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Original article

THE EFFECTS OF SOME PHENANTHROLINE RUTHENIUM (II) COMPLEXES ON A549 CELL PROLIFERATION

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Abstract

In recent years, ruthenium (II) complexes have increasingly attracted the interest of researchers because of their high antitumor activities that are usually related to DNA binding. Here, we synthesized two ruthenium (II) complexes, using imidazo[4,5-f][1,10]phenanthroline (ip) and 2-phenylimidazo[4,5-f][1,10]phenanthroline (pip) that were characterized by spectroscopic and elemental analyses. First of all, we investigated the ability of these complexes to produce lethal effects in human lung carcinoma, A549 cells. The cytotoxicity results evaluated by the MTT assay, revealed that the IC_{50} for Ru(II) complexes of imidazo[4,5-f][1,10]phenanthroline $[Ru(ip)_3](PF_6)_2$ and 2-phenylimidazo[4,5-f][1,10]phenanthroline $[Ru(pip)_3](PF_6)_2$ after 24 h of incubation with A549 cells was approximately 32 and 46 μg/ml, respectively. Interestingly, it was observed that these complexes showed weak cytotoxic effects in normal human lung cells (IC_{50} > 80 ug/ml). $[Ru(pip)_3](PF_6)_2$ has a potent inhibitory effects on A549 compared to other cells as measured by BrdU labeling assay. Treatment of DNA with $[Ru(pip)_3](PF_6)_2$induced DNA binding activity, which was demonstrated by a viscosity assay. In summary, Ru(II) complexes of $[Ru(pip)_3](PF_6)_2$ showed significant cytotoxic and anti-proliferative effects on A549 cells, suggesting that this complex may have potential therapeutic agent, and therefore, this must be further investigated by other in vitro bioassays for the development of therapeutic agents.

Key words: *Ru(II) complex, Phenanthroline, Cell viability, DNA binding, A549.*

Bazı Fenantrolin Rutenyum (II) Komplekslerinin A549 Hücre Çoğalması Üzerine Etkileri

Ruthenium (II) komplekslerinin DNA bağlanması ile ilgili olan yüksek antitumor aktiviteleri nedeni ile bu bileşikler son yıllarda araştırmacıların yoğun ilgisini çekmektedir. Bu çalışmada imidazo[4,5-f][1,10]fenantrolin (ip) and 2-fenilimidazo[4,5-f][1,10]fenantrolin (pip) ligand olarak kullanılarak, iki rutenyum (II) kompleksi sentezlenmiş olup, bu bileşikler spektroskobik ve elemental analiz yöntemleri ile karakterize edilmiştir. İlk olarak, bu komplekslerin insan akciğer karsinoma, A549 hücreleri üzerine olan ölümcül etkileri araştırılmıştır. MTT deneyi ile elde edilen sitotoksik sonuçlar göstermiştir ki, A549 hücrelerinin 24 saat imidazo[4,5-f][1,10]fenantrolin Ru(II) kompleksi $[Ru(ip)_3](PF_6)_2$ ve 2-fenilimidazo[4,5-f][1,10]fenantrolin Ru(II) kompleksi $[Ru(pip)_3](PF_6)_2$ ile inkübasyonu sonucunda IC_{50} değerlerinin sırasıyla yaklaşık olarak 32 ve 46 μg/ml'dır.İlginçtir ki, bu komplekler normal insan akciğer hücrelerine karşı düşük sitotoksik etkiye sahiptirler (IC_{50} > 80 ug/ml).BrdU bağlanma deneyi sonucunda, $[Ru(pip)_3](PF_6)_2$ kompleksinin diğer hücrelere kıyasla A549 hücreleri üzerinde güçlü baskılayıcı etkiye sahip olduğu görülmüştür. $[Ru(pip)_3](PF_6)_2$ kompleksinin dana timus DNA'sı ile etkileşimi sonrasında DNA bağlanma aktivitesinin arttığı viskozite deneyi ile tespit edilmiştir.Özet olarak, $[Ru(pip)_3](PF_6)_2$ kompleksi anlamlı sitoksik etki ve A549 hücre çoğalması üzerine inhibitor bir etki göstermiştir. Bu kompleksin güçlü tedavi edici bir etkiye sahip olabileceği ve bu nedenle tedavi edici ajanların geliştirilmesi için farklı in vitro biyoassaylar kullanarak araştırılması gerekliliği varsayılmaktadır.

Anahtar kelimeler: *Ru (II) kompleks, Fenantrolin, Hücre canlılığı, DNA bağlanması, A549.*

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INTRODUCTION

Nucleic acid-binding metal complexes are currently being investigated in many laboratories because of their utility as DNA structural probes, DNA-dependent electron-transfer probes and potential anticancer drugs (1, 2). Recently, there has been increased attention focused on the binding properties of polypyridyl complexes of ruthenium with biomolecules like DNA, RNA or polynucleotides (3, 4).

