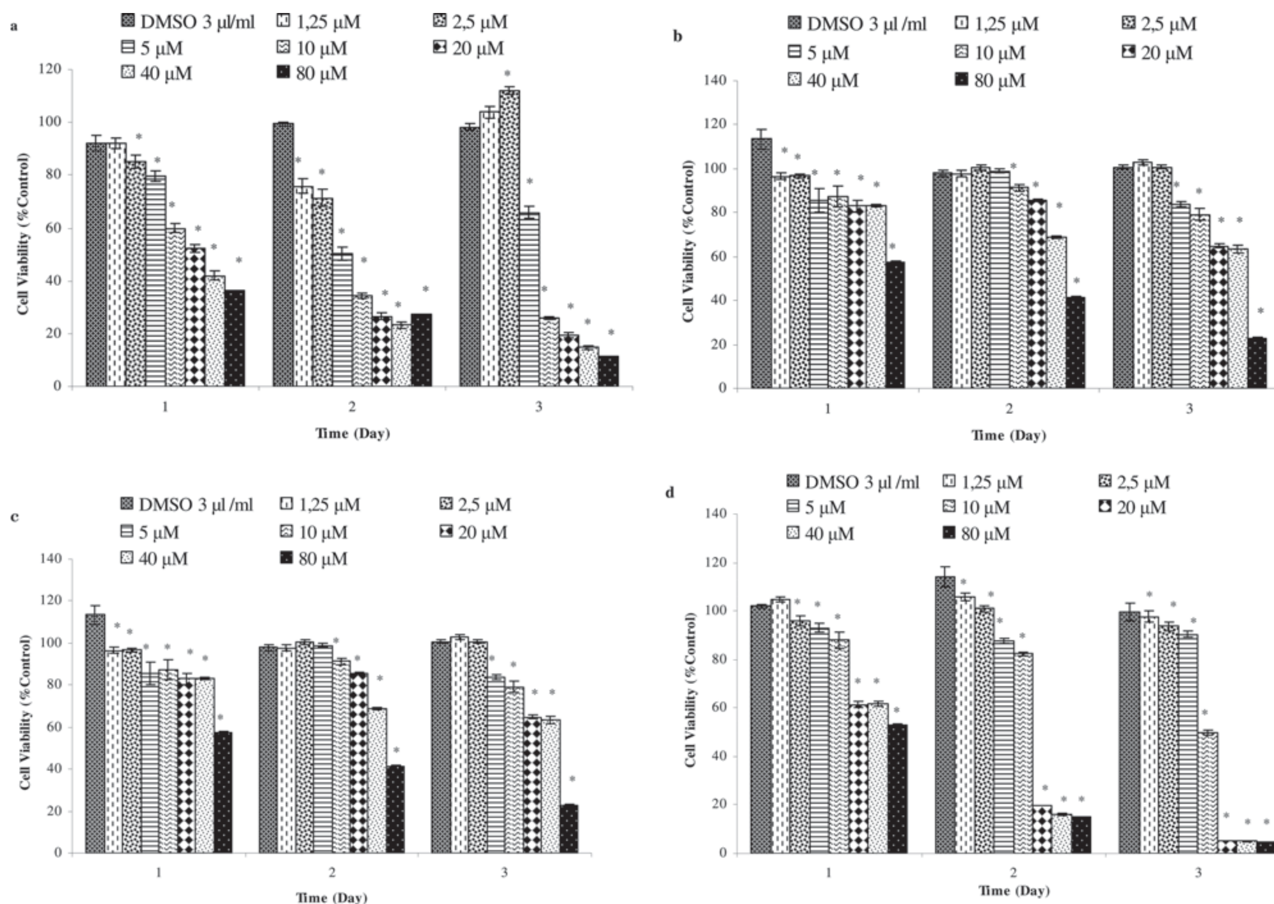

Figure 1. (A–D). Comparison between [Au(phen)Cl2]Cl and Pt(phen)Cl2 cytotoxic activity on A549 and V79 379A cell lines as determined in the MTT assay. Dose-response curves of the antiproliferative effect of compounds for MTT assays performed after 24, 48 and 72 h exposures. The results are expressed as the mean ± SD. *Indicates significant difference from the control group by the Tukey test ($p < 0.05$). (A) [Au(phen)Cl2]Cl on A549, (B) Pt(phen)Cl2 on A549, (C) [Au(phen)Cl2]Cl on V79 379A, (B) Pt(phen)Cl2 on V79 379A cells.

line at 24 h. For instance, percentage of alive healthy cells was 73.2%, whereas cancer cells were 59.9% at 10 µM concentration end of 24 h.

