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Novel ruthenium and palladium complexes as potential anticancer molecules on SCLC and NSCLC cell lines

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Abstract

Lung cancer is one of the major causes of cancer-related deaths in the world. Non-small-cell lung cancer (NSCLC) is the most common type of lung cancer, and small-cell lung cancer (SCLC) is the most aggressive subtype of lung cancer. Proper therapies for SCLC have not yet been developed. However, new molecules have been designed and big innovation in treating SCLC has been achieved. Platinum-based antitumor drugs like cisplatin and carboplatin have several disadvantages including side effects, cisplatin-resistant tumors and limited solubility in aqueous media. Thus, two novel chiral aminoalcohol-based bis(phosphinite) ligands containing (η^6-p-cymene)-Ru(II)-phosphinite and bis(phosphinite)–Pd(II) complexes were synthesized and evaluated for anticancer activity. In this study, the results showed that complex **1** has the strongest cytotoxic effects on SCLC and NSCLC cell lines. On the other hand, cisplatin, ruthenium and palladium complexes are capable to induce apoptosis. Especially, complexes **1** and **2** can induce apoptosis for both SCLC and NSCLC. When compared to the qRT-PCR and TUNEL results, we obtained a significant correlation between apoptotic index and p21, Bax gene expressions. This work revealed the potential of the synthesized complexes as anticancer agents with cytotoxic and pro-apoptotic activity as leading compounds for further anticancer researches.

Keywords SCLC · NSCLC · Phosphinite · Palladium · Ruthenium · p21 · Bax

Introduction

Transition metal complexes may play a variety of important roles in many biological processes (Patel et al. 2013) such as cell division and gene expression, as well as carcinogenesis (Heydari et al. 2017). Among the synthesized compounds, organometallic- and inorganic-based compounds are very useful molecules. Organophosphorus compounds, especially phosphines (Gümgüm et al. 2007; Durap et al. 2008), aminophosphines (Gümgüm et al. 2006; Biricik et al. 2007a), phosphinites (Karakas et al. 2018; Al-Bayati et al. 2018), etc., are excellent versatile organometallic ligands that are easily prepared and form a variety of complexes with various transition metals including platinum (Durap et al. 2008), ruthenium (Karakas et al. 2018; Ok et al. 2014), palladium (Deepthi et al. 2012; Karakas et al. 2016), gold (Lazarevic et al. 2017) and copper (Biricik et al. 2007b) metal centers. In the literature, most studies showed that organometallic and inorganic compounds have potential anticancer effects (Mjos and Orvig 2014; Demkowicz et al. 2016; Wani et al.

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2016) Their anticancer activity is currently a hot topic of cancer research.

In the last decades, ruthenium(II) complexes bearing chelating phosphine and phosphinite ligands have been received much attention due to their application in the field of homogeneous catalysis (Lindner et al. 2003; Arauj et al. 2005; Baysal et al. 2017). These compounds have also been successfully tested as anticancer, antibacterial and antioxidant agents. Biological properties of Ru ion similar to Fe mimicking in binding to a variety of serum proteins such as transferrin and albumin (Deshpande and Kumbhar 2005; Richert and Budzisz 2010). Ruthenium complexes generally show low systemic toxicity and antitumor activity. Chemical properties of Ru complexes under physiological conditions are dependent on the two oxidation states (+2 and +3) (Lazarevic et al. 2017). Therefore, organometallic Ru complexes have been considered to be an alternative to platinum and the organometallic half-sandwich ruthenium(II) complexes in type of [(η^6-arene)RuL] (arene: *p*-cymene, benzene, etc.) have been extensively studied in recent years (Debreczeni et al. 2006; Liu et al. 2006; Pettinari et al. 2014; Ellahioui et al. 2019).

Pd(II) and Pt(II) complexes share similar properties like structural and thermodynamic analogy (Ruiz et al. 2008a; Rocha et al. 2010). In recent years, newly synthesized palladium complexes have been a very popular field of research due to their similarity to traditional platinum-based anticancer molecules (Moghadama et al. 2016). There is a huge tendency to study the antitumor activity of Pd(II) complexes on many cancer cell lines (Miklásová et al. 2012; Ruiz et al. 2008b). In addition, palladium has higher lability than platinum analogs. Bidentate ligands like amino acids that are present in biological systems have been utilized to synthesize palladium anticancer complexes (Anzellotti et al. 2006). In order to obtain effective Pd(II) complexes, stabilization of the N–Pd interaction with the amine carrier ligand is necessary to decrease the excessive reactivity. Usage of sterically hindered amines increases the lipophilicity of the complexes, thus making easier for complexes to enter the cells (Fanelli et al. 2016). Especially, phosphine-based palladium(II) complexes have been studied for their valuable bioactivities (Priyarega et al. 2011; Shabbir et al. 2017). The cytotoxicity of the palladium(II) complexes was comparable to that of platinum-based drugs; it is mediated by apoptosis. According to the literature, Pd(II) complexes can be potential candidates for anticancer therapy (Fanelli et al. 2016).

Lung cancer is one of the major causes of cancer-related deaths in the world. Non-small-cell lung cancer (NSCLC) is the most common type of lung cancer and represents about approximately 80–85% of lung cancers (James et al. 2018). Small-cell lung cancer (SCLC) is the most aggressive subtype of lung cancer, and the overall survival at 5 years is approximately 5–10% (Fiorentino et al. 2016). Reducing power, radical scavenging, DNA binding, antibacterial activity and DNA cleavage activity of ionic liquid-based Ru(II)-phosphinite complexes were reported (Meriç et al. 2019). However, to the best of our knowledge, there are no study on the use of chiral aminoalcohol-based bis(phosphinite) ligands and their transition metal complexes in anticancer activity. Herein, for the first time, synthesis and potential anticancer properties of chiral aminoalcohol-based bis(phosphinite) ligands containing (η^6-*p*-cymene)-Ru(II)-phosphinite and bis(phosphinite)—Pd(II) complexes were explored.