In this regard, ruthenium (II) complexes have attracted a great deal of attention because of their spectroscopic and electrochemical properties (5-7). Many ammine complexes of ruthenium (II) tend to bind selectively to imine sites in biomolecules because of their nitrogen lone pairs that are available for metal ion coordination. Consequently, ruthenium complexes often selectively conjugate the histidyl nitrogen of imidazole on proteins and the N7 site on the imidazole ring of purine nucleotides (8). Ru(II) complexes bind to DNA mainly through three types of weak interactions: electrostatic-dominated binding, involving interactions between the cationic metal complex and the negatively charged phosphates of the DNA; ligand π-stacking interactions that are characterized by intercalation of an extended electron-deficient planar aromatic ring system (two or three six-membered rings), adjacent between the base pairs, through the major or the minor groove of the nucleic acid helix; and groove binding, in which the metal complex binds in the DNA grooves, associated by hydrogen bonds and/or van der Waals interactions along the groove of the duplex. In contrast to the intercalation mode, the groove binding does not significantly perturb the DNA structure (9). Tris (phenanthroline) complexes of ruthenium (II) display enantiomer selectivity in binding to DNA, which can be served as spectroscopic probes in solution to distinguish right- and left-handed DNA helices (10). In particular, ruthenium (II) complexes with polypyridyl ligands, due to a combination of easily constructed rigid chiral structures spanning all three spatial dimensions and a rich photo physical repertoire, have attracted considerable attention (11).

Ruthenium (II) complexes are also increasingly attracting the interest of researchers as potential antitumor and antimetastatic drugs. Some of the Ru (II) complexes demonstrated high *in vitro* cytotoxicity in HeLa, HepG2, BEL-7402 and CNE-1 tumor cells (12). Ruthenium (II) complexes showed significantly lower host toxicity (13); therefore novel antitumor properties of these, complexes seemed to be more interesting in comparison to platinum complexes.

Based on these observations, in this article, we report the synthesis and characterization of ruthenium (II) complexes of imidazo[4,5-f][1,10]phenanthroline, $[Ru(ip)_3](PF_6)_2$ and 2-phenylimidazo[4,5-f][1,10]phenanthroline, $[Ru(pip)_3](PF_6)_2$. We also investigated the effects of these complexes on DNA-binding, cytotoxicity and proliferation of A549 lung cancer cells. Because the toxicity of drugs used in the clinic is so important and impacts on their clinical success, we also investigated the cytotoxic activity of these two ruthenium (II) complexes on normal human lung cells (WI38).

EXPERIMENTAL

Synthesis of the molecules

$RuCl_3.(H_2O)n$, LiCl and 1,10-phenanthroline-5,6-dione were purchased from Aldrich organics. All the reagents were used without further purification. The compounds were checked for purity by thin layer chromatography (TLC) on silica gel 60 F_{254} (Merck, U.K). Elemental analyses were performed on a CHNS-O Carlo Erba EA 1108 elemental analyzer. The infrared spectra were recorded as KBr discs on a Shimadzu IR-470 infrared spectrophotometer in the 4000–200 cm^{-1} region. ^{1}H-NMR spectra (δ, ppm, Hz) were recorded on a Bruker DPX300 spectrometer with $(CD_3)_2SO$ as the solvent at room temperature and tetramethylsilane (TMS) as the internal standard. ES-MS spectras were recorded on a Jeol SX 102 mass spectrometer.

Synthesis of the ligands

The ligands imidazo[4,5-f][1,10]phenanthroline (ip) and 2-phenylimidazo[4,5-f][1,10]phenanthroline (pip) were synthesized according to the procedure published previously (14, 18-20). 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 conch aqueous ammonia, immediately resulting in a yellow or light-yellow precipitate, which was washed with water, acetone, and diethyl ether respectively, and then dried in desiccator. The pure products were obtained by recrystallization. Purification of the compounds was controlled by thin layer chromatography, IR and ^{1}H NMR.

Imidazo [4,5-f][1,10] phenanthroline: Selected IR data (KBr disk ν_{max}/cm^{-1}): 3437, 3114 (Ar N-H, C-H), 1616, 1419, 1391 (C=N, C=C). ^{1}H NMR $(CD_3)_2SO$ δ (ppm): 13.14(br, 1H), 9.06-9.03 (br, 1H), 8.87-8.83 (dd, 2H), 7.89-7.85 (m, 2H), 6.90-6.88 (dd, 2H).

2-phenylimidazo [4,5-f][1,10] phenanthroline: Selected IR data (KBr disk ν_{max}/cm^{-1}): 3420, 3114 (Ar N-H, C-H), 1609, 1433, 1398 (C=N, C=C). ^{1}H NMR $(CD_3)_2SO$ δ (ppm): 13.5(br, 1H), 9.02(q, 2H), 8.93(q, 2H), 8.25-8.10 (m, 3H), 7.82-7.64 (m, 4H).

Synthesis of RuL_2Cl_2 complexes

A mixture of $RuCl_3$. (H_2O) n (1 mmol), an appropriate phenanthroline derivative (2 mmol), LiCl (7 mmol) and DMF (8 ml) was refluxed under argon for 8 h, during which the color of the solution changed to dark. The reaction mixture was cooled, acetone was added and the mixture was incubated 24 h in 0°C. The precipitate was filtered, washed with water and diethyl ether, and dried in vacuum desiccator (14, 18-20). Purification of the compounds was monitored by thin layer chromatography, IR and ^{1}H NMR.