A similar cytotoxicity for 1.25–2.5–5–10 µM concentrations of the Pt(phen)Cl_2 were compared to two cell lines at the first day. IC_{50} value was approximately 80 µM in the first day, between 40 and 80 µM in the second and third day for A549 cells. But concentrations of IC_{50} were 80 µM after 24 h and between 10 and 20 µM after 48 h and 10 µM after 72 h for V79 379A cells. It was found that the cytotoxicity of Pt(phen)Cl_2 was significantly effective on V79 379A cells at 20 µM following 72 h although half the amount of A549 cells were killed by the same concentrations (20 µM). The results of the cytotoxic activity *in vitro* expressed as 40 µM concentration of this compound inhibited a proliferation rate of the cancer cells by 36.6% and healthy cells by 95.1% at the third day.

These dose-dependent growth inhibitory effects were also confirmed in the colony formation assay.

Soft agar colony forming assay

To further analyze whether the compounds have antineoplastic effects or not, cells were subjected to soft agar colony formation with compounds. CFA was performed using a double-layer soft agar method. The colony

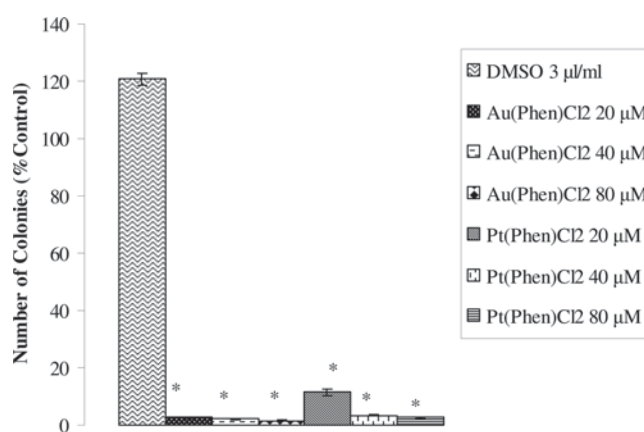

Figure 2. Percentage soft agar colony forming efficacy of A549 cells treated with Pt(phen)Cl2 and [Au(phen)Cl2]Cl (20–40 and 80 µM). Results of mean three different experiments. *Indicates significant difference from the control group by the Tukey test ($p < 0.05$).

formation assay was developed to detect the growth inhibitory effects of drugs on the anchorage-independent growth of colonies[18]. [Au(phen)Cl_2]Cl and Pt(phen) Cl_2 treatments were found to exert a concentration-dependent inhibitory effect on the CFA of A549 cells (Figure 2). A significant difference was observed in the

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size and morphology of the colonies. Number of colonies in the [Au(phen)Cl_2]Cl group was significantly lower than Pt(phen)Cl_2, but size of colonies in the Pt(phen)Cl_2 group was smaller. [Au(phen)Cl_2]Cl at minimum concentration of 20 μM for this assay caused inhibition (97.5%) of colony number of A549 cells more effectively than Pt(phen)Cl_2 (88.6%). While 40 μM doses of [Au(phen)Cl_2]Cl reduced colony number by 98.1%, cut down colony inhibition by 96.84% for Pt(phen)Cl_2. Doses of 80 μM also caused decreases in colony inhibition from 97.47 to 98.7%, respectively, for [Au(phen)Cl_2]Cl and Pt(phen)Cl_2.

