Gold(III) compounds-mediated inhibition of lung cancer cell proliferation

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Research on chemotherapeutics for lung cancer is crucial for designing a new therapeutic strategy against malignant lung tumors. Although radiotherapy and chemotherapy, which are not selective for cancer cells and exert toxic effects on healthy cells, have a limited advantage, they are the primary treatment modalities for non-small lung cancer. In addition to cytotoxicity, resistance of chemotherapeutics results in failure of treatment. This is why it is of utmost importance to focus on the creation of new chemotherapeutics without toxicity for the successful treatment and improved survival of cancer patients. New gold(III) and Pt(II) compounds were synthesized with a heterocyclic ligand using 2-phenylimidazo[4,5-f][1,10] phenanthroline as a ligand and bis-1,4-di[([1,10] phenanthroline-5-il)amino]-2-buten as a bridge molecule. The characterization of the compounds was carried out using a variety of spectroscopic methods (^{1}H NMR, IR, MS, and elemental analysis). Their antiproliferative, antitumoral, and apoptotic activities were determined. IR spectra and NMR results confirmed the formation of dinuclear heterocyclic complexes for two metal complexes. Cytotoxicity studies on lung cancer cells (A549) and healthy cells (CHL) showed a marked increase in cytotoxicity with the use of gold(III) complexes, and especially [Au(L)B] $(PF_6)_2$ showed higher cytotoxic and apoptotic features than cisplatin at lower concentrations in cancer cells. These findings have been supported by results from DAPI staining and colorimetric measurement of the caspase-3 enzyme in both cell lines. Compounds showed selective toxicity on the cancer cells. In the light of the high efficacy of our newly synthesized gold complexes, they might be good and promising anticancer agents compared with cisplatin. ***Anti-Cancer Drugs*** **27:225–234**

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Introduction

Currently, lung cancer is the most common cause of cancer-related deaths; it accounts for more than one million worldwide annual deaths. It is one of the most malignant types of human cancer and about 70% of patients die as a result of cancer metastasis. Approximately 85% of individuals diagnosed with lung cancer have non-small lung cancer, such as adenocarcinoma, which remains an aggressive cancer with a poor prognosis [1,2]. Therefore, research on chemotherapeutics for lung cancer cells is crucial for designing a new therapeutic strategy against malignant lung tumors. Although radiotheraphy and chemotherapy, which are not selective for cancer cells and exert toxic effects on healthy cells, have a limited advantage, they remain the primary treatment modilaties for non-small lung cancer. In addition to the cytotoxicity of chemotherapeutics, resistance of chemotherapeutics results in the failure of cancer treatment. Primary or acquired resistance of many tumors has led researchers to focus on searching new and strong strategies to enhance the efficacy of chemotherapy [3]. Although cisplatin and carboplatin, which are well known and the most effective anticancer drugs, are used widely, their resistance as well as toxic effects on healthy tissue have led to a need to create more effective new metal complexes with less toxicity. Metal complexes provide a broad platform for drug design in the field of anticancer therapy [4]. A relationship between metals and cancer treatment has been widely accepted by researchers. As the biological activity of proteins and enzymes bind up their metal center, many metal complexes have been considered potential chemotherapeutic agents and wide-ranging studies on the transition metal complexes of the heterocyclic ligand have been carried out. In addition to various oxidation states of metal ions, metal complexes have different geometries that can alter the chemical reactivity and coordination number [4,5]. The importance of metals or metal-containing compounds for the treatment of cancer has been known since the 16th century [6,7]. Among all the metals, platinum and gold still remain attractive compounds with potential anticancer activities. An optimal design to regulate the chemical and physical behavior of biologically gold complexes by the inclusion of appropriate ligands in well-known gold complexes with anticancer properties can be successful [8]. Altaf and colleagues recently reported on gold(III) dialkyl/diaryldithiocarbamato complexes as well as tetrakis(thione)platinum(II) complexes

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(2015), Mustafa and colleagues reported on (1,2-diaminocyclohexane)(1,3-diaminopropane)gold(III) chloride complexes, and Al-Jaroudi and colleagues reported that mixed diamine ligand gold(III) complexes (2014) and mono and binuclear phosphanegold(I) dithiocarbamate complexes (2015) have attractive anticancer properties *in vitro* [9–13].

The present work was designed to continue research in the field of cytotoxic activity of some gold(III) complexes. We synthesized, analyzed, and investigated the anticancer activity of $[Au(L)B](PF6)_2$ and $[(Au(L)B)Pt]Cl_2$ complexes (L: 2-phenylimidazo[4,5-f][1,10]phenanthroline used as ligands, and B: bis-1,4-di[([1,10] phenanthroline-5-il)amino]-2-buten used as bridge) on lung carcinoma cells (A549) and healthy lung fibroblast cells by measuring cytotoxic [3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay] and apoptotic effects [biochemical measurement of the caspase-3 enzyme and 4,6-diamidino-2-phenylindole (DAPI) staining] of compounds.