Experimental

Materials

Bis(phosphinite) ligands 1 and 2 (Karakas et al. 2016), bis(phosphinite) palladium(II) complexes (Karakas et al. 2016) and bis(phosphinite) ruthenium(II) complexes (Karakas et al. 2018) were prepared according to procedures in our previous works. Synthesis of bis(phosphinite) palladium(II) complexes **(1** and **2)** and bis(phosphinite) ruthenium(II) complexes **(3** and **4)** were carried out under dry argon atmosphere using Schlenk line technique. All solvents were dried and distilled under argon prior to use. ^{31}P-{^{1}H} NMR (162.0 MHz), ^{1}H (400.1 MHz) and ^{13}C (100.6 MHz) spectra were evaluated by a Bruker AV400 spectrometer, with tetramethylsilane (TMS) as an internal reference for ^{13}C NMR and ^{1}H NMR or 85% H_3PO_4 as an external reference for ^{31}P-{^{1}H} NMR.

Synthesis of bis(phosphinite) ligands 1 and 2

(2R)-1-[benzyl({[6-({benzyl[(2R)-2-hydroxypropyl]amino}methyl)pyridin-2-yl]methyl}) amino]propan-2-ol and (2R)-1-[benzyl({[3-({benzyl[(2R)-2-hydroxypropyl]amino}methyl) phenyl]methyl})amino]propan-2-ol (1.5 mmol) were dissolved in dry toluene (20 mL) under an argon atmosphere. Then, trimethylamine (Et_3N) (3 mmol) and monochlorophosphine ($ClPPh_2$) (3 mmol) were added dropwise into this mixture and stirred at room temperature for 1 h. The white precipitate (triethylammonium chloride) was filtered under argon, and the remaining part was dried in vacuo to produce a white viscous oily compound.

Ligand 1

^{31}P-{^{1}H} NMR ($CDCl_3$, δ, ppm): 106.86 (s, O-**P**-(C6H5)2); ^{1}H-NMR (δ ppm, $CDCl_3$): 1.26 (d, 2H, $J = 6.20$ Hz, –CHC**H**$_3$); 2.56 (br, 2H, –CHC**H**$_2$ (a)); 2.80 (br, 2H, –CHC**H**$_2$

(b)); 3.63–3.75 (m, 4H, –NC$\underline{H}_2$Ph + –NC$\underline{H}_2$); 4.11–4.15 (m, 2H, –C$\underline{H}$CH$_3$); 7.20–7.54 (m, 33H, aromatic protons of ligand); ^{13}C-NMR (δ ppm, CDCl$_3$): 20.85 (–CH$\underline{C}$H$_3$); 59.45 (–NC$\underline{H}_2$Ph + –NC$\underline{H}_2$Py); 75.34 (–$\underline{C}$HCH$_3$); 60.86 (–CH$\underline{C}$H$_2$); 120.96, 126.88, 128.19, 128.21, 128.45, 128.97, 129.07, 130.00, 130.25, 130.52, 130.69 (aromatic carbons); 136.50, 142.61, 158.94 (*i-c*arbons).

Ligand 2

^{31}P-{^{1}H}- NMR (δ ppm, CDCl$_3$): 108.05 (s, O-**$\underline{P}$**-(C6H5)2); ^{1}H-NMR (δ ppm, CDCl$_3$): 1.24–1.30 (m, 6H, –CHC$\underline{H}_3$); 2.54–2.58 (m, 2H, –CHC$\underline{H}_2$ (a)); 2.72–2.76 (m, 2H, –CHC$\underline{H}_2$ (b)); 3.57–3.63 (m, 4H, –NC$\underline{H}_2$Ph + –NC$\underline{H}_2$); 4.20 (br, 2H, –C$\underline{H}$CH$_3$); 7.23–7.50 (m, 34H, aromatic protons of ligand); ^{13}C-NMR (δ ppm, CDCl$_3$): 139.24, 139.42, 142.65 (*i*-carbons of phenyl); 126.80, 127.57, 128.14, 128.20, 128.32, 128.43, 128.95, 129.42, 130.06, 130.25, 130.34, 130.58 (aromatic carbons of phenyl); 75.53 (d, *J* = 23.1 Hz, –$\underline{C}$HCH$_3$); 60.50 (–CH$\underline{C}$H$_2$); 59.08 (–NC$\underline{H}_2$Ph + –NC$\underline{H}_2$); 20.92 (–CH$\underline{C}$H$_3$).

Synthesis of dichlorobis(phosphinite)Pd(II) complexes (1 and 2)

Bisphosphinite (1.5 mmol) and Pd(cod)Cl$_2$ (1.5 mmol) were dissolved in 30 mL of dichloromethane under an argon atmosphere. The reaction mixture was stirred for 1 h at room temperature. The solution was concentrated to ca. 2 ml and adding 15 ml petroleum ether caused precipitation of the dark yellow solid. The supernatant was removed and the solid was washed with hexane/diethyl ether (1:1) and then dried by vacuum to obtain bis(phosphinite) palladium(II) complexes (**complexes 1–2)** as a final product.