Apoptosis

DAPI staining

The significant antiproliferative activity showed by the two promising compounds Pt(phen)Cl_2 and [Au(phen)Cl_2]Cl prompted to evaluate the apoptotic activity of A549 and V79 379A cells. The apoptotic morphology in cellular bodies and the chromatin condensation were also confirmed by DAPI staining. During cell apoptosis, an early biochemical event is the activation of an endogenous nuclear endonuclease, causes the degradation of genomic DNA[23]. Apoptosis is characterized by chromatin condensation, apoptotic bodies, membrane blepping and cytoplasmic condensation[20,23,24]. Both test compounds induced a dose-dependent apoptotic cell death (Figures 3 and 4). For compound [Au(phen)Cl_2]Cl, the presence of apoptotic bodies was observed, which suggested that these compounds induced death by apoptosis especially in cancer cells. The cells treated with Pt(phen)Cl_2 compound demonstrated similar morphological changes on both cell lines. This data corroborated with MTT results.

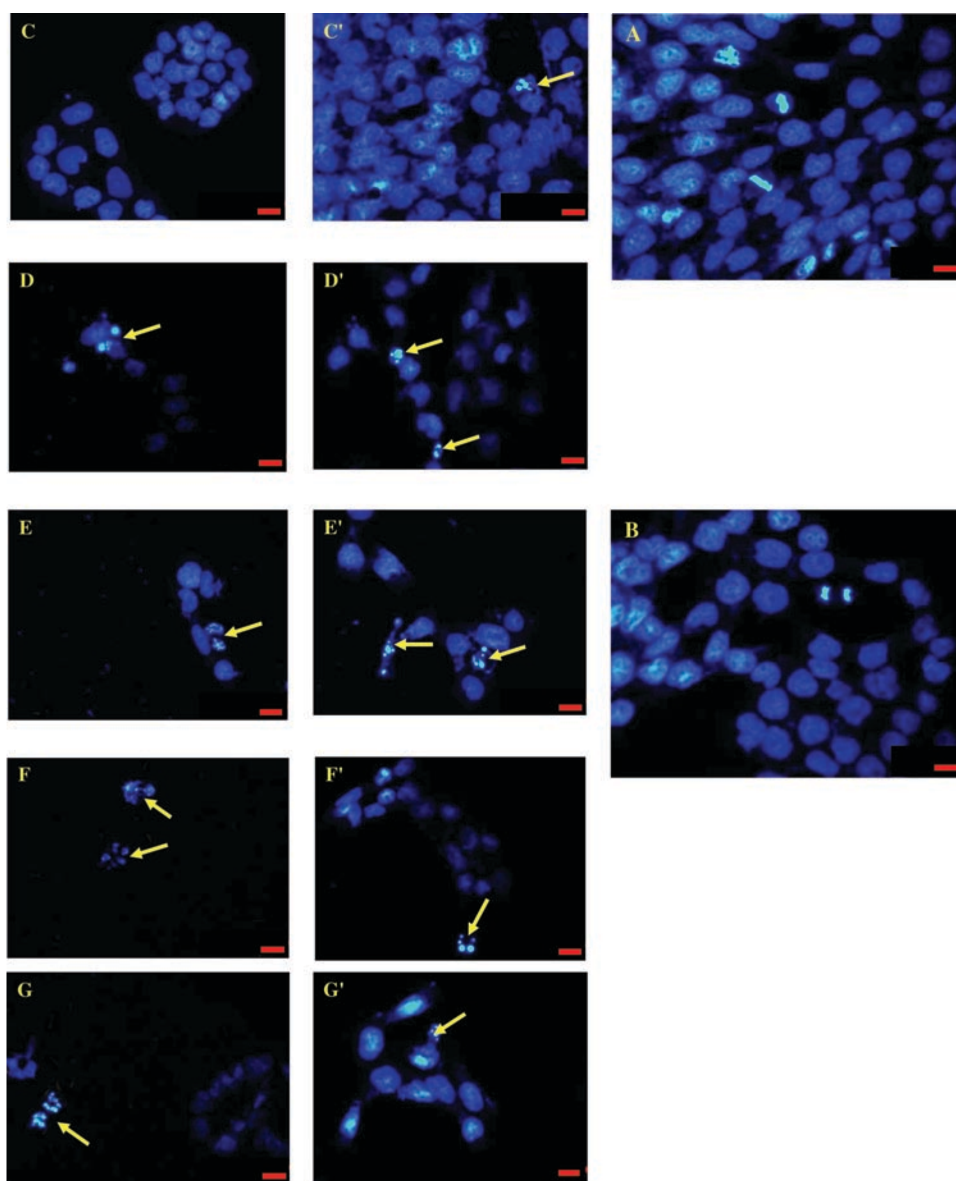

Figure 3. Chromatin condensation and observation of apoptotic bodies on V79 379A cell cultures evaluated after 12 h, stained with DAPI. (A) Control. (B) Cells treated with DMSO. (C-D-E-F-G) Cells treated with Pt(phen)Cl2 2,5-5-10-20-40-80 μM, respectively. (C'-D'-E'-F'-G'-) Cells treated with [Au(phen)Cl2]Cl 2,5-5-10-20-40-80 μM, respectively. Arrows show classical apoptotic bodies. Scale bars 20 μm.