Materials and methods

Chemical synthesis

$NaAuCl_4{\cdot}2H_2O$ and ligands were purchased from Sigma-Aldrich, Munich, Germany. All other reagents were used as purchased from commercial suppliers without further purification. The compounds were checked for purity by thin-layer chromatography (TLC) on a silica gel 60 F_{254} (Merck, Darmstadt, Germany). Melting points were determined using a Gallenkamp MPD350.BM2.5 digital melting point apparatus (Anadolu University, Eskişehir, Turkey) and were uncorrected. Spectroscopic data were recorded on the following instruments: elemental analyses were carried out on a CHNS-O Carlo Erba EA 1108 elemental analyzer (Anadolu University); IR using a Shimadzu 470 IR spectrophotometer (Anadolu University); ^{1}H NMR spectra [δ(ppm) Hz] were run on a Varian (300 MHz) spectrometer (Anadolu University) in $CDCl_3$ or d_6-DMSO; and fast atom bombardment was recorded using a Finnigan Mat 95 mass spectrometer (Anadolu University) with meta-nitrobenzylalcohol as the matrix. The compounds were checked for purity by TLC on silica gel 60 F_{254} (Merck).

In this research, $[Au(L)(B)]PF_6$ and $[Au(L)(B)]PtCl_2$ complexes were synthesized using 2-phenylimidazo [4,5-f][1,10]phenanthroline (L:pip) as a ligand and bis-1,4-di[([1,10] phenanthroline-5-il)amino]-2-buten (B) as a bridge molecule.

The ligand 2-phenylimidazo[4,5-f][1,10]phenanthroline (pip) (L) was synthesized according to the procedure published already [14–17]. A mixture of 1,10-phenanthroline-5,6-dione (1 mmol), appropriate aldehyde derivative (1.4 mmol), glacial acetic acid (8 ml), and ammonium acetate (1.6 g) was refluxed for 1 h; after cooling, this mixture was diluted with water and neutralized with concentrated aqueous ammonia, immediately yielding a yellow or light-yellow precipitate, which was washed with water, acetone, and diethyl ether, respectively, and then dried in a dessicator. The pure products were obtained by recrystallization. The bridge compound bis-1,4-di[([1,10]phenanthrolin-5-il) amino]-2-buten (B) was obtained using the prevalent literature method [17].

A mixture of $NaAuCl_4{\cdot}2H_2O$ (1 mmol), ligand (pip) (1 mmol), and EtOH-H_2O (10 ml) was refluxed under argon for 8 h. The reaction mixture was cooled, the precipitate was filtered and washed with water and diethylether, and dried in a vacuum dessicator. Purification of the compounds was monitored by TLC and ^{1}H NMR.

$[Au(L)B](PF_6)_2$ complexes were synthesized according to the classical literature method. $Au(pip)Cl_2$ (1 mmol) and bis-1,4-di[([1,10] phenanthroline-5-il)amino]-2-buten (B) (1 mmol) in MeOH were heated to reflux under argon for 6 h. After cooling to room temperature, MeOH was evaporated. The complex was precipitated by the addition of saturated aqueous NH_4PF_6. The product was filtered and washed with water and diethylether. The complex was dried in vacuo for 24 h and stored in a desiccator. Recrystallization from acetonitrile/diethylether was used for purification.

The mixture of $[Au(L)B](PF_6)_2$ solution in EtOH and K_2PtCl_4 in H_2O was stirred at room temperature for 12 h. The precipitate was filtered and washed several times with water and diethylether, and dried in a vacuum dessicator.

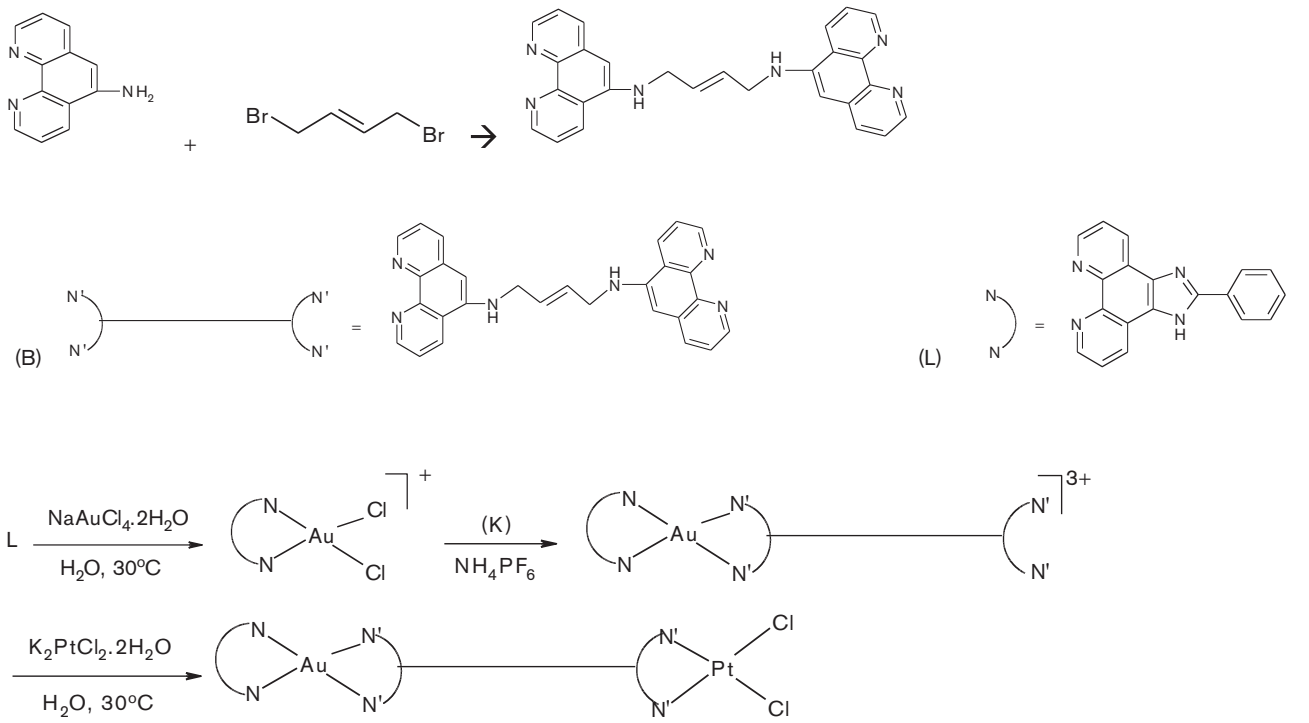
Purification of all the compounds was performed by TLC and characterization of the intermediate and final compounds arising from this work was carried out by a variety of spectroscopic methods, which include ^{1}H NMR, IR, MS, and elemental analysis. Synthesis methods are provided in Figure 1.