Complex 1

^{31}P-{^{1}H} NMR (CDCl$_3$, δ, ppm): 108.22 (s, O-**$\underline{P}$**-(C6H5)2); ^{1}H NMR (CDCl$_3$, δ, ppm): 1.28 (m, 6H, –CHCH$_3$); 2.50 (broad, 4H, –CHCH$_2$); 3.11–3.82 (m, 8H, –NCH$_2$Ph + –NCH$_2$); 4.10 (m, 2H, –CHCH$_3$); 7.22–7.54 (m, 33H, aromatic protons); ^{13}C NMR (CDCl$_3$, δ, ppm): 19.84 (–CHCH$_3$); 59.52 (–NCH$_2$Ph + –NCH$_2$Py); 74,93 (–CHCH$_3$); 60.35 (–CHCH$_2$); 120.62, 126.75, 127.40, 127.46, 127.75, 128.43, 128.96, 131.78, 131.99, 132.45, 132.85, 133.40 (aromatic carbons); 136.23, 140.60, 159.78 (*i*-carbons).

Complex 2

^{31}P-{^{1}H} NMR (CDCl$_3$, δ, ppm): 103.76 (s, O-**$\underline{P}$**-(C6H5)2); ^{1}H NMR (CDCl$_3$, δ, ppm): 7.20–7.58 (m, 34H, aromatic protons), 3.87 (broad, 2H, –CHCH3), 3.21–3.29 (m, 8H, –NCH$_2$Ph + –NCH$_2$), 2.37–2.42 (m, 4H, –CHCH$_2$), 0.84–0.90 (m, 6H, –CHCH$_3$). ^{13}C NMR (CDCl$_3$, δ, ppm): 138.02, 138.25 (i-carbons), 127.24, 127.86, 128.37, 128.60, 128.73, 130.98, 131.69, 131.86, 132.07, 132.42, 132.72, 133.12 (aromatic carbons), 74.84 (–CHCH$_3$), 59.51 (–CHCH$_2$), 57.96 (NCH$_2$Ph + –NCH$_2$), 19.47 (–CHCH$_3$).

Synthesis of (η^6-p-cymene)-Ru(II)-phosphinite complexes (3 and 4)

Bisphosphinite (1.5 mmol) and [Ru(η^6-*p*-cymene)(μ-CI)Cl]$_2$ (1.5 mmol) were dissolved in 30 mL of dichloromethane under argon atmosphere and stirred for 1 h at room temperature. Final product concentrated to ca. 2 ml under reduced pressure. Addition of 15 ml petroleum ether caused precipitation of a tile-red solid. The supernatant was removed and the solid was washed with hexane/diethyl ether (1:1) and then dried in vacuum to obtain ruthenium(II) complexes (**complexes 3–4)** as a final product.

Complex 3

^{31}P-{^{1}H}- NMR (δ ppm, CDCl$_3$): 110.32 (s, O-**$\underline{P}$**-(C6H5)2); ^{1}H NMR (δ, ppm, CDCl$_3$): 1.05–1.13 (m, 18H, –CH(C$\underline{H}_3$)$_2$ *p*-cymene + –CHC$\underline{H}_3$); 1.83 (s, 6H, –C$\underline{H}_3$ *p*-cymene); 2.33–2.40 (m, 2H, –NC$\underline{H}_2$Ph(b)); 2.56–2.72 (m, 4H, –NC$\underline{H}_2$Ph(a) + –C$\underline{H}$(CH$_3$)$_2$ *p*-cymene); 3.27 (d, 2H, *J* = 13.7 Hz, –NC$\underline{H}_2$ (b)); 3.40 (d, 2H, *J* = 14.5 Hz, –CHC$\underline{H}_2$ (b)); 3.56 (d, 2H, J = 13.72 Hz, –NC$\underline{H}_2$ (a)); 3.62 (d, 2H, *J* = 14.5 Hz, –CHC$\underline{H}_2$ (a)); 4.63 (br, 2H, –C$\underline{H}$CH$_3$); 5.15 (br, 6H, aromatic protons of *p*-cymene); 5.25 (br, 2H, aromatic protons of *p*-cymene); 7.25–7.901 (m, 33H, aromatic protons); ^{13}C NMR (δ ppm, CDCl$_3$): 17.43 (–$\underline{C}$H$_3$ of *p*-cymene); 20.46 (–CHC$\underline{H}_3$); 21.77, 21.95 (–CH($\underline{C}$H$_3$)$_2$ of *p*-cymene); 29.97 (–$\underline{C}$H(CH$_3$)$_2$ of *p*-cymene); 59.22–59.63 (–N$\underline{C}$H$_2$ + N$\underline{C}$H$_2$Ph); 60.75 (–$\underline{C}$HCH$_2$); 74.36 (–CH$\underline{C}$H$_2$); 87.41, 88.14, 89.56, 90.60 (aromatic carbons of *p-cymene*); 97.45, 111,23 (*i*-carbons of *p*-cymene); 121.00, 126.76, 127.65, 127.77, 128.06, 128.98, 130.83, 132,84, 132.95, 133.11, (aromatic carbons); 137.630, 138.12, 139.40, 158.98 (*i-carbons*); m/z: 1415.25 [M–H$^+$] $C_{71}H_{81}N_3O_2P_2Ru_2Cl_4$ (MA: 1414.23).