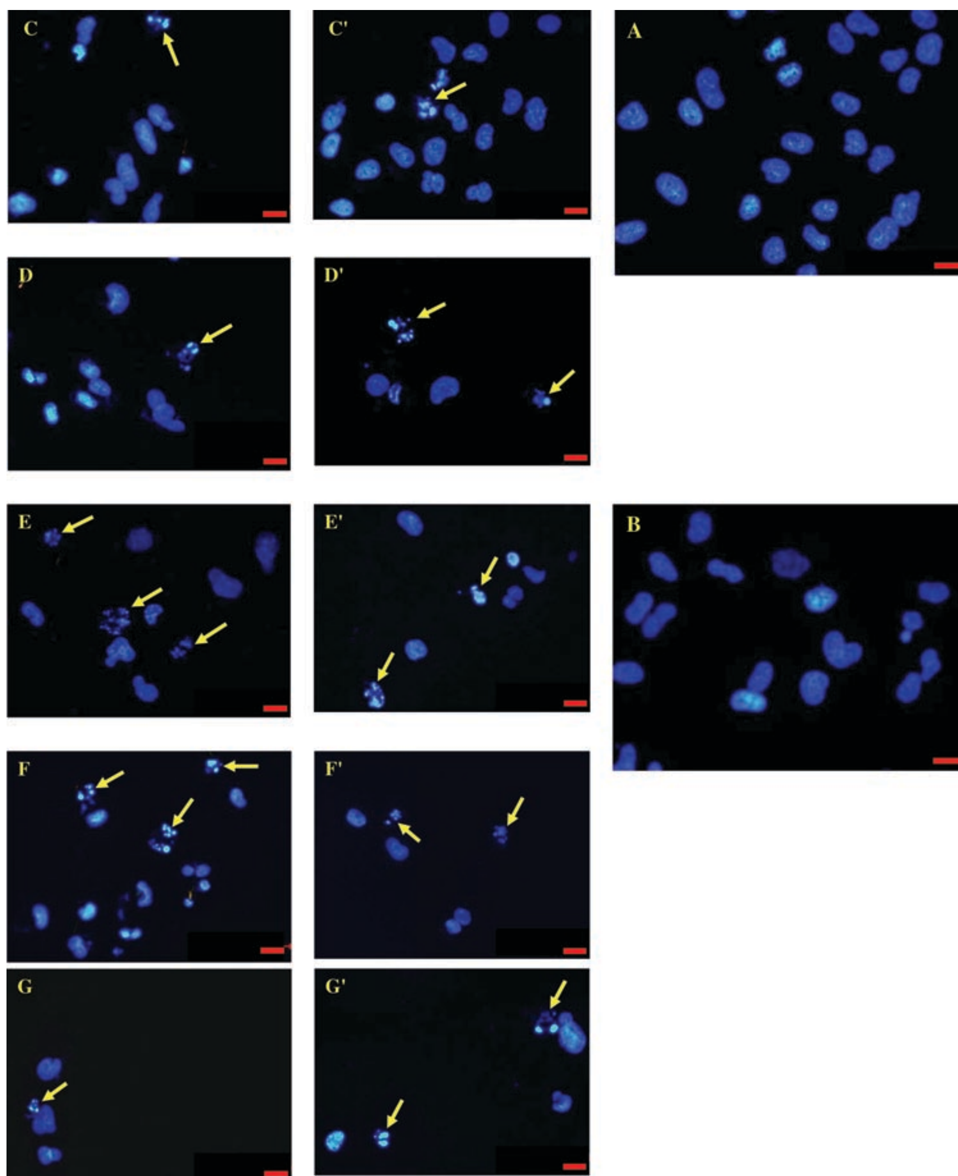

Figure 4. Chromatin condensation and observation of apoptotic bodies on A549 cell cultures evaluated after 12 h, stained with DAPI. (A) Control. (B) Cells treated with DMSO. (C-D-E-F-G) Cells treated with Pt(phen)Cl2 2,5-5-10-20-40-80 µM, respectively. (C'-D'-E'-F'-G') Cells treated with [Au(phen)Cl2]Cl 2,5-5-10-20-40-80 µM, respectively. Arrows show classical apoptotic bodies. Scale bars 20 µM.

Determination of caspase-3 activity

Based on the increase of apoptosis in cells treated with compounds $[Au(phen)Cl_2]Cl$ and $Pt(phen)Cl_2$, the next aim of the study was to determine whether these test compounds cause apoptosis in the V79 379A and A549 cells via mitochondria. Caspase-3 is a well-known executor enzyme in apoptosis pathway[20]. These cells were incubated with 40 µM the test compounds for 12 h and measured caspase-3 activity. The induction of apoptotic activity via mitochondrial pathway was more pronounced in the cancer cells compared to normal cells for all of test compounds. Activity