$[Au(L)B](PF_6)_2{\cdot}2H_2O$: Selected IR data (KBr disk ν_{max}/cm^{-1}): 3450, 3070, 3016 (Ar N–H, C–H), 1628, 1601, 1447 (C=N, C=C), 289 (M–N). ^{1}H NMR (300 MHz) (DMSO-d_6) δ(ppm): 3.36–3.39 (4H, m), 4.06–4.15 (2H, m), 7.56–8.15 (10H, m), 8.30–8.55 (12H, m), 8.60–8.73 (14H, m), 8.97–9.33 (2H, m), 10.80 (1H, bs), 10.89 (1H, bs). MS (ES): *m*/*z*: 1111 [M + 1].

$[Au(L)B]PtCl_2{\cdot}2H_2O$: Selected IR data (KBr disk ν_{max}/cm^{-1}): 3450, 3070, 3016 (Ar N–H, C–H), 1628, 1601, 1447 (C=N, C=C), 325 (M–Cl), 289 (M–N). ^{1}H NMR (300 MHz) (DMSO-d_6) δ(ppm): 3.36–3.39 (4H, m), 4.06–4.15 (2H, m), 7.65–8.24 (22H, m), 8.67–8.78 (14H, m), 9.27–9.45 (2H, m), 10.85–10.91 (2H, bm). MS (ES): *m*/*z*: 1377 [M + 1].

The stability of gold(III) anticancer agents is therefore an important determinant of clinical efficacy. Our complexes were very stable and they could easily be dissolved in dimethyl sulfoxide (DMSO) at small volumes.

Fig. 1



Synthesis of the compounds.

Cells and cell culture assays

CHL (Chinese hamster lung fibroblast like) and A549 (human lung carcinoma epithelial like) cell lines were obtained from the Institute for Fermentation (Osaka, Japan). CHL cells were maintained as a monolayer in Dulbecco's modified Eagle medium (Sigma) containing 10% (v/v) fetal bovine serum (Sigma), penicillin-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. CHL and A549 cells were incubated at 37°C in a humidified atmosphere of 5% (v/v) CO_2 in air.

Cytotoxicity assay

MTT was used to evaluate the viability of cells [18]. Compounds were dissolved freshly in DMSO and screened at a range of concentrations in fresh liquid medium using cancerous and normal cells. Both cell lines were cultured in medium with 10% fetal calf serum in the presence of 2.5–40 μmol/l of $[Au(L)B](PF_6)_2$ and $[(Au(L)B)Pt]Cl_2$. After 24, 48, and 72 h incubation, the MTT assay was performed as described previously by Koparal *et al.* [19]. Cisplatin was used as a positive control as it was used for the treatment or palliation of many types of cancers including non-small cell lung, ovarian, and testicular tumors [20]. All experiments were repeated at least three times.

DAPI staining

Nuclear staining was performed using DAPI. After 24 h of seeding, cells were treated with various doses of $[Au(L)B](PF_6)_2$, $[(Au(L)B)Pt]Cl_2$, and cisplatin for 12 h. The assay was performed as described previously [21]. Fluorescent images were viewed using an Olympus fluorescence microscope (Olympus IX70; Olympus, Tokyo, Japan).

Colorimetric caspase-3 assay

The enzymatic activity of caspase-3 induced by compounds was investigated using a colorimetric assay kit (R&D Systems, Minneapolis, Minnesota, USA) 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

Table 1 IC_{50} values of compounds

IC_{50} values of test compounds	24 h	48 h	72 h
A549/[Au(L)B]$(PF_6)_2$	Between 20 and 30 μmol/l	5 μmol/l	Below 2.5 μmol/l
CHL/[Au(L)B]$(PF_6)_2$	Between 5 and 10 μmol/l	Below 2.5 μmol/l	Below 2.5 μmol/l
A549/[(Au(L)B)Pt]Cl_2	About 20 μm	Below 2.5 μmol/l	2.5 μmol/l
CHL/[(Au(L)B)Pt]Cl_2	About 10 μm	Below 2.5 μmol/l	2.5 μmol/l
A549/cisplatin	Above 20 μm	About 20 μmol/l	About 20 μmol/l
CHL/cisplatin	Above 20 μm	20 μmol/l	Below 20 μmol/l

at a wavelength of 405 nm using an ELISA reader (ELX808IU; Bio-Tek, Winooski, Vermont, USA).

Statistical data analysis

The SPSS software (SPSS Inc., Chicago, Illinois, USA) was used for the statistical analyses of the MTT assays. Data were evaluated using one-way analysis of variance, followed by the Tukey test. A value of P less than 0.05 was considered significant.