Complex 4

^{31}P-{^{1}H}- NMR (δ ppm, CDCl$_3$): 110.24 (s, O-**$\underline{P}$**-(C6H5)2); ^{1}H NMR (δ, ppm, CDCl$_3$): 0.98–1.05 (m, 18H, –CH(C$\underline{H}_3$)$_2$ of *p*-cymene + –CHC$\underline{H}_3$); 1.77 (s, 6H, –C$\underline{H}_3$ of *p*-cymene); 2.32 (d, 2H, *J* = 8.8 Hz, –CHC$\underline{H}_2$ (b)); 2.44 (d, 2H, *J* = 8.7 Hz, –CHC$\underline{H}_2$ (a)); 2.56 (br, 2H, –C$\underline{H}$(CH$_3$)$_2$ of *p*-cymene); 3.14 (d, 4H, *J* = 13.4 Hz, –NC$\underline{H}_2$Ph); 3.50 (d, 4H,

J=13.5 Hz, –NC$\underline{H}_2$); 5.08–5.30 (m, 8H, aromatic protons of *p*-cymene); 4.55 (br, 2H, –C$\underline{H}$CH$_3$); 7.06–7.85 (m, 34H, aromatic protons); ^{13}C NMR (δ ppm, $CDCl_3$): 17.41 (–$\underline{C}$H$_3$ of *p*-cymene); 20.49 (–CHC$\underline{H}_3$); 21.97, 21.70 (–CH($\underline{C}$H$_3$)$_2$ of *p*-cymene); 30.08 (–$\underline{C}$H(CH$_3$)$_2$ of *p*-cymene); 59.24–59.00 (–N$\underline{C}$H$_2$ + N$\underline{C}$H$_2$Ph + –$\underline{C}$HCH$_2$); 74.40 (–CH$\underline{C}$H$_3$); 87.22, 88.15, 89.59, 91.00 (aromatic carbons of *p*-cymene); 97.32, 110,91 (*i*-carbons of *p*-cymene); 126.68, 127.39, 127.66, 127.79, 128.03, 128.85, 129.31, 130.78, 130,86, 132.94, (aromatic carbons); 137.35, 137.89, 139.43, 139.62 (*i*-carbons); m/z: 1413.20 [M-H$^+$] $C_{72}H_{82}N_2O_2P_2Ru_2Cl_4$ (MA: 1413.36).

Cell culture and cell proliferation assay

NSCLC and SCLC cell lines were obtained from Dr. Jun YOKOTA (IGTP, Barcelona). NSCLC cell line H1975, HCC78 and SCLC cell line H209, N417 were cultured in RPMI 1640 supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin in a humidified of 5% CO_2 at 37 °C. Cell proliferation was determined by using a CellTiter-Glo kit (Promega, USA) which is a homogenous method to determine the number of viable cells. Cells were counted with an automated cell counter, seeded in 96 well plates 3×10^3 cell/well. Different concentrations (1, 10, 30, 50, 75, 100, 200 µg/mL) of ruthenium and palladium complexes were added per well. On the other hand, to compare the effect of synthesized complexes to the cisplatin we treated cells with different concentrations (0.1, 0.5, 1, 10, 30, 50, 100 µg/mL) of cisplatin (CAS 15663-27-1). Afterward, cells were cultured in 96 well plates for 48 h. IC_{50} values were calculated by regression analysis.

Apoptosis assay

To evaluate the potential apoptotic effects of the synthesized molecules on H1975, HCC78, H209 and N417 cells, cell lines were treated with cisplatin, ruthenium and palladium complexes and survival rates were evaluated based on their IC_{50} values. Cell lines were incubated in a humidified of 5% CO_2 at 37 °C at 24 h. At the end of the incubation period, in situ Apoptosis Detection Kit (Millipore, USA) was used to detect apoptotic cells in situ by the indirect TUNEL method. The results of TUNEL assay were analyzed by using fluorescence microscopy.

Real-time polymerase chain reaction

Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was applied to determine apoptosis and proliferation-dependent mRNA expression changes in ruthenium and palladium complexes treated for 48 h. At the end of the incubation, treated and untreated cell lines were washed with ice-cold phosphate-buffered saline (PBS), and then, total RNA from the cell lines was isolated as described previously (Tokgun et al. 2015). Quantitative real-time qRT-PCR analysis performed by using SYBR green (Qiagen, Germany) reagent and Corbett Rotorgene (Qiagen, Germany) instrument according to the manufacturer's protocol. The relative mRNA expression levels were calculated using the comparative Ct method. Expression levels of Bax and p21 were evaluated. The transcript level of the ß-actin gene was used as the endogenous reference. The results were assessed with delta delta CT formulas ($2^{-\Delta CT}$).

Statistical analysis

All experiments were repeated independently to confirm the results by performing in three replicates. The Student's t test is used to calculate a p value based on the difference in the mean values between two groups of data and $p \leq 0.05$ considered to be statistically significant.

Results and discussion

Synthesis of bis(phosphinite) ligands and their complexes

Coordination chemistry studies of the P-donor ligands have become center of attention through their unique applications in bioinorganic chemistry (Dong et al. 2007; Appelt et al. 2017). Recently, the preparation of ionic liquid-based Ru(II)-phosphinite (James et al. 2018) and phosphine containing heterobimetallic ruthenium(II) complexes and their applications were reported (Appelt et al. 2017). Attracting features of this type of compound prompted us to focus on chiral aminoalcohol-based bis(phosphinite) ligands (Fig. 1). In the present study, two bis(phosphinite) palladium(II) complexes (**1**–**2**) and half-sandwich (η^6–*p*-cymene)-Ru(II)-phosphinite complexes (**3**–**4**) were prepared using the different synthetic strategies as shown in Fig. 2. These bis(phosphinite) ligands and their Ru(II) and Pd(II) complexes were originally synthesized by our research group (Karakas et al. 2016, 2018). $Pd(cod)Cl_2$ was used in the preparation of bis(phosphinite) palladium(II) complexes (**1**–**2**) through the replacement of cod (1,5-cyclooctadiene) by the phosphinites (Fig. 2). Complexes **1**–**2** were isolated in good yields as bright yellow solids. Air stable red compounds (**3**–**4**) occur in high yields by the reaction of [Ru(*p*-cymene) (µ-Cl)Cl]$_2$ with one equivalent of bis(phosphinite) ligands in CH_2Cl_2 at room