$[Ru(ip)_2Cl_2]$: Selected IR data (KBr disk ν_{max}/cm^{-1}): 3420, 3114, 3020 (Ar N-H, C-H), 1658, 1590, 1467 (C=N, C=C) 439 (M-Cl). ^{1}H NMR $(CD_3)_2SO$ δ(ppm) :7.55 (4H, t), 7.77 (2H, q), 8.10-8.35 (4H, m), 8.86 (2H, d), 10.50 (2H, d). MS (ES): m/z: 612 [M+1].

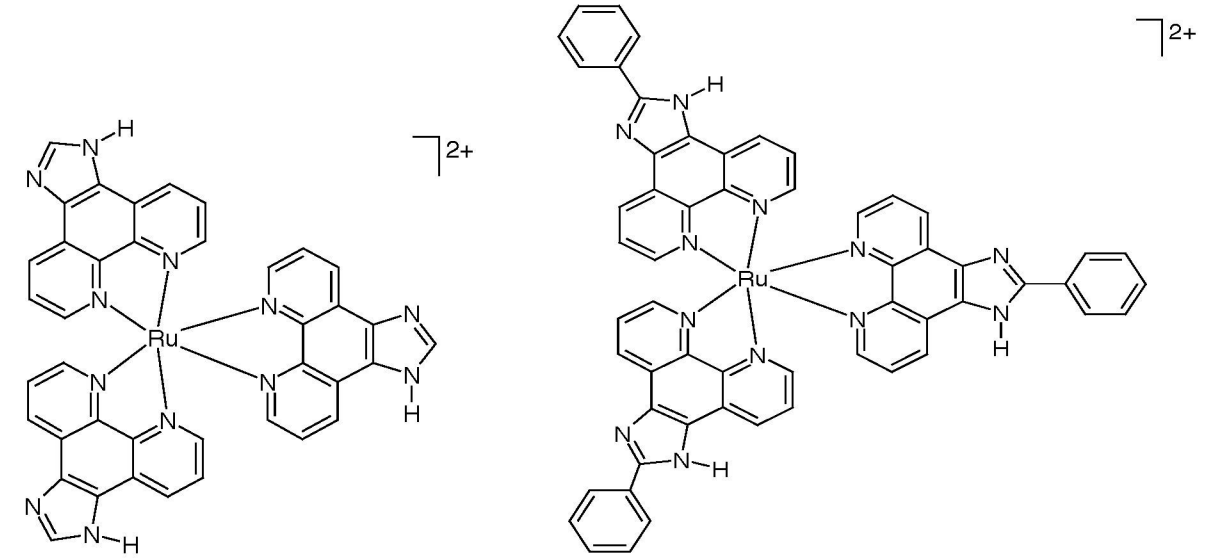
$[Ru(pip)_2Cl_2]$: Selected IR data (KBr disk ν_{max}/cm^{-1}): 3450, 3070, 3016 (Ar N-H, C-H), 1628, 1601, 1447 (C=N, C=C), 417 (M-N). ^{1}H NMR $(CD_3)_2SO$ δ(ppm) :7.55 (4H, t), 7.70-7-85 (6H, m), 8.20-8.40 (8H, m), 8.50-8.56 (4H, m), 10.50 (2H, d). MS (ES): m/z:764 [M+1].

Synthesis of $[Ru(L)_3](PF_6)_2$ complexes

$[Ru(L)_3](PF_6)_2$ complexes were synthesized according to published methods (14,18-20). $Ru(L)_2Cl_2$ (1 mmol) and an appropriate L (1 mmol) in Me-OH was heated to reflux under argon for 6 h. After cooling to room temperature, Me-OH was evaporated. The complex was precipitated as a darkly colored solid by the addition of saturated aqueous NH_4PF_6. The product was filtered and washed with water and diethyl ether. The complex was dried in a vacuum for 24 h and stored in a desiccator. Recrystallization from appropriate acetonitrile/diethyl ether or acetone/toluene was used for purification. The compound was characterized by IR (infrared), ^{1}H-NMR and MASS spectroscopic data and elemental analyses.

$[Ru(ip)_3](PF_6)_2$: Selected IR data (KBr disk ν_{max}/cm^{-1}): 3420, 3108, 3081 (Ar N-H, C-H), 1625, 1576, 1439 (C=N, C=C), 286 (M-N). ^{1}H NMR $(CD_3)_2SO$ δ(ppm): 8.55-8.14 (12H, m), 8.98-8.95 (6H, m), 9.95-9.80 (3H, dd), 10.30 (1H, bs), 10.41 (1H, bs), 10.48 (1H, bs). MS (ES): m/z: 758 [M+1]. Elemental Analysis, Calcd: C, 43.06; H, 2.22; N, 15.45. Found: C, 43.31; H, 2.52; N, 15.83%.

$[Ru(pip)_3](PF_6)_2$: Selected IR data (KBr disk ν_{max}/cm^{-1}): 3450, 3070, 3023 (Ar N-H, C-H), 1628, 1604, 1448 (C=N, C=C). ^{1}H NMR $(CD_3)_2SO$ δ(ppm): 7.95-7.54 (21H, m), 8.98-8.35 (12H, m), 10.80 (1H, bs), 10.89 (1H, bs), 10.92 (1H, bs). MS (ES): m/z: 990 [M+1]. Elemental Analysis, Calcd: C, 52.02; H, 2.75; N, 12.77. Found: C, 52.31; H, 2.52; N, 12.93%.