Results

Chemistry

In the current work, we described the synthesis and characterization of Au(III) and Pt(II) dinuclear complexes. The structure of the compounds obtained was elucidated by spectral data. [Au(L)(B)]PF_6 and [Au(L)(B)]$PtCl_2$ complexes were synthesized using 2-phenylimidazo[4,5-f][1,10]phenanthroline (L:pip) as a ligand and bis-1,4-di[([1,10] phenanthroline-5-il)amino]-2-buten (B) as a bridge molecule. The characterization of the intermediate and final compounds obtained from this work was carried out using a variety of spectroscopic methods, which included ^{1}H NMR, IR, MS, and elemental analysis. IR spectra of the ligands showed that two bands were different from the spectrum of gold complexes. In general, the carbonyl stretches were relatively insensitive to changes in the metal center and its coordination environment on the carbonyl stretch was a secondary effect. In the ^{1}H NMR spectra, the peaks of gold complexes' aromatic protons were observed as multiplets. Because of greater aromatic planar and stronger deshielding effect, the phenanthroline protons showed large downfield shifts. The proton resonance on the nitrogen atom of the imidazole rings of pip was not observed because the proton was active and exchanged quickly between the two nitrogens of the imidazole rings. As the N atoms were higher in number, with stronger electronegativity in the intercalated ligand ip or pip than in the ancillary ligand phen, the protons in the intercalated ligand had more electron-deficient characteristics than those in the ancillary ligand. Therefore, there were more downfield chemical shifts for the respective protons in the intercalative ligand. The results of elemental analyses and especially the M + 1 values in the ES/MS spectra are as expected.

Inhibition of proliferation by new metal complexes on cells

In our biological evaluation, the cytotoxicity of newly synthesized compounds was compared with cisplatin, the approved Pt drug in the clinic. The inhibitory concentration 50 (IC_{50}), is defined by the FDA as the concentration required to reduce the size of the cell population by 50%. Under our experimental conditions, cisplatin was more cytotoxic than our newly designed compounds in two cell lines tested, as highlighted by IC_{50} values (Table 1). [Au(L)B]$(PF_6)_2$ and [(Au(L)B)Pt]Cl_2 treatment showed a significant dose-dependent decrease in cell viability. [(Au(L)B)Pt]Cl_2 induced higher response on cells tested at the lowest concentrations for 24 h than the 72 h exposure period. After 24 h of exposure, [(Au(L)B)Pt]Cl_2 at concentrations of 5, 10, and 20 μmol/l induced inhibition of A549 cell proliferation by 22, 41, 40, and 69%, respectively. The cytotoxic effect of [Au(L)B]$(PF_6)_2$ at a minimum concentration of 2.5 μmol/l to A549 and CHL cells resulted in less than 20% inhibition of cell proliferation after 24 h of exposure (Fig. 2).

