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RESEARCH ARTICLE

Synthesis of 2-Substitutedbenzimidazolium Tetrachloroplatinate(II) Compounds and Their Cytotoxic Activities on Different Cell Lines

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Synthesis of 2-Substitutedbenzimidazolium Tetrachloroplatinate(II) Compounds and Their Cytotoxic Activities on Different Cell Lines

SUMMARY

The aim of the study was the synthesis of novel platinum compounds having benzimidazole ligands and screening for their in vitro cytotoxic activity on human cervical carcinoma HeLa, human lung carcinoma A549, and human lung epithelial Beas-2B cell lines. 2-Substituted benzimidazole ligands were synthesized by using appropriate aldehydes and o-phenylenediamine. Subsequently, 2-substituted benzimidazole ligands and potassium tetrachloroplatinate(II) (K_2PtCl_4) were used to synthesize 2-isopropylbenzimidazole tetrachloroplatinate(II) (K1) and 2-(1-methylpropyl)benzimidazole tetrachloroplatinate(II) monohydrate (K2). HRMS, IR, elemental analysis, ^{1}H-NMR, and melting point were used to characterize the synthesized compounds. Cytotoxic activities against HeLa, A549, and Beas-2B cells after 48 h and 72 h incubation of the platinum compounds were investigated via MTT assay. Cisplatin and carboplatin were used as reference drugs. The cytotoxic activity results showed that K2 platinum compound displayed 53.42%±2.21 (at 160 μM) on HeLa, 88.16%±0.22 (at 160 μM) on A549 and 92.09%±0.57 (at 160 μM) on Beas-2B after 48 h incubation, K2 displayed 27.42%±2.03 (at 160 μM) on HeLa, 93.95%±0.53 (at 160 μM) on A549 and 91.99±0.22 (at 160 μM) on Beas-2B after 72 h incubation. Both of the platinum compounds have higher cell inhibitory effects than reference drug carboplatin after 48 h incubation for tested cells.

Key Words: *Benzimidazole, platinum complexes, cytotoxic activity, HeLa, A549, Beas-2B.*

2-Sübstitüebenzimidazolyum Tetrakloroplatinat(II) Bileşiklerinin Sentezi ve Çeşitli Hücre Hatlarındaki Sitotoksik Etkileri

ÖZ

Bu çalışmanın amacı, benzimidazol ligandı taşıyan yeni platin bileşiklerinin sentezi ve bu bileşiklerin insan servikal karsinoma HeLa, insan akciğer karsinoma A549 ve insan sağlıklı akciğer epitel Beas-2B hücre hatları üzerindeki in vitro sitotoksik etkilerinin araştırılmasıdır. 2-Sübstitüe benzimidazol ligandları uygun aldehit türevleri ve o-fenilendiamin kullanılarak sentezlenmiştir. Ardından, 2-sübstitüe benzimidazol ligandları ve potasyum tetrakloroplatinat(II) (K_2PtCl_4) kullanılarak 2-izopropilbenzimidazol tetrakloroplatinat(II) (K1) and 2-(1-metilpropil)benzimidazol tetrakloroplatinat(II) monohidrate (K2) sentezlenmiştir. Sentezlenen bileşikler HRMS, IR, elementel analiz, ^{1}H-NMR ve erime noktası kullanılarak karakterize edilmiştir. Sentezlenen platin bileşiklerinin HeLa, A549 ve Beas-2B hücre hatlarına karşı MTT testi kullanılarak sitotoksik etkileri araştırılmıştır. Referans ilaç olarak sisplatin ve karboplatin kullanılmıştır. Sitotoksik aktivite çalışmalarında, bileşik K2'nin 160 μM konsantrasyonda 48 saatlik inkübasyonları sonrasında inhibisyon değerleri HeLa hucrelerinde %53.42±2.21, A549 hucrelerinde %88.16±0.22 ve Beas-2B hucrelerinde %92.09±0.57 bulunmuştur. Her iki platin bileşiğinin de test edilen hücrelere karşı 48 saat inkübasyon sonucunda referans ilaç olan karboplatine göre daha etkili olduğu görülmüştür.

Anahtar Kelimeler: *Benzimidazol, platin kompleksleri, sitotoksik aktivite, HeLa, A549, Beas-2B.*

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INTRODUCTION

According to World Health Organization's (WHO) report, an estimated 9.6 million people died of cancer in 2018, and cancer is the second leading cause of death worldwide (WHO, 2019). Lung, colorectal, prostate, liver, and stomach cancer are the most common types of cancer in men, although the most common types of cancers in women are breast, cervical, lung, colorectal, and thyroid cancer. Cervical cancer is the fourth most common cancer among women worldwide, accounting for 7.5 percent of all cancer deaths in women in 2018, with a reported 570.000 new cases (WHO, 2019; WHO, 2020).