of caspase-3 was observed to increase by 34% after the treatment with $[Au(phen)Cl_2]Cl$ compound on cancer cell line, whereas the level of this enzyme was higher (84%) for $Pt(phen)Cl_2$ in treated cancer cells as compared to nontreated cells (Figure 5). This data clearly indicated that the complexes in the study induce apoptosis by caspase-mediated mechanism. Moreover, the results of caspase-3 assay confirm with DAPI staining photographs.

Discussion

The widespread clinical use of cisplatin in cancer chemotherapy and its side effects (for example, higher toxicity for other tissues) has prompted studies for new chemotherapeutic agents. Various studies showed that some Au(III) and Pt(II) complexes have more effective activity on some cancer types than cisplatin[10,25]. Au(III) complexes, with their metal centres being isoelectronic and isostructural to Pt(II), are thus promising candidates as anticancer agents[10]. The phenomenon of gold

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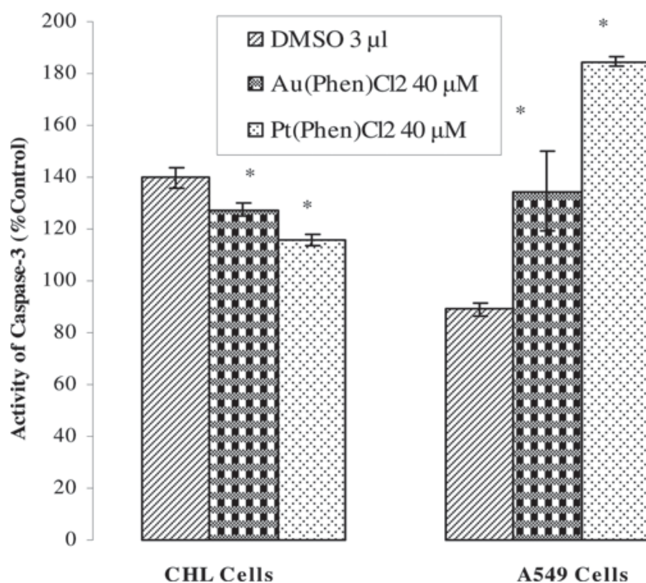