$[Ru(ip)_3]^{2+}$ $[Ru(pip)_3]^{2+}$

Figure 1. Chemical structures of the ruthenium (II) complexes.

Cell culture

The human cancer cell line, A549 were obtained and cultured as recommended by American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were maintained in Ham's F12K medium (Sigma-Aldrich, UK) supplemented with 10% fetal bovine serum (FBS, Gibco, U.K) as adherent monolayers. Cells were incubated at 37 °C under 5% CO_2/95% air in a humidified incubator. The normal human lung cell line WI38 was also used. WI38 cells obtained from ATCC (Rockville, MD, US) were cultured in Eagle's Minimum Essential Medium supplemented with 10% FBS. WI38 cells were routinely subcultured using 0.25% trypsin-EDTA solution (Sigma-Aldrich, UK).

Stock solutions of ruthenium (II) complexes were prepared in dimethyl sulfoxide (DMSO). Final concentrations were prepared with fresh cell culture medium (DMSO concentration < 0.1 %).

MTT assay

The effects of ruthenium (II) complexes on the viability of A549 and WI38 cells were studied by the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl-2H-tetrazolium bromide] (Sigma-Aldrich, UK) assay as described by Mossman (15). A549 and WI38 cells (2×10^4) were seeded in 96-well micro plates both in the presence and absence of different concentrations of $[Ru(L)_3](PF_6)_2$ complexes (25.6 ng, 128 ng, 640 ng, 3.2 µg, 16 µg, or 80 µg per ml) for 24 h or 48 h at 37 °C in a 5% CO_2/95 % air atmosphere. Then, 20 µL of MTT (5 mg/ml) was added to each well. Following 2 h incubation, 200 µl of DMSO was added. Cells were incubated at 25 °C for an addition 10 min and then the absorbance was read on a Bio-Tec (ELX 808 IU) ELISA reader at a wavelength of 540 nm.

The signal generated is directly proportional to the number of viable (metabolically active) cells in the wells. The values of the blank wells were subtracted from each well of treated and control cells, the % viability was determined as follows: Viability (%) = (Absorbance of the treated well) / (Absorbance of the control wells) × 100. Each concentration was tested in three different experiments run in triplicate. The IC_{50} (compound concentration (µg/ml) that produces a 50 % reduction in cellular viability) was obtained from the dose-dependent curves.

Cell proliferation assay

To investigate the effects of $[Ru(ip)_3](PF_6)_2$ and $[Ru(pip)_3](PF_6)_2$ on cell proliferation, A549 cells were incubated with various concentrations (40, 30, 20, 10, 5 or 2.5 µg/ml) of the complexes for 24 h. After each time point, the cells were labeled with 10 µl of BrdU solution at 37 °C for 2 h and then

fixed by the addition of Fixodent solution for 30 min at room temperature. After removing the Fixodent solution, cells were treated with 100 µl of anti-BrdU working solution for 90 min at room temperature. After that, the cells were incubated with the substrate solution until the color is sufficient for photometric detection that was predetermined. The absorbance of the samples was measured in an ELISA reader (Organom, Technical) at 492 nm. Paclitaxel® (Sigma-Aldrich, U.K) was used as a positive control agent.

Viscosity measurements

Viscosity experiments were carried out using an Ubbelohde viscometer maintained at a constant temperature of 30 ± 0.1 °C in a temperature bath. Rod-like DNA samples with approximately 200 bp in length were prepared by ultrasonicating calf thymus DNA (ct, Sigma) for 30 min to minimize complexities arising from DNA flexibility (16). Flow time was measured with a digital stopwatch. Each sample was measured three times, and an average flow time was calculated. Data were presented as $(\eta/\eta_0)^{1/3}$ versus the binding ratio (17), where η is the viscosity of ct DNA with the Ru (II) complexes and η_0 is the viscosity of CT DNA alone. On the other hand, binding of ethidium bromide to ct DNA was used as a positive control in this assay.

Statistical analysis

Statistical analysis of the results was performed by using a one-way analyses of variance (ANOVA) test for multiple comparisons with a control. Differences were considered statistically significant for $p < 0.05$, relative to control.

RESULTS

IR spectrums of the $[Ru (L)_3] (PF_6)_2$ complexes showed two bands between 3450-3020 cm^{-1} due to ν(N-H), 1658-1439 cm^{-1} due to ν(C=N) which were different from the spectrum of ligands. Comparing the spectra of the metal complexes with imidazophenanthroline or phenylimidazophenanthroline, it was determined that the bands centered at approximately 1390 and 1620 cm^{-1} were C=N, C=C stretches on the ligand. In general, these stretches were relatively insensitive to changes in the metal center.