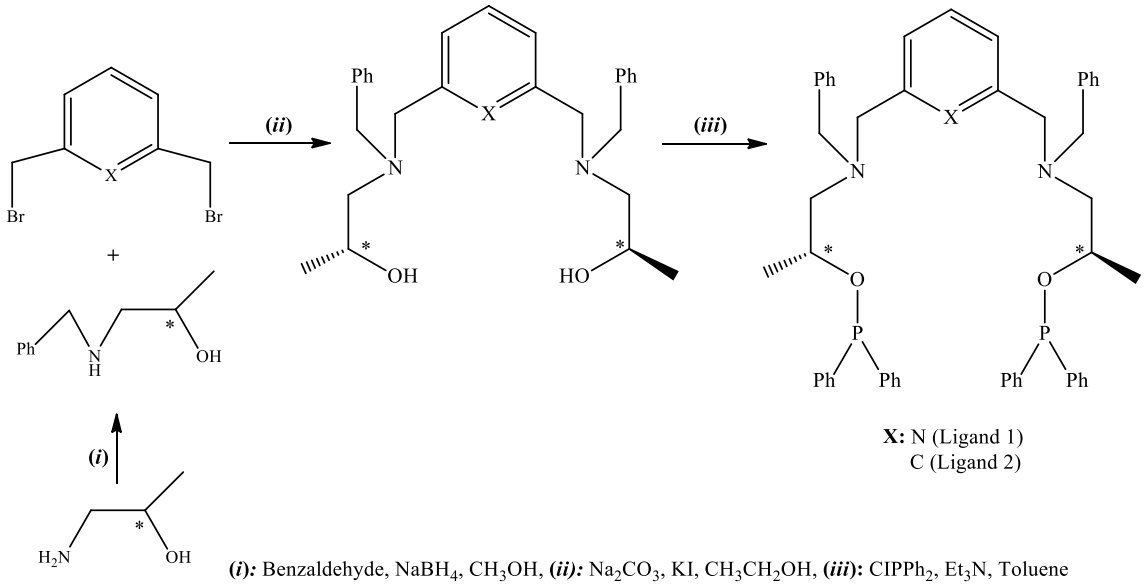

Fig. 1 Synthesis of bis(phosphinite) ligand 1 and 2

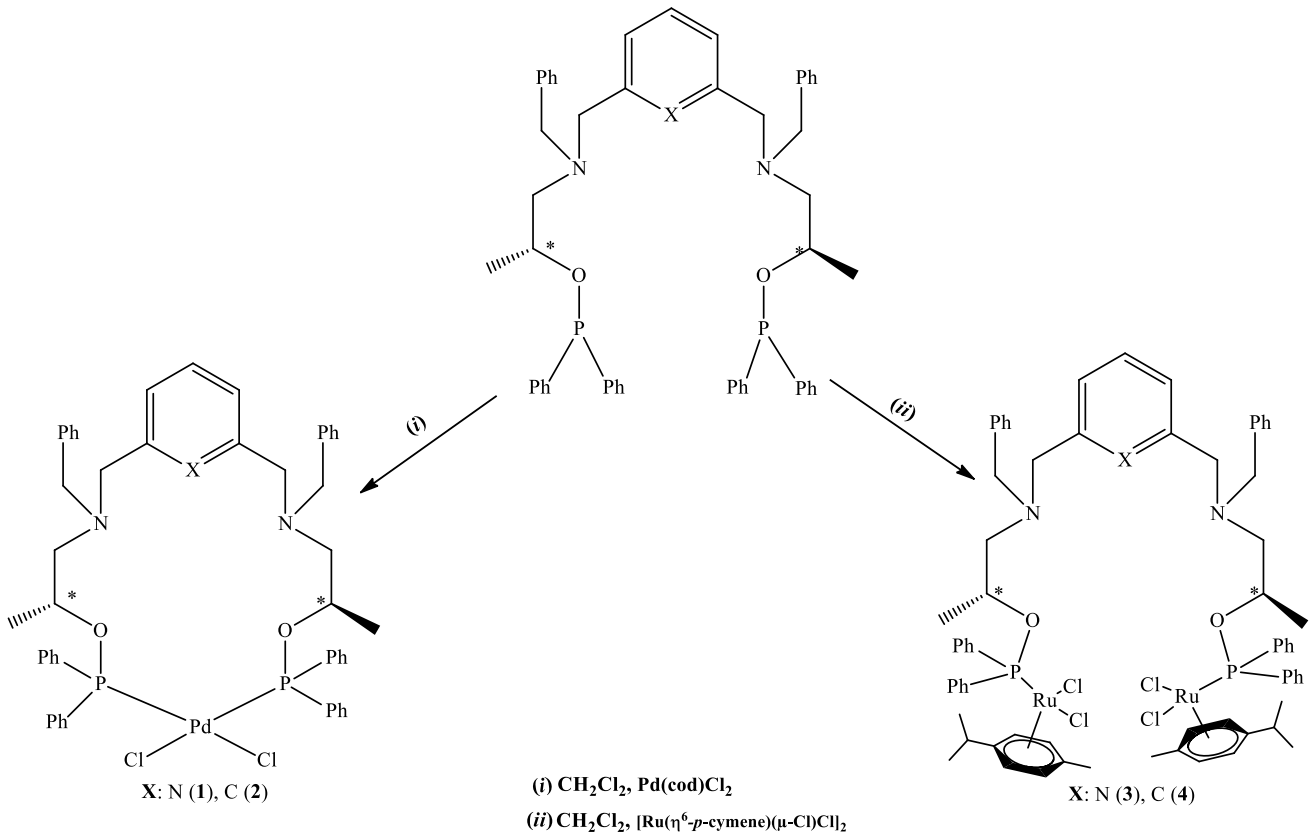

Fig. 2 Synthesis of bis(phosphinite)Pd(II) complexes **(1, 2)** and (η^6-p-cymene)-Ru(II)-phosphinite complexes (**3**–**4**)

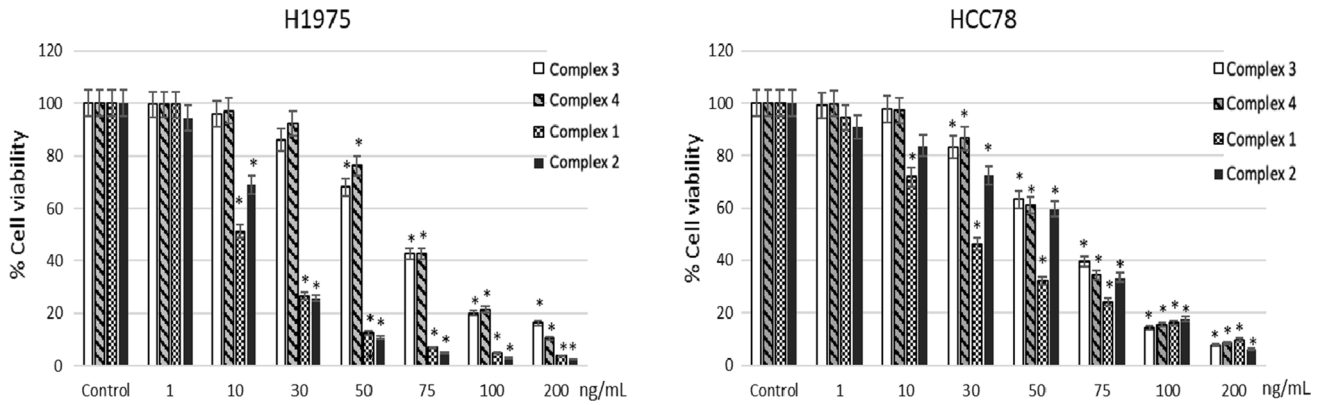

Fig. 3 Cytotoxic effects of the complexes on NSCLC cells. Cell death was determined by the luminometric method. The Student's t test (*$p < 0.001$) was used to evaluate the significance of cytotoxic effects of the complexes

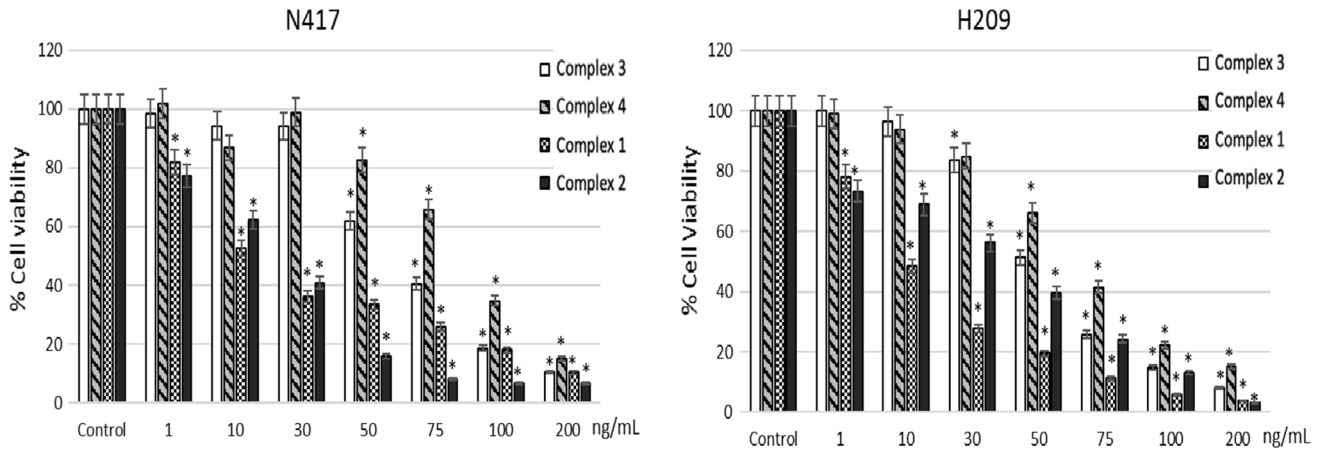

Fig. 4 Cytotoxic effects of the complexes on SCLC cells. Cell death was determined by the luminometric method. The Student's t test (*$p < 0.001$) was used to evaluate the significance of cytotoxic effects of the complexes

temperature (Fig. 2). Both complexes have high melting points and are highly soluble in MeCN, MeOH, CH_2Cl_2, $CHCl_3$, DMF and DMSO, but exhibit very poor solubility in water. The bis(phosphinite)-Ru(II) and Pd(II) complexes are stable for a month in ambient air and moisture. The complexes were characterized by nuclear magnetic resonance (NMR) spectroscopy.