Figure 5. A549 and V79 379A cells were treated with [Au(phen)Cl2]Cl (40 μM) and Pt(phen)Cl2 (40 μM) compounds for 12 h. Caspase-3 activity was determined using caspase assay kit obtained from R&D and used according to the protocol of the manufacturer. Data are expressed as the mean ± SD of three independent experiments. The significance was determined using Tukey test (*$p < 0.05$ vs. untreated control).

in medicine dates back to antiquity although the chemistry and antitumor activity of gold(III) complexes have increased considerably in recent decades because significant biological activities were found for new and old gold complexes[25,26]. Henderson et al. (1998) synthesized a number of organogold(III) complexes that displayed interesting antibacterial, antitumor and antifungal properties. Several gold(III) complexes were investigated, and many of them turned out to exhibit relevant cytotoxic or DNA binding properties by Abbate, Marcon, Messori and co-workers[27]. Buckley et al. (1996) first reported some organogold(III) complexes endowed with significant cytotoxic and anticancer properties[28]. Guo et al. (2003) revealed cytotoxicity of three gold complexes [Au(Quinpy)Cl]Cl, [Au(Quingly)Cl]Cl and [Au(Quinala)Cl]Cl with three AueN bonds. Researchers reported that cytotoxicity of [Au(Quinpy)Cl]Cl against A549 cells is about 3 times higher than that of cisplatin. In addition, Ronconi et al. (2005) synthesized a series of gold(III) complexes with AueS bonds, most of which are much more cytotoxic *in vitro* than cisplatin. Recently, a series of gold(III) complexes with one AueS and two AueN bonds have cytotoxicity against HeLa cell line, and show higher cytotoxicity than cisplatin[29]. On the other hand, it is known that many platinum compounds exhibit very remarkable cytotoxic and anticancer activity. Zhang and co-worker (2008) demonstrated that tri-functional mononuclear platinum(II) complexes and new type binuclear platinum complexes have cytotoxic and antitumor effects against leukemia, colon carcinoma, breast carcinoma, bladder carcinoma and gastricarcinoma cell lines[4]. Moreover, in spite of the fact that there is a scarcity of data concerning the anticancer activity of 1,10-phenanthroline (phen) and its derivatives, some metals with bidentate ligand are known to have anticancer activity against many kinds of cancer[14,15,28,30]. For example, the chemotherapeutic potential of 1,10-phenanthroline (phen) was evaluated using two human carcinoma cell lines (A-498 and Hep-G2) by Deegan et al. (2006) in addition; phen and three metal-phen complexes were found cytotoxic and had DNA synthesis inhibition capability with metal complexes. Several studies demonstrated that the complexes of Cu and phen have apoptotic effect[30]. Additionally, the palladium(II) complex of the unsubstituted 1,10-phenanthroline, $Pd(phen)_2(H_2O)(NO_3)_2$ did not show any activity, which indicates the dominant effect of the electron donor amino group in the 5th position in this complex in study of Wesselinova et al. (2009)[28]. But Abbate and co-workers (2000) reported that incubation of the A2780 tumour cell line and either sensitive or resistant to cisplatin with $[Au(phen)Cl_2]Cl$ resulted in strong cytotoxicity and antitumor effect[27].

It is known that a number of researchers have focused considerable attention on determining appropriate chemotherapeutic agent for treatment of various cancer types. Ideally, chemotherapeutic drugs should specifically target only neoplastic cells and should decrease tumour burden by inducing cytotoxic and/or cytostatic effects in tumour cells with minimal "collateral damage" to normal cells[27]. In the present study, $[Au(phen)Cl_2]Cl$ and $Pt(phen)Cl_2$ were synthesized. The compounds were assayed for cytotoxic and apoptotic activities against A549 and V79 379A cells and were found effective. MTT assay showed that $[Au(phen)Cl_2]Cl$ are more toxic than $Pt(phen)Cl_2$ against cancer cells (A549) (Figure 1). Additionally, compounds of $[Au(phen)Cl_2]Cl$ had low antiproliferative activity on healthy cells (V79 379A) compared to cancer cells (A549).