The ^1H chemical shifts were assigned and comparison with those of similar compounds (14, 18-20). In the ^1H-NMR spectra, the peaks of ruthenium (II) complexes aromatic protons were observed as approximately 8.14-10.12 ppm as multiples. Due to greater aromatic planar and stronger deshielding effect, the phenanthroline protons showed large downfield shifts. In addition, the proton resonance on the nitrogen atom of the imidazole rings of imidazo[4,5-f][1,10]phenanthroline and 2-phenylimidazo[4,5-f][1,10]phenanthroline were not observed, because metal coordination causes electron deficiency in the ligand and, as a result, these NH protons are very active and easy to be exchanged between the two imidazole nitrogen atoms in solution. A similar example has been reported in the literature (18, 19). Considering there is a greater content of N atoms with strong electronegativity in the intercalated ligand imidazo[4,5-f][1,10]phenanthroline and 2-phenylimidazo[4,5-f][1,10]-phenanthroline. The proton on the nitrogen atom of the imidazole, resonating at ca. δ 13.14 /13.5 for ip and pip as a broad singlet and unobserved for Ru(II) complexes, exchanges quickly between the two nitrogens of the imidazole ring, characteristic of an active proton.

Elemental analyses results and especially the M+ 1 value in ES/MS spectra were as expected.

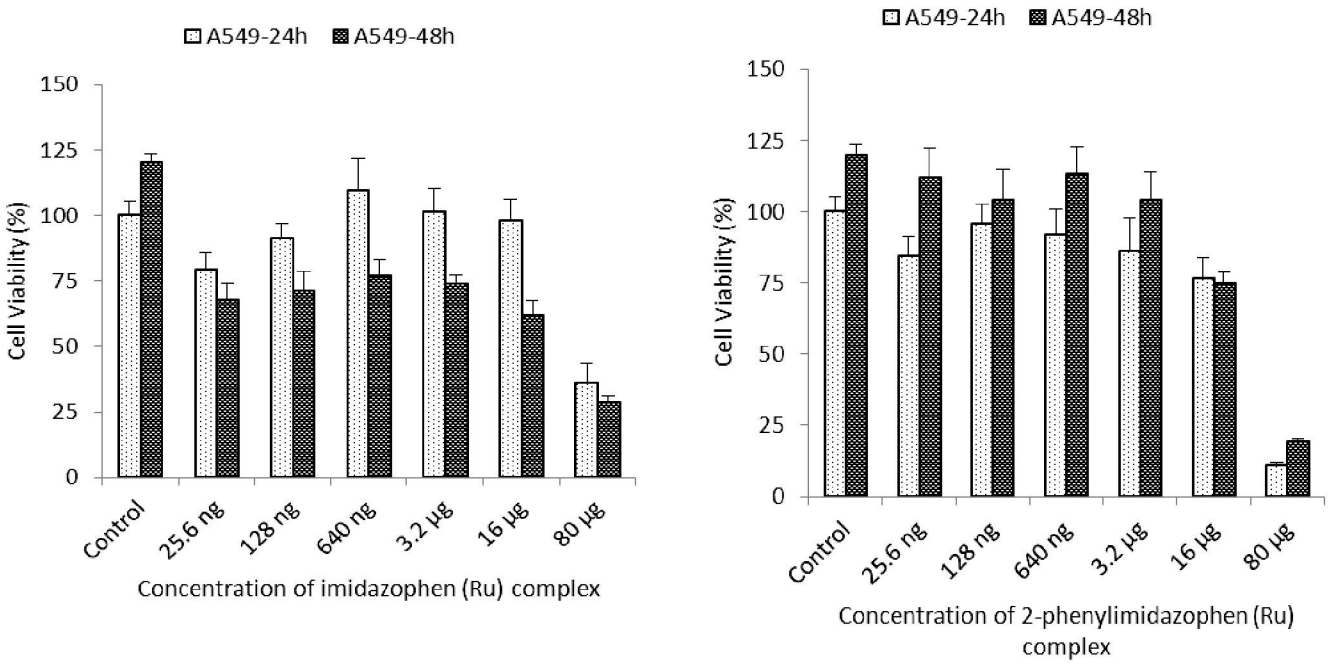


Figure 2. Effects of $[Ru(L)_3](PF_6)_2$ complexes on the viability of A549 cells. Cells were treated either 24 h or 48 h in the presence of $[Ru(L)_3](PF_6)_2$ complexes. Cell viability was assessed by the MTT colorimetric method. Data show the mean ± SD of three independent experiments.

The IC_{50} values of $[Ru(ip)_3](PF_6)_2$ and $[Ru(pip)_3](PF_6)_2$ were approximately 32 and 46 µg/ml after 24 h of exposure, respectively. In contrast to A549 cells, weak cytotoxic effects were observed in normal human WI38 cells (IC_{50} > 80 µg/ml, Figure 3).