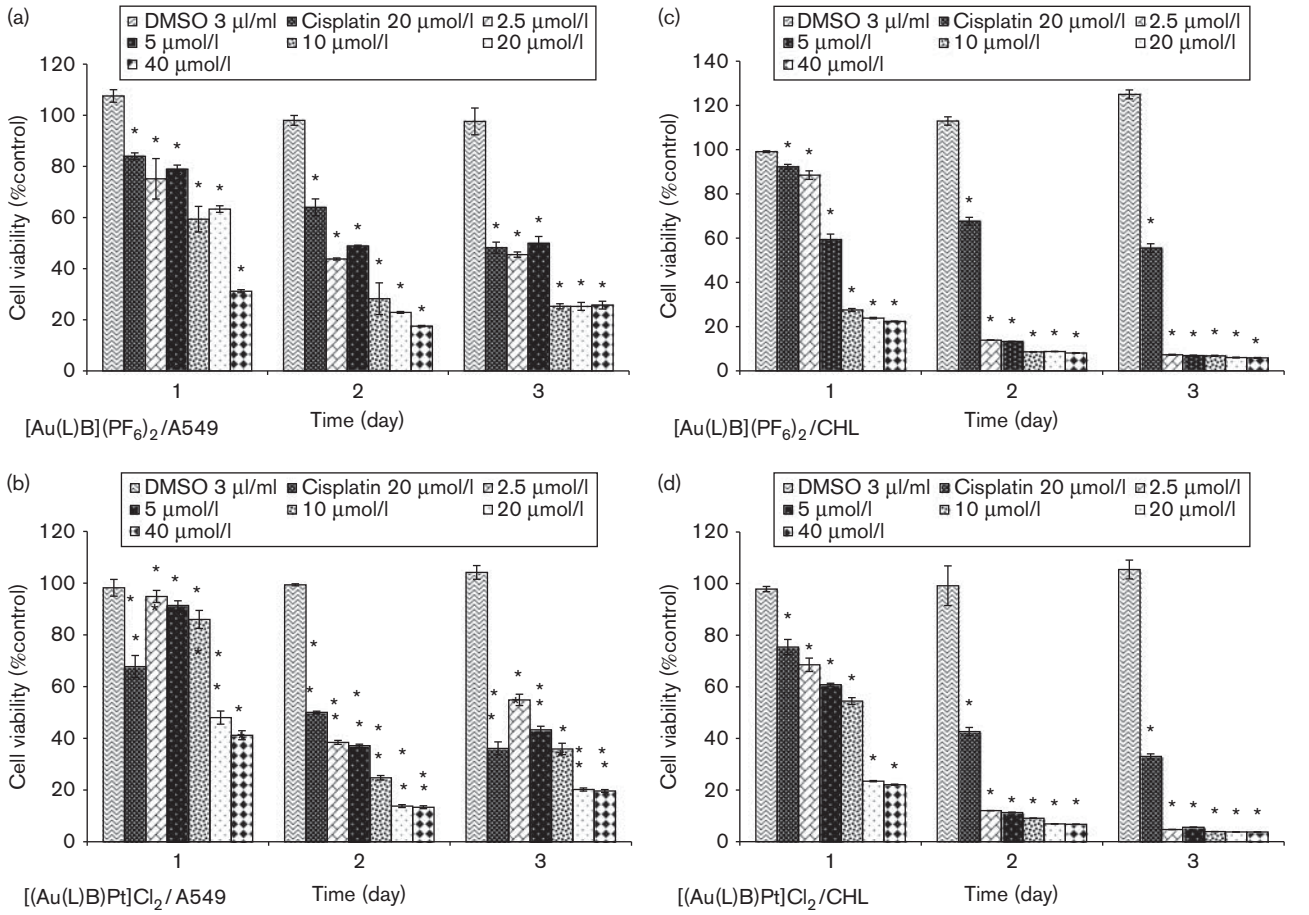
Detection of apoptotic bodies

Nuclear chromatin condensation and nuclear fragmentation are a typical characteristic of cell death, especially in apoptosis. Both of the cell lines were exposed to compounds and cisplatin for 12 h and then cells were stained with DAPI. Condensed nuclear material can be stained with DAPI and appears as bright dots, as shown in Figs 3 and 4. Cisplatin is known to induce apoptotic cell death in cancer cells [22]. Consistent with our results, as shown in Figs 3 and 4, DAPI staining showed that apoptotic morphological features such as cell shrinkage, condensation, and apoptotic bodies (arrows) were present in cisplatin-, [Au(L)B]$(PF_6)_2$- and [(Au(L)B)Pt]Cl_2-treated cells, whereas no significant changes in the control and solvent group were observed.

Detection of apoptosis by caspase-3 enzyme activity

Caspase-3 is an important apoptotic marker involved in the mitochondria-dependent intrinsic apoptotic pathway and activated at the late phase. To address whether compounds induce apoptosis, we measured caspase-3 activity in CHL and A549 cells in the presence of [Au(L)B]$(PF_6)_2$, [(Au(L)B)Pt]Cl_2 and cisplatin. Measurement of the colorimetric caspase-3 level indicated an increased level of caspase-3 at 12 h in CHL and A549 cells (Fig. 5).

Fig. 2



(a–d) Comparison of the cytotoxic activity of $[Au(L)B](PF_6)_2$ and $[(Au(L)B)Pt]Cl_2$ on A549 and CHL 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. *Significant difference from the control group by the Tukey test ($P < 0.05$). (a) $[Au(L)B](PF_6)_2$ on A549, (b) $[(Au(L)B)Pt]Cl_2$ on A549, (c) $[Au(L)B](PF_6)_2$ on CHL, (d) $[(Au(L)B)Pt]Cl_2$ on CHL cells. DMSO, dimethyl sulfoxide; MTT, 3-(4,5-dimethyl-2-thiazol)-2,5-diphenyl-2H-tetrazolium bromide.

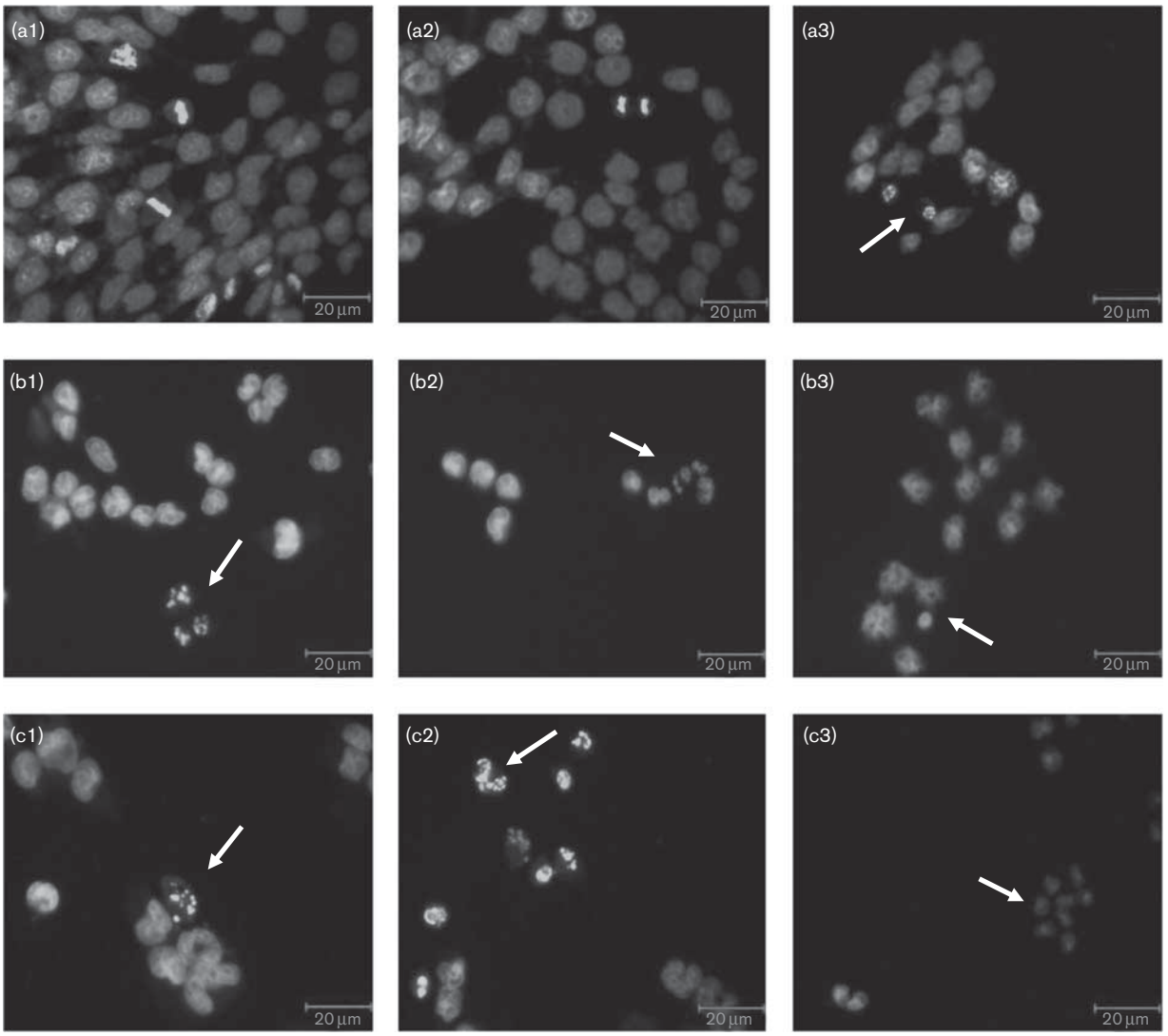
The caspase-3 activity of cancer cells treated with $[(Au(L)B)Pt]Cl_2$ for 12 h was found to be two times higher than healthy cells. The results showed that cisplatin exerted the same effect in both cell lines, which was lower than our new compounds and also indicated that $[Au(L)B](PF_6)_2$ stimulated caspase-3 activity in healthy cells was more than that in the cancer cells.