The cytotoxic effect of the bis(phosphinite)Pd(II) (1–2) and (η^6–p-cymene)-Ru(II)-phosphinite complexes (3–4) on lung cancer cells

Luminometric CellTiter-Glo analysis was performed to determine the specific cytotoxic activity of each complex. NSCLC and SCLC cells were incubated with different concentrations (1, 10, 30, 50, 75, 100, 200 µg/mL) of ruthenium and palladium complexes for 48 h (Figs. 3 and 4, respectively).

Table 1 IC_{50} values for each molecule for each cell line

	NSCLC		SCLC	
	H1975	HCC78	N417	H209
Complex 1	10.43 ± 0.85	19.85 ± 1.54	8.17 ± 1.43	6.52 ± 1.24
Complex 2	15.17 ± 0.75	48.48 ± 4.96	12.15 ± 3.53	22.21 ± 6.94
Complex 3	55.38 ± 4.12	52.38 ± 3.92	53.65 ± 3.02	44.97 ± 2.52
Complex 4	66.67 ± 3.84	50.85 ± 2.98	136 ± 8.8	51.6 ± 3.74

Afterward, the half-maximal inhibitory concentration (IC_{50}) has been calculated by using regression analysis. We obtained that complex **1** has the strongest cytotoxic effects on both SCLC and NSCLC cell lines, while complex **4** has the lowest cytotoxic effects on both cell lines.

Table 2 IC_{50} values for cisplatin for each cell line

	NSCLC		SCLC	
	H1975	HCC78	H1975	HCC78
Cisplatin	16.72 ± 0.98	1.87 ± 0.24	14.23 ± 1.01	0.89 ± 0.05

When the results of cytotoxic effects of the ruthenium and palladium complexes were compared, it was observed that palladium has more cytotoxic activity than ruthenium (Table 1).

On the other hand, the cytotoxicity results clearly indicate that complexes **1**–**3** are more cytotoxic than complexes **2**–**4** in dose-dependent manner. The cytotoxic activity of each complex are also compared with cisplatin via treating cells with different concentrations of cisplatin. Depending on the CellTiter-Glo results, we calculated IC50 values for cisplatin and we obtained that cisplatin is an effective molecule, especially against to complexes 1 and 2 in H209 and HCC78 cell lines (Table 2). These results revealed that complexes 1 and 2 are more effective proliferation inhibitors for fast growing cells such as H1975 and N417. This study has shown for the first time that ruthenium and palladium complexes could induce apoptosis in both human small- and non-small-cell lung carcinoma cell lines. Among the anticancer agents containing metals, ruthenium and palladium complexes have become the most promising therapeutic molecules. Recent studies in the literature showed that ruthenium polypyridyl and ruthenium(II) salicylate complexes could inhibit cancer cell proliferation (Chen et al. 2019; Jiang et al. 2019). Ru(III) (d^5, paramagnetic) and Ru(II) (d^6, diamagnetic) oxidation states are available for ruthenium species in physiological solution. These two oxidation states are of important for their biological activity. In both oxidation states, Ru has good affinity for sulfur and nitrogen ligands and coordinated with octahedral geometry. Most of the Ru compounds have a low level of toxicity, and some of the Ru complexes have the capacity to be selective elimination of cancer cells. Therefore, ruthenium has been considered to be a strong alternative to platinum complexes (Deshpande and Kumbhar 2005). On the other hand, in the literature, there are some reports which mentioned that palladium complexes are intercalated with DNA, thereby exerting a significant effect on in vitro antitumor activity (Farhangian et al. 2017). In the literature, recent studies show that ruthenium complexes are more cytotoxic than palladium ones (Onar et al. 2019). However, we thought that in our study these differences come from the differences of the side groups of molecules that designed in our study.

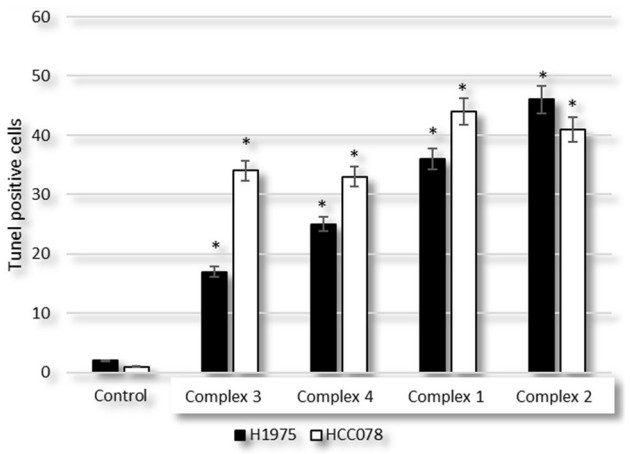

Fig. 5 Complexes induce apoptosis in NSCLC cell lines. Apoptotic cells were analyzed by terminal transferase dUTP Kit (Millipore) system. The Student's t test (*$p < 0.001$) was used to evaluate the significance of the TUNEL results