After Rosenberg et al. discovered the antiproliferative effect of cisplatin (cis-diamminedichloroplatinum(II)) serendipitously (Rosenberg et al., 1965; Rosenberg et al., 1969), platinum compounds as anticancer agents received attention. Cisplatin, carboplatin, and oxaliplatin (Figure 1.), which have been marketed worldwide, have become the most effective medicines for the treatment of cancers. Platinum compounds, especially cisplatin, are used for neoadjuvant chemotherapy in cervical cancer (Biersack, 2017). DNA is the primary target of anticancer activity for platinum compounds and *in vitro* studies have shown that the N7 position of guanine is more preferable to attack over the other bases in DNA (Ghosh, 2019). Although cisplatin has a great success in the treatment of several tumor diseases, it has several side effects and restrictive effects, including ototoxicity, nephrotoxicity, myelosuppression, and neurotoxicity (Dasari & Bernard Tchounwou, 2014; Daugaard & Abildgaard, 1989; Pérez et al., 1999). Thus, therapeutic use of cisplatin is limited by especially both tumor resistance and toxicological considerations (Jamieson & Lippard, 1999). Owing to the need for platinum compounds, which have less toxicity, good water solubility, fewer side effects, and a broader spectrum of activity, several metal compounds have been synthesized and investigated for their biological effects since the discovery of cisplatin.

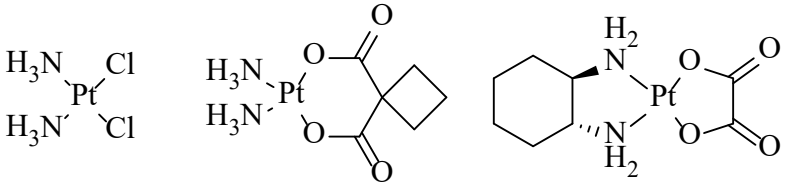


Figure 1. Structures of platinum drugs approved for clinical use worldwide.

Benzimidazole nucleus is one of the important pharmacophores in medicinal chemistry, and benzimidazole compounds have anticancer, antihistaminic, antihypertension, antibacterial, antiviral, and antifungal properties (Gaba & Mohan, 2016; Narasimhan et al., 2012). The presence of benzimidazole core can help to improve solubility. Moreover, the benzimidazole core can bind a wide range of macromolecules in biological systems due to its ability to act as a proton donor or acceptor (Beltran-Hortelano et al., 2020). Platinum compounds containing N-donor ligands such as benzimidazole show better biological efficiency with less toxicity (Al-Khathami et al., 2019; Facchetti & Rimoldi, 2019). According to literature data, benzimidazoles with substituents at the C2 position have activity a variety of cancer cell types (Gozelle et al., 2019; Nashaat et al., 2020; Doğan et al., 2021). Several platinum compounds bearing substituted benzimidazole ligands were reported previously (Zeyrek et al., 2017; Eren et al., 2018; Eren et al., 2019; Gozelle et al., 2019; Niknam et al., 2019; Özçelik et al., 2019).