In an attempt to explore the molecular mechanism underlying the observed cytotoxic response, DAPI staining and caspase-3 colorimetric enzyme assays were conducted. DAPI staining data of the present study revealed that $[Au(phen)Cl_2]Cl$ and $Pt(phen)Cl_2$ induced apoptosis on both dose-dependent cell lines (Figures 3 and 4). Especially, $[Au(phen)Cl_2]Cl$ was capable of leading to apoptotic cell death in cancer cells. In order to understand the mechanism, colorimetric caspase-3 assay was carried out as further biological study. Caspase-3 assay showed that induction of apoptosis occurred in V79 379A and A549 cells via mitochondrial pathway (Figure 5). Chemotherapy was based on apoptotic cell death mechanism and some cancer cells were killed by including Pt(II) and Au(III) compounds via apoptosis like cisplatin[32]. The results of researches about cancer have demonstrated, that these metal complexes are really appropriate drug materials for treatment of various cancer types because apoptosis and cytotoxicity are closely related with chemotherapy. It is commonly believed that the cytotoxic effects of metal complexes are consequences of DNA binding[5,7]. This cytotoxicity results

in internucleosomal DNA fragmentation, a hallmark of apoptosis[8,10]. Many studies revealed that platinum-based compounds induced DNA and damages of other cellular components and also apoptotic DNA fragmentation with bio-molecules such as DNA and proteins. Au(III) compounds are active via mechanisms that differ from those of the Pt(II) antitumor agents such as cisplatin. Mitochondria may be the major target for at least some of these Au(III) compounds. Relevant antimitochondrial effects were demonstrated in some cases, eventually leading to cell apoptosis[7,8,10,11,33].

The colony formation assay was developed to detect the growth inhibitory effects of drugs on the anchorage-independent growth of colonies[18]. There are many studies about the antitumoral effects of Au(III) and Pt(II) compounds, but any of them were carried out for CFA assay. In the present study, the antineoplastic activity of [Au(phen)Cl_2]Cl and Pt(phen)Cl_2 complexes were evaluated CFA assay. Results demonstrated that compounds affect cellular CFA of cancer cell line. For example, the number of colonies in the group of [Au(phen)Cl_2]Cl at a concentration of 20 µM was 2.5% and it was found 11.4% in the group of Pt(phen)Cl_2 at the same concentration compared to control group (Figure 2).

Abbate and co-workers (2000) reported that the biological properties of [Au(phen)Cl_2]Cl might be derived, at least partially, from free phenanthroline[27]. Studies showed that both metal-free phen and metal-phen complexes affected mitochondrial function, retarded synthesis of cytochromes b and c, and uncoupled cellular respiration[30].

All observations of the present study demonstrated that compounds have very significant cytotoxic and apoptotic effects. Cytotoxic and apoptotic effects of these compounds might be resulted from phenanthroline reinforced with Au(III) and Pt(II) metals. Therefore, [Au(phen)Cl_2]Cl and Pt(phen)Cl_2 complexes may be promising lead candidates for cancer treatment like cisplatin. In brief, these results suggest that phen and three metal-phen complexes may have a therapeutic role for the successful treatment and management of cancer.

Conclusion

In this study, [Au(phen)Cl_2]Cl and Pt(phen)Cl_2 were synthesized and the structure elucidation of the synthesized compounds was performed by IR, ^{1}H-NMR and MASS spectroscopic data and the results of elemental analyses. In addition, their antiproliferative, antitumoral and apoptotic activities were determined. As a result of pharmaceutical tests, it has been pointed out that these compounds ([Au(phen)Cl_2]Cl and Pt(phen)Cl_2) have cytotoxic, apoptotic effects and they are capable to suppress tumour formation in dose-dependent manner. All the tests showed that [Au(phen)Cl_2]Cl is very effective in inhibiting cell growth on A549 cells. Consequently, the [Au(phen)Cl_2]Cl may be used in the development of therapeutic agents for lung cancer. Further research is currently under progress in our laboratory to know the mechanism of inhibition and the modifications of the compounds with different organometallics to improve the potency.

Declaration of interest

This study was supported by Anadolu University (Project No: 070304) and NOVARTIS.

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