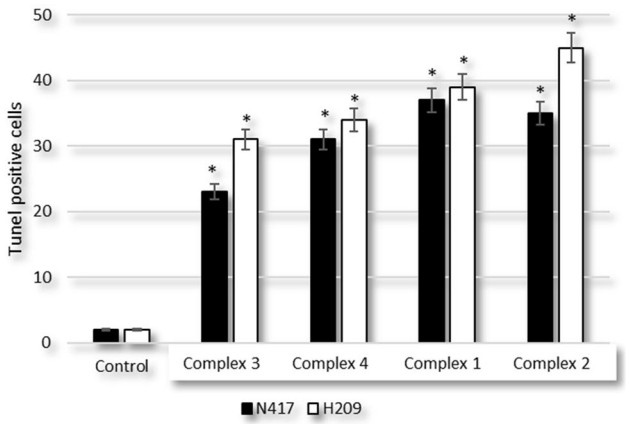

Fig. 6 Complexes induce apoptosis in SCLC cell lines. Apoptotic cells were analyzed by terminal transferase dUTP Kit (Millipore) system. The Student's t test (*$p < 0.001$) was used to evaluate the significance of the TUNEL results

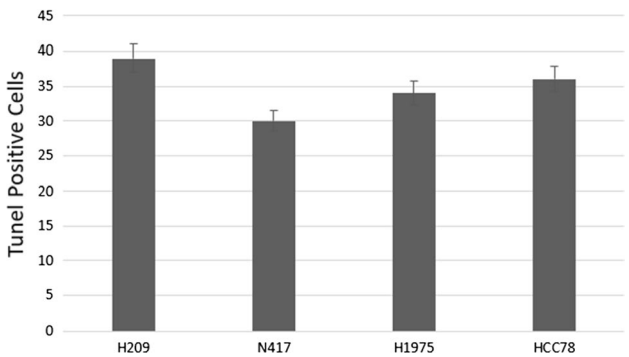

Fig. 7 Cisplatin induce apoptosis in lung cancer cell lines. Apoptotic cells were analyzed by terminal transferase dUTP Kit (Millipore) system. The Student's *t* test (*$p < 0.001$) was used to evaluate the significance of the TUNEL results

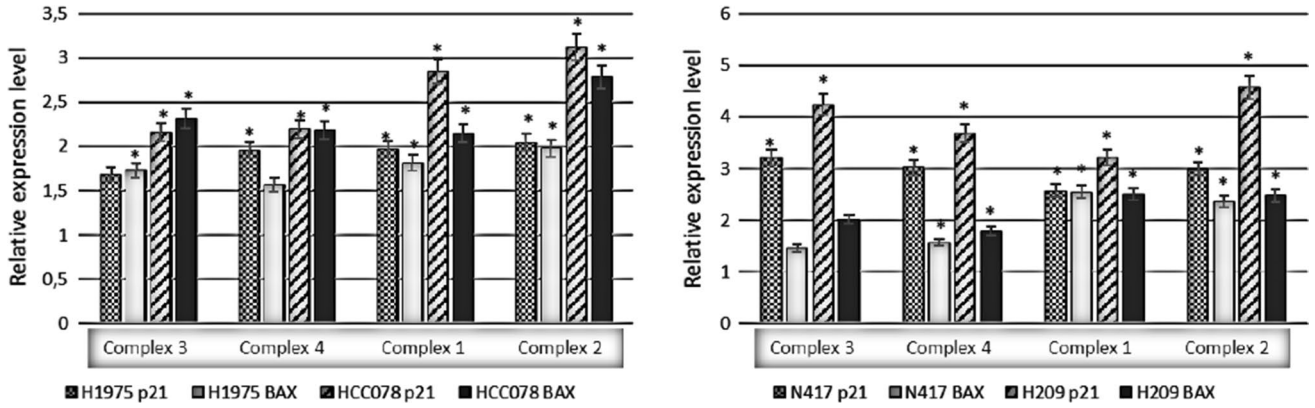

Fig. 8 Complexes induce p21 and bax mRNA expressions. The Student's *t* test (*$p < 0.001$) was used to evaluate the significance of the qRT-PCR results

Bis(phosphinite)Pd(II) (1–2) and (η^6-p-cymene)-Ru(II)-phosphinite complexes (3–4) induce apoptosis on lung cancer cell lines

To obtain the cell death mechanism after treatment with ruthenium and palladium complexes and to determine apoptotic cell index, we performed the indirect TUNEL method. DNA fragmentation of apoptotic cells has been detected by using the TUNEL assay. The TUNEL assay has been the most widely used for identifying apoptotic cells in situ. Our results indicate that cisplatin, ruthenium and palladium complexes are able to induce apoptosis in each cell line. Especially, complexes **1** and **2** can induce apoptosis for both SCLC and NSCLC (Figs. 5, 6 and 7, respectively).

When the effects of Pd and Ru side groups were compared, we obtained that Pd has stronger effect on induction of apoptosis.

In a similar manner, the TUNEL assay and qRT-PCR results clearly showed that there is a significant correlation between apoptotic cell index and p21, Bax gene expressions (Fig. 7). In the literature, some researchers mentioned that the type of cell death in response to Pd(II) complexes was shown to be through apoptotic pathways which is similar to our results (Kacar et al. 2014; Antunovic et al. 2015).

The TUNEL assay results showed that complex **2** strongly induces apoptosis in the lung cancer cells compared to the other complexes (Fig. 8).

Conclusions

In conclusion, the results show that the complexes are more efficient in their action on both NSCLC and SCLC cell lines. We synthesized four complexes and showed their potential cytotoxic effects. Importantly, they also successfully inhibited the cellular viability and induce apoptosis at lower concentrations. Previous works have shown that ruthenium complexes are more cytotoxic than palladium complexes in general. However, it should be remembered that this effect can change depending on the additional side groups. These findings obviously demonstrate that four complexes are very promising antitumor agents and support further investigations concerning its molecular targets in lung cancers.

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