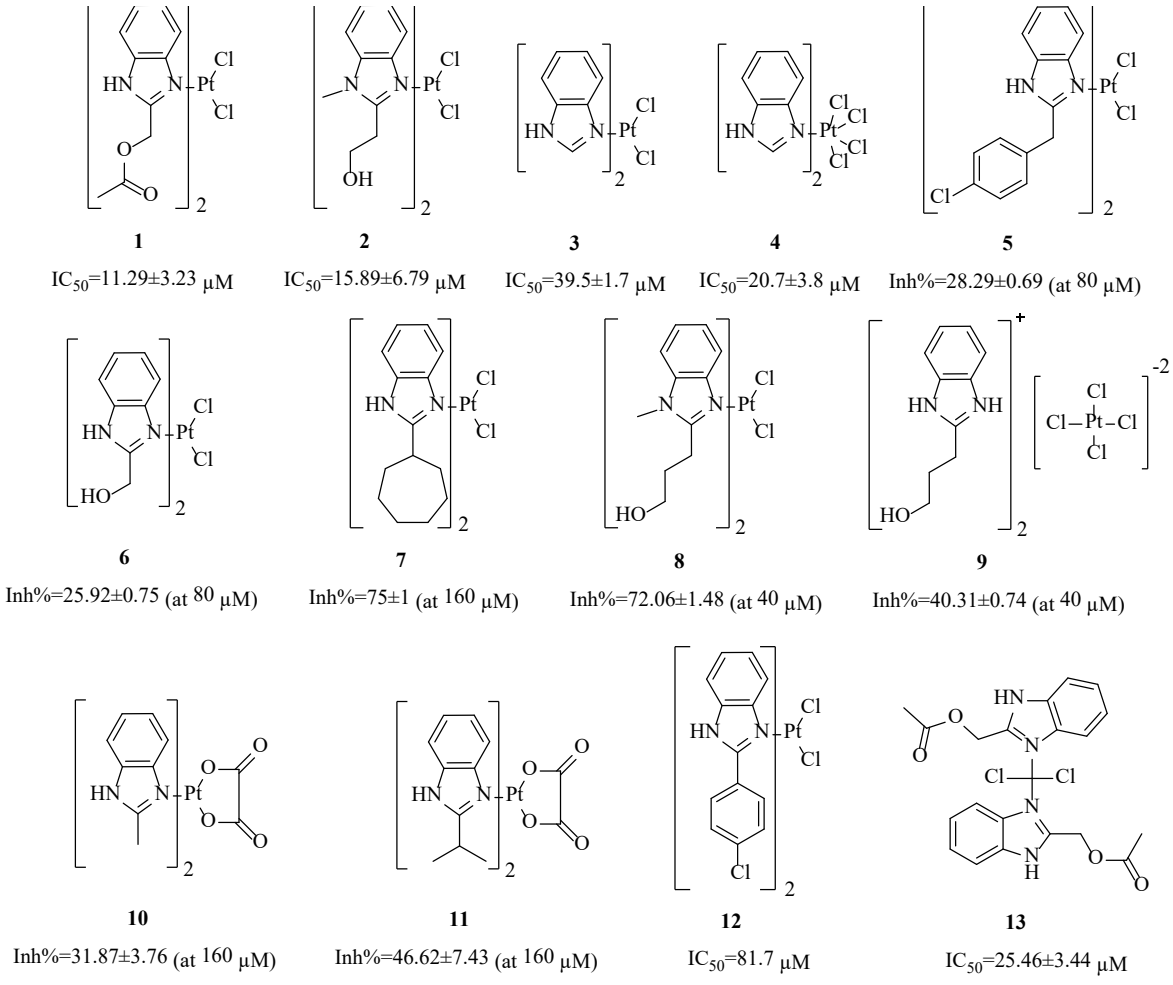
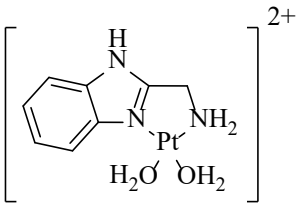


Figure 2. Platinum compounds bearing benzimidazole ligands against HeLa cell line.

In previous studies, several platinum compounds bearing substituted benzimidazole ligands against human cervical carcinoma HeLa cell line have been described, including *cis*-[dichloro-bis(2-acetoxymethylbenzimidazole)platinum(II)] (**1**), *cis*-[dichloro-bis(1-methyl-2-(2'-hydroxyethyl)benzimidazole)platinum(II)] (**2**) (Gümüş et al., 2009), *cis*-[dichloro-bis(benzimidazole)platinum(II)] (**3**), [tetrachloro-bis(benzimidazole)platinum(IV)] (**4**) (Utku et al., 2010), *cis*-[dichloro-bis(2-(4-chlorobenzyl)benzimidazole)platinum(II)] (**5**) (Özçelik et al., 2012), *cis*-[dichloro-bis(2-hydroxymethylbenzimidazole)platinum(II)] (**6**) (Utku et al., 2014), *cis*-[dichloro-bis(2-cycloheptylbenzimidazole)platinum(II)] (**7**) (Özçelik et al., 2015), *cis*-[dichloro-bis(1-methyl-2-(3' - hydroxypropyl) benzimidazole) platinum(II)] (**8**), 2 - (3' - hydroxypropyl) benzimidazolium tetrachloroplatinate (II) (**9**) (Eren et al., 2018), oxalate - bis (2-methylbenzimidazole) platinum(II) (**10**), oxalate-bis (2 - isopropylbenzimidazole)platinum(II) (**11**) (Gozelle et al., 2019), *cis*-[dichloro-bis (2 - (4 - chlorophenyl) benzimidazole) platinum(II)] (**12**) (Özçelik et al., 2019), *trans*-[dichloro-bis(2-acetoxymethylbenzimidazole)platinum(II)] (**13**) (Eren et al., 2019) (Figure 2.).