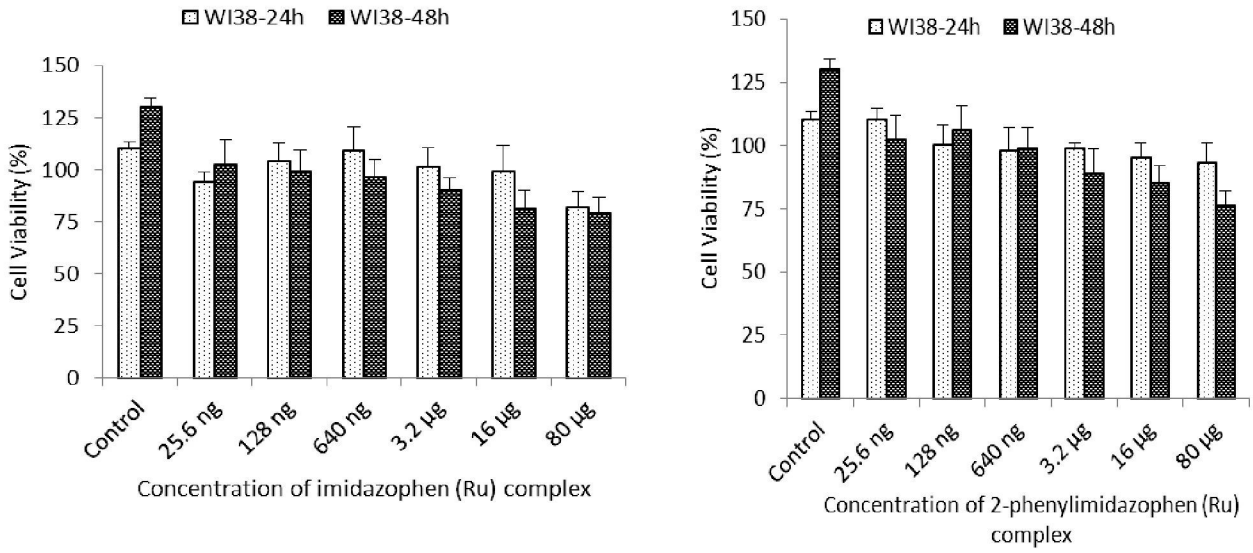


Figure 3. Effects of $[Ru(L)_3](PF_6)_2$ complexes on the viability of WI38 cells. Cells were treated with either 24 h or 48 h in the presence of $[Ru(L)_3](PF_6)_2$ complexes. Cell viability was assessed by the MTT colorimetric method. Data show the mean ± SD of three independent experiments.

Effects of [Ru(L 3](PF6)2 complexes on A549 and WI38 cell viability

To investigate the antitumor effects of $[Ru(L)_3](PF_6)_2$ complexes, the viability of A549 cells was studied using the MTT assay after treatment of cells with $[Ru(ip)_3](PF_6)_2$ and $[Ru(pip)_3](PF_6)_2$ complexes for either 24 or 48 h. The results of the MTT assay showed that $[Ru(ip)_3](PF_6)_2$ reduced the viability of A549 cells at 25.6 ng, 128 ng and 80 µg concentrations after 24 h incubation. The $[Ru(pip)_3](PF_6)_2$ also reduced the viability of A549 cells at 25.6 ng and 80 µg concentrations for 24 h (Figure 2).

Effects of $[Ru(L)_3](PF_6)_2$ complexes *on A549 cells proliferation*

Cell proliferation was examined by a BrdU labeling cell proliferation kit based on DNA replication. The results showed a concentration-dependent increase in inhibition of A549 cell proliferation after these cells were treated with either $[Ru(ip)_3](PF_6)_2$ or $[Ru(pip)_3](PF_6)_2$. These results indicated that $[Ru(pip)_3](PF_6)_2$ is capable of inhibiting A549 cells proliferation even at non-cytotoxic concentrations. As shown in Figure 4, treatment with $[Ru(pip)_3](PF_6)_2$ at concentrations ranging from 2.5 to 40 µg/ml had substantial effects on cell proliferation (inhibition of proliferation was between 22-88 %) which were similar to the inhibiton of the Paclitaxel (inhibition ranging between 15-92 %) after 24 h (Figure 4).