Discussion

We previously reported our investigation on Au(III) and Pt(II) complexes of 1,10-phenanthroline (phen), namely, $[Au(phen)Cl_2]Cl$ and $Pt(phen)Cl_2$ against lung cancer and dinuclear $[Au(L1)(B)Pt]Cl_2$ and $[Ru(L1)_2(B)Pt]Cl_2$ complexes including 1,8-diaminonaphthalene (L) as a ligand and bis-1,4-di[([1,10]phenanthroline-5-il)aminomethyl]cyclohexane (B) as a bridge on an *H-ras* oncogene-activated cancer cell line. It was clearly observed that gold complexes had better anticancer activity in both studies [21,23]. Here, the investigation was extended to a new series of gold(III) complexes. All test compounds and cisplatin showed a concentration-dependent cytotoxic effect in both cell lines (Fig. 2). The cell proliferation reduced significantly after 24 h of treatment of test compounds. The MTT results showed that especially $[Au(L)B](PF_6)_2$ induced inhibition in cancer cell growth, with a lower toxic effect on healthy cells. On the basis of the cytotoxicity results, it might be suggested that when PF_6 was incorporated into gold complexes instead of the Pt, the resulting gold complexes tended to show an enhanced anticancer effect with fewer side effects *in vitro*. Under the same biological conditions, cisplatin showed similar toxicity in both healthy and cancer cells with a higher examination concentration (the IC_{50} value was above 20 µmol/l for 24 h).

It is well known that commonly used anticancer drugs trigger apoptosis to kill cancer cells [3]. On the basis of

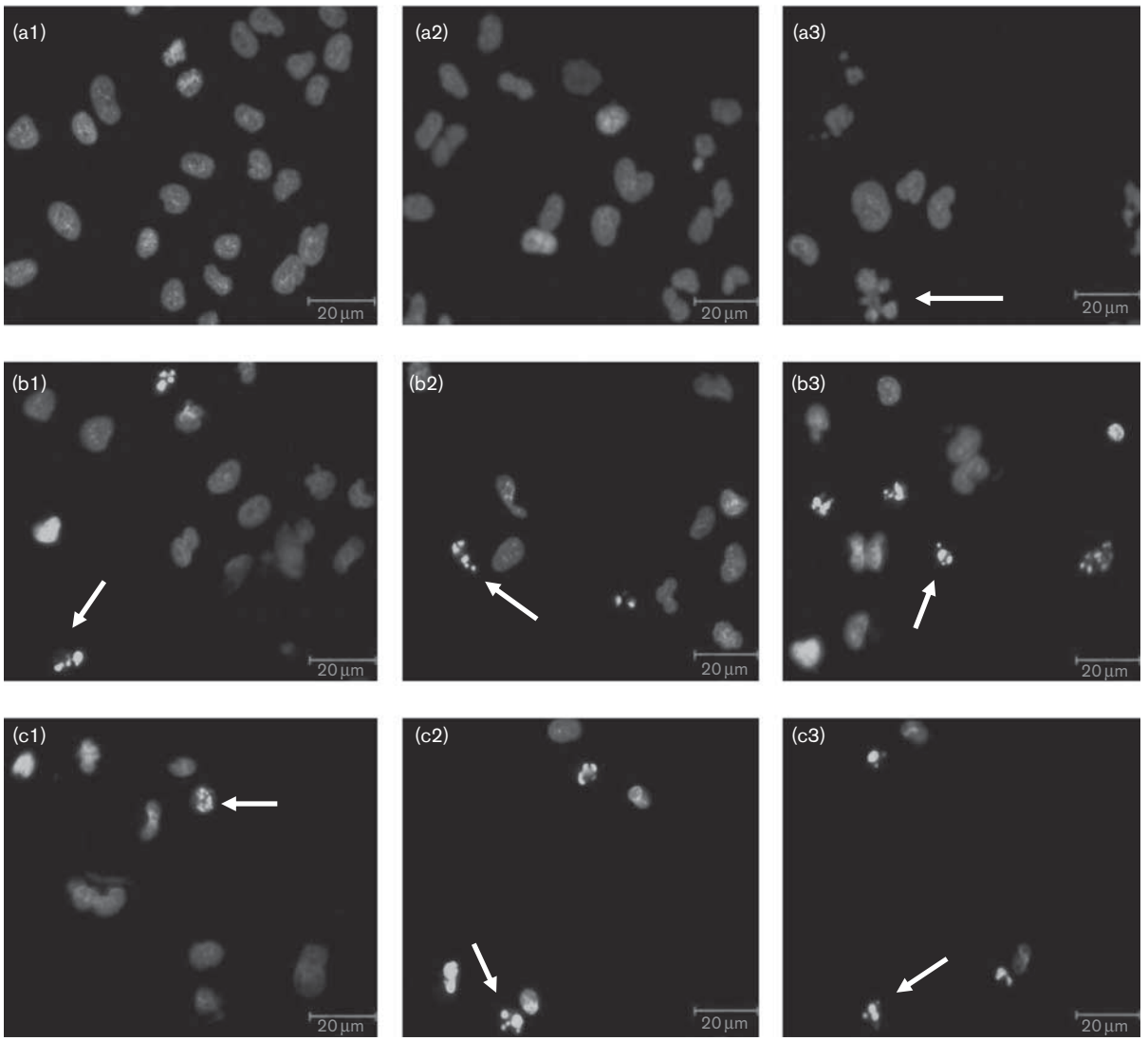
Fig. 3



Chromatin condensation and observation of apoptotic bodies on CHL cell cultures evaluated after 12 h, stained with DAPI. (a1) Control. (a2) Cells treated with DMSO. (a3) Cells treated with cisplatin 40 μmol/l. (b1–b3) Cells treated with $[Au(L)B](PF_6)_2$ 10–20–40 μmol/l, respectively. (c1–c3) Cells treated with $[(Au(L)B)Pt]Cl_2$ 10–20–40 μmol/l, respectively. Arrows show classical apoptotic bodies. Scale bars 20 μm. DAPI, 4,6-diamidino-2-phenylindole; DMSO, dimethyl sulfoxide.

this idea, apoptotic effects of $[Au(L)B](PF_6)_2$ and $[(Au(L)B)Pt]Cl_2$ complexes were evaluated microscopically and biochemically *in vitro*. DAPI staining is a reliable apoptotic assay in the field of chemotherapeutic research and it helps to detect the apoptotic changes at the DNA level [24]. It should be noted that a significant number of cells showing signs of apoptosis such as condensation, apoptotic bodies, and fragmentations were observed in $[(Au(L)B)Pt]Cl_2$ and $[Au(L)B](PF_6)_2$-treated groups of cells. Caspase-3 is another detection method for apoptosis. The caspase-dependent apoptotic process is related to two pathways of anticancer drug-induced apoptosis: the intrinsic (a mitochondria-dependent pathway) and extrinsic pathway (a death receptor-dependent pathway). The death receptor activation pathway uses transmembrane death receptors and their ligands, which finally activate caspase-8. Activated caspase-8 initiates the direct cleavage and activation of the downstream effectors caspase-3 and caspase-7, leading to apoptosis. The mitochondria-dependent pathway involves cleavage of proapoptotic proteins by caspase-8 releasing cytochrome C in the mitochondria. Released cytochrome C activates caspase-3 and caspase-9, inducing apoptosis, with morphological, biochemical, and mitochondrial changes. Caspase-3 is associated with apoptosis and induces a large number of morphological changes characteristic of cells undergoing apoptosis [25–27]. The caspase-3 activity increased significantly with $Au(L)B)Pt]Cl_2$ treatment in

Fig. 4

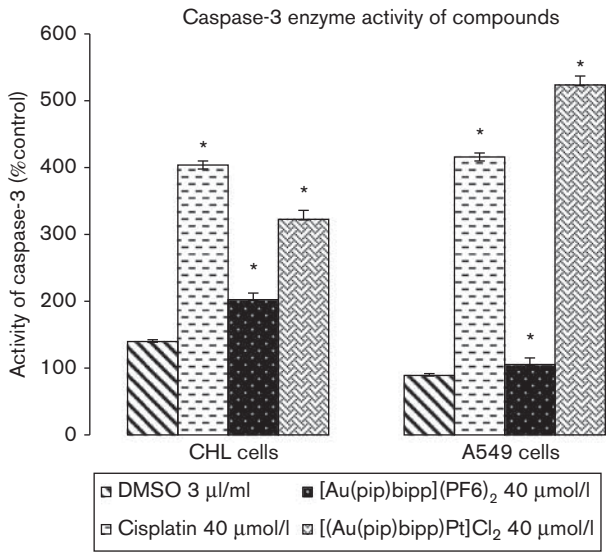


Chromatin condensation and observation of apoptotic bodies on A549 cell cultures evaluated after 12 h, stained with DAPI. (a1) Control. (a2) Cells treated with DMSO. (a3) Cells treated with cisplatin 40 µmol/l. (b1–b3) Cells treated with [Au(L)B]$(PF_6)_2$ 10–20–40 µmol/l, respectively. (c1–c3) Cells treated with [(Au(L)B)Pt]Cl_2 10–20–40 µmol/l, respectively. Arrows show classical apoptotic bodies. Scale bars 20 µm. DAPI, 4,6-diamidino-2-phenylindole; DMSO, dimethyl sulfoxide.

cancer cells compared with the [Au(L)B]$(PF_6)_2$ and cisplatin. The results revealed that cisplatin exerted the same effect in both cell lines, which was lower than that of our new compounds. It was also found that [Au(L)B]$(PF_6)_2$ stimulated caspase-3 activity in healthy cells more than the cancer cells. These results revealed that apoptosis is a major mechanism whereby gold complexes induce cell death. In addition to our previous studies, on the basis of the present results, induction of apoptosis by gold complexes might be through the caspase-3 pathway on human lung cancer cells.