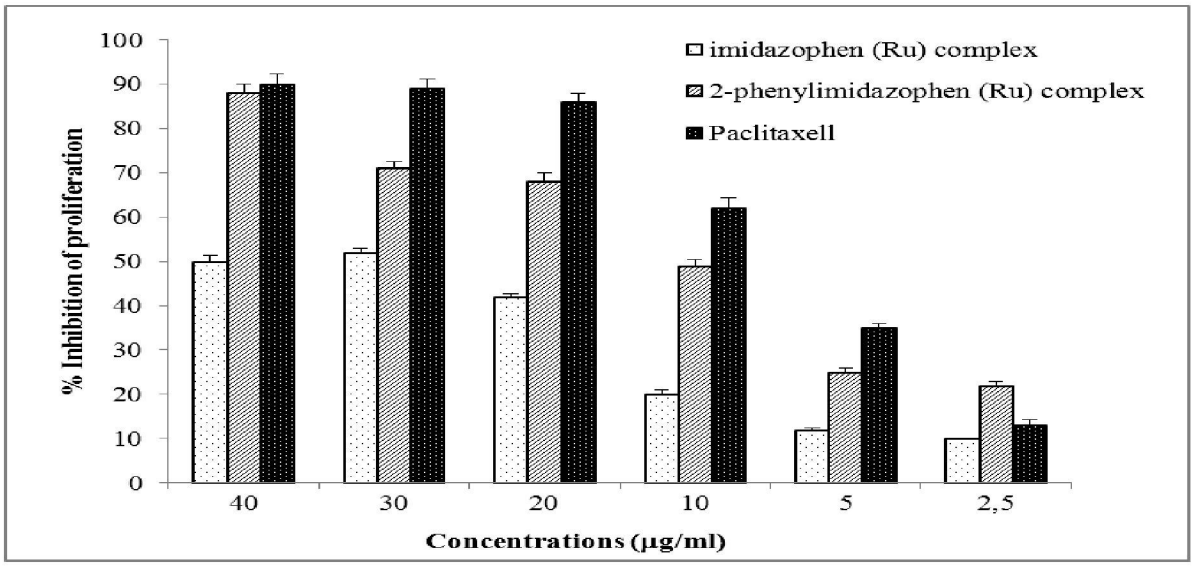


Figure 4. The proliferation effect of the $[Ru(ip)_3](PF_6)_2$ and $[Ru(pip)_3](PF_6)_2$ complexes and Paclitaxel® *in vitro* on A549 tumor cells. Tumor cells were incubated in the presence of drugs and after 24 h at 37°C, the inhibition of proliferation was assessed by the BrdU incorporation method. Data show the mean ± S.D. of three independent experiments.

Viscosity studies

The effects of $[Ru(ip)_3](PF_6)_2$ and $[Ru(pip)_3](PF_6)_2$ on the viscosity of CT DNA are shown in Figure 5. From these results, it is clear that increasing concentrations (1/R ([Ru]/ [DNA] = 0-0.08) of $[Ru(ip)_3](PF_6)_2$ caused the relative viscosity of DNA to increase. However, the evaluation $[Ru(pip)_3](PF_6)_2$ activity showed a significant increase in relation to the ethidium bromide used as a positive control.

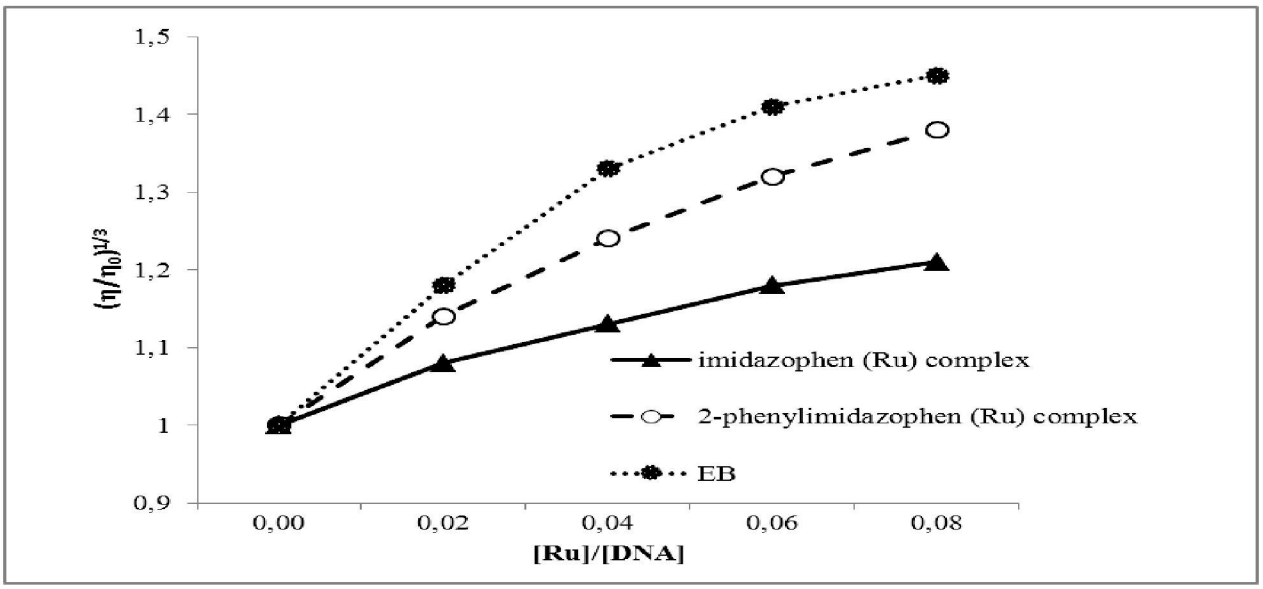


Figure 5. Effects of increasing amounts of $[Ru(ip)_3](PF_6)_2$ and $[Ru(pip)_3](PF_6)_2$ complexes and ethidium bromide on the relative viscosities of calf thymus DNA at 30.0±0.1 ^{0}C, [DNA]= 0.5 mM.

DISCUSSION

Studies on the synthesis and biochemical evaluation of new compounds containing metal ions are an area of growing interest. Some derivatives of various metals such as platinum, rhodium, and ruthenium have been investigated and have shown some promising results (19). Especially, ruthenium complexes have been proposed for potential chemotherapeutic activity (20), which correlates well with DNA binding affinity (21). In addition, some derivatives have surprisingly low general toxicity compared to platinum compounds (22).