Several publications have shown that gold(III) compounds had more cytotoxic activity than cisplatin. Messori *et al.* [28] studied Au complexes and DNA interactions, and gold(III) was found to be effective against Pt(II)-resistant cell lines with fewer negative effects. While studying interactions between Au complexes and guanine, Onkto and co-workers (cited in another study by Messori *et al.* [29]) observed ligand substitution reactions for some Au complexes. Mirabelli *et al.* (1986) reported that gold(III) ions could induce DNA strand breaks [30]. The antiproliferative and cytotoxic effects of series of gold(III) compounds were synthesized and analyzed on HeLa and A549 cells by Wang *et al.* [31] and by Maiore *et al.* [32] on cisplatin-resistant cells. In addition to the literature, the presented data indicate that gold(III) complexes with specific ligands

Fig. 5



A549 and CHL cells were treated with $[Au(L)B](PF_6)_2$ (40 µmol/l), [(Au(L)B)Pt]Cl_2 (40 µmol/l), and cisplatin (40 µmol/l) compounds for 12 h. Caspase-3 activity was determined using the caspase assay kit obtained from R&D Systems 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 the Tukey test (*$P < 0.05$ vs. untreated control). DMSO, dimethyl sulfoxide.

incorporated into the structural design may have the potential to overcome mechanisms including resistance and side effects to cisplatin.

Researchers have been attempting to find a perfect match to gold and ligand complexes with fewer side effects [33–39]. Antitumor activities of new gold(III) complexes with 1,2-diaminocyclohexane were estimated in prostate cancer and gastric carcinoma by Al-Jaroudi *et al.* [40]. Pantelić and co-workers reported the anticancer activity of gold(III) complexes with some esters of (*S*,*S*)-ethylenediamine-*N*,*N*′-di-2-propanoic acid against human cervix adenocarcinoma (HeLa), human myelogenous leukemia (K562), and human melanoma (Fem-x) tumor cell lines as well as against a noncancerous human embryonic lung fibroblast cell line (MRC-5). They suggested that one of the complexes showed higher activity and selectivity than cisplatin [41].

Besides gold(III)-containing and platinum(II)-containing compounds, 1,10-phenanthroline has been reported to be cytotoxic to tumor cells [42–45]. DNA-binding properties, cytotoxic, and antiproliferative activities were investigated by different laboratories. Although it is well known that 1,10-phenanthroline 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, in the literature, a few studies have reported on its anticancer activity. A gold complex with 6,6-dimethyl-1,10-phenanthroline was synthesized and characterized, and DNA binding and cytotoxic activities were determined [46]. Recently, a phenanthrolin-dicarbocylate ester has been found to show promising anticancer activity with selective DNA-binding activity against several human cancer cell lines [47]. The chemotherapeutic potential of 1,10-phenanthroline and three of its transition metal complexes was determined on two different cancer cells by Deegan *et al.* [48] and they suggested that phen and three metal–phen complexes may have been successful therapeutics. A silver(I) complex of phenanthroline was found to be more potent than cisplatin on HepG2 cells [49]. Gold(III) complexes with bidentate ligands based on the 1,10-phenanthroline and 2,2′-bipyridine scaffolds showed promising anticancer activity against a variety of tumor cell lines [50]. Interestingly, phenanthroline-based small molecules were used as a cellular imaging and cancer screening agent by Sergeeva and colleagues. Their studies showed that these compounds have selective activity to esophageal cancer cells and can be used as selective markers in intracellular cancer diagnostics. They have remarkable cytotoxicity [51].

Consequently, the various metal-based compounds showed marked anticancer activity. It seems that apoptosis is the main mechanism responsible for the inhibition of cancer cell proliferation. However, exact identification of the molecular mechanism and selectivity of the compounds requires further studies to improve the results of chemotherapy and quality of life of the patients. Together with literature-based data, our findings provide a new insight into the effect and selective activity of gold (III) compounds with phenanthroline.

Conclusion

In this study, $[Au(L)B](PF_6)_2$ and [(Au(L)B)Pt]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. Also, their antiproliferative, antitumoral, and apoptotic activities were determined. Generally, $[Au(L)B](PF_6)_2$ and [(Au(L)B)Pt]Cl_2 showed better anticancer properties in A549 cells with lower cytotoxicity on healthy cells, whereas cisplatin showed similar cytotoxic effects on both cancer and healthy cells. The IC_{50} value was around 20–30 µmol/l in A549 cells and 5–10 µmol/l in CHL cells for $[Au(L)B](PF_6)_2$ and 20 µmol/l in A549 cells and 10 µmol/l in CHL cells for [(Au(L)B)Pt]Cl_2 as shown in Table 1. In the light of high efficacy of our newly synthesized gold complexes, they might be good and promising anticancer agents compared with the cisplatin. Further research is currently ongoing in our laboratory to determine the mechanism of inhibition and the modifications of the compounds with different organometallics to improve the potency.

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Conflicts of interest

There are no conflicts of interest.

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