For the investigation of new ruthenium (II) complexes that may have anticancer properties, we tested the effects of $[Ru(L)_3](PF_6)_2$ complexes on the A549 cell line. Determination of cell viability by the MTT assay in drug-treated or control cells is the widely- used method in cell screening studies. Here, using the MTT viability assay, we found that two $[Ru(L)_3](PF_6)_2$ complexes significantly (64.4%; 80 µg/ml of $[Ru(ip)_3](PF_6)_2$, 35.9 % and 16 µg/ml of $[Ru(pip)_3](PF_6)_2$, 76.3% after 24 h incubation, $p < 0.05$) reduced the viability of A549 cells in a concentration-dependent manner relative to untreated cells. The IC_{50} value of $[Ru(ip)_3](PF_6)_2$ and $[Ru(pip)_3](PF_6)_2$ was approximately 32 and 46 µg/ml, respectively. While the modest antitumor activity of ruthenium (II) complexes such as cis-Cl_2 $(DMSO)_4$ ruthenium were studied relatively early, significant activity has been seen only recently with ruthenium (II) complexes stabilized with heteroaromatic ligands (23). The experimental results indicate that both $[Ru(ip)_3](PF_6)_2$ and $[Ru(pip)_3](PF_6)_2$ complexes are very effective at reducing A549 cell viability. However, the presence of either imidazole- or 2-phenylimidazole- groups existence does not have a significant effect on cell viability with these complexes. Our cytotoxicity results confirmed previous observations that [1,10]phenanthroline ruthenium (II) complexes demonstrate high *in vitro* cytotoxicity against HeLa (cervical), HepG2 (hepatocellular) and A549 (lung) cells (24). On the other hand, ruthenium (II) complexes as antitumor drugs have attracted great attention; ruthenium-sulfoxide complex, NAMI-A has entered clinical trials due to its good antimetastatic activity (25). However, cytotoxicity studies of some ruthenium-based compounds showed either no activity or very weak cytotoxic activity against a chronic human myelogenous leukemia cell line (26).

Lately, the main direction in developing effective cancer preventive approaches have centered on the use of cytotoxic chemical compounds against cancer cells. For this reason, we investigated the effects of cytotoxicity of ruthenium (II) complexes on WI38 normal human lung cells. We found that the cytotoxicity of $[Ru(ip)_3](PF_6)_2$ and $[Ru(pip)_3](PF_6)_2$ on the tested A549 cancer cells was much

stronger than their cytotoxicity on normal WI38 cells (IC_{50} > 80 µg/ml). These results suggest that the cytotoxic activity of these ruthenium (II) complexes might be selective to tumor cells.

Several reports have indicated that ruthenium complexes inhibited cell proliferation in a number of human cancer cell lines such as NCl-H460, Sf-268, HeLa and MCF-7 (27-29). In the present study, we investigated the effects of these two $[Ru(ip)_3](PF_6)_2$ and $[Ru(pip)_3](PF_6)_2$ complexes on A549 lung cancer cells and on WI38 normal lung cancer cells. Our results showed clearly that $[Ru(pip)_3](PF_6)_2$ significantly induced the inhibition of A549 cell proliferation (up to 50 %) even at a non-cytotoxic concentration (10 µg/ml) relative to Paclitaxel, used as a positive control (63 % inhibition). However, $[Ru(ip)_3](PF_6)_2$ showed lower inhibition (at IC_{50} value; 50 % inhibition) of A549 cell proliferation as compared to $[Ru(pip)_3](PF_6)_2$ and Paclitaxel. The results showed clearly that $[Ru (pip)_3](PF_6)_2$ is a potent cell proliferation inhibitory complex in A549 cells compared to other.

There appears to be a correlation between cytotoxicity and DNA binding for the representative ruthenium amine (30) and tetracationic ruthenium (II) (31) anticancer compounds in the cell cultures. Additionally, consistent with DNA binding *in vivo*, a number of amine and heterocyclic complexes of ruthenium have shown inhibition of DNA replication, binding to nuclear DNA (30) and reduction of RNA synthesis (32). Recent study has showed that ruthenium (II) polypyridyl complex binds to DNA by intercalation and that antitumor activity of this complex could be related to its interaction with DNA (33). Based on this evidence, we next examined whether cell proliferation inhibition caused by $[Ru(pip)_3](PF_6)_2$ was due to its ability to interact with DNA. The results showed that with increasing the amounts of $[Ru(pip)_3](PF_6)_2$, the relative viscosities of ct DNA solution increased steadily, which were similar to the results with ethidium bromide. The increased degree of viscosity, which may depend on its affinity to DNA, followed the order of ethidium bromide > $[Ru(pip)_3](PF_6)_2$ > $[Ru(ip)_3](PF_6)_2$. However, the large increase in the relative viscosity values for $[Ru(pip)_3](PF_6)_2$ revealed that this complex might be better intercalated than $[Ru(ip)_3](PF_6)_2$, which is consistent with our results from the cell proliferation assay.

CONCLUSION

The data presented here clearly show that the cytotoxic activity of $[Ru(pip)_3](PF_6)_2$ is selective to tumor cells, as verified by investigation of cytotoxic activity of this ruthenium (II) complex towards lung carcinoma and normal lung cells. Furthermore, $[Ru(pip)_3](PF_6)_2$ showed a potent inhibitory effect on A549 cell proliferation compared to $[Ru(ip)_3](PF_6)_2$ that might be relative to its high DNA binding ability. Thus, these results provide a base for further research characterizing the antitumor properties of $[Ru(pip)_3](PF_6)_2$.

ETHICAL APPROVAL

No human subjects or experimental animals were used in the experiments conducted for this article. Human lung carcinoma (A549) and human normal lung fibroblast (WI38) cells were purchased from ATCC and cultured *in vitro*.

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