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IC_{50}=24.6±0.7 μM

Figure 3. Platinum compound bearing 2-aminomethylbenzimidazole against A549 cell line.

Cytotoxic activity of 2 - Aminomethylbenzimidazol containing diaqua platinum(II) compound (**14**) (Figure 3.) was investigated against human lung carcinoma A549 cell line (Mitra et al., 2016).

In this study, we synthesized two novel platinum compounds with 2-isopropylbenzimidazole and 2-(1-methylpropyl)benzimidazole and then investigated their cytotoxic activities on HeLa, A549, and human lung epithelial Beas-2B cells.

MATERIAL AND METHODS

Chemistry

All chemical compounds and solvents were reagent grade and purchased locally from Merck and Sigma-Aldrich. All chemicals and solvents were used without additional purification. Pre-coated aluminum thin layer chromatography (TLC) was used to monitor the reactions. Dragendorff reagent, iodine vapor, and ultraviolet light were used for TLC visualization methods. The molecular weight of the carrier ligands was assessed by electrospray ionization mass spectrometry (ESI+) in-house on a Waters LCT Premier XE Q-TOF Mass Spectrometer, also coupled to an AQUITY Ultra Performance Liquid Chromatography system (Waters Corporation, Milford, MA, USA). The purity of the final compounds was determined to be >97% by UPLC via an ultraviolet light detector. FTIR spectra were measured on a Perkin Elmer Spectrum 400 FTIR/FTNIR spectrometer (Perkin Elmer, Inc., Waltham, MA, USA) and were reported in cm^{-1} units. Elemental analyses were performed with a LECO-932 CHNS analyzer (LECO Corporation, St. Joseph, MI, USA), and proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a Varian Mercury 400 MHz Fourier-Transform (FT)-NMR spectrometer (Agilent Technologies, Palo Alto, CA, USA) by using TMS as the internal standard (Ankara University, Faculty of Pharmacy). The relative integrals of peak areas agreed with those expected for the assigned structures. All chemical shift values were expressed in ppm (δ). Melting points were recorded via an SMP-II Digital Melting Point Apparatus (Schorpp Geraetetechnik, Überlingen, Germany) and are uncorrected.

*2-Isopropylbenzimidazole (**L1**).* Synthesis and detailed structural analyses of ligand **L1** were carried out previously reported (Gozelle et al., 2019).

*R,S-2-(1-methylpropyl)benzimidazole (**L2**).* Synthesis and detailed structural analyses of ligand **L2** were carried out previously reported (Gozelle et al., 2019).

*2-Isopropylbenzimidazolium Tetrachloroplatinate(II) (**K1**).* A solution of K_2PtCl_4 (300.0 mg, 0.72 mmol) in 0.5 N HCl was added to a stirred solution of **L1** (200.0 mg, 1.25 mmol) in 0.5 N HCl dropwise over 30 min at rt. The reaction mixture was stirred at 50 °C for 11 days and protected from light. The resulting precipitate was filtered off and washed with cold water, cold ethanol, cold acetone, and diethyl ether several times before being dried in vacuo.

Infrared (IR) [(Attenuated total reflection (ATR)]: ν (cm^{-1}) 3101, 3063, 2969. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 1.47 (d, *J*=6.8 Hz, 12H), 3.44-3.53 (m, 2H), 7.55 (dd, *J*=6.0 and 3.2 Hz, 4H), 7.96 (dd, *J*=6.4 and 3.2 Hz, 4H). Anal. calcd for $C_{20}H_{26}N_4Cl_4Pt$: C 36.43; H 3.97; N 8.50; found C 36.19; H 4.18; N 8.57. mp >360 °C. Yield 20%. High-resolution mass spectrometry (HRMS) (m/z) calculated for **K1** [M+H] 161.1079, found 161.1077.

*2-(1-Methylpropyl)benzimidazolium Tetrachloroplatinate(II) Monohydrate (**K2**).* A solution of K_2PtCl_4 (300.0 mg, 0.72 mmol) in 0.5 N HCl was added to a stirred solution of **L2** (218.0 mg, 1.25 mmol) in 0.5 N HCl dropwise over 30 min at rt. The reaction mixture was stirred at 50 °C for 6 days and protected from light. The resulting precipitate was filtered off and washed with cold water, cold ethanol, cold acetone, and diethyl ether several times before being dried in vacuo.

IR (ATR): ν (cm^{-1}) 3098, 3034, 2950. ^{1}H-NMR (DMSO-d_6, 400 MHz) δ: 0.88 (t, *J*= 7.4 Hz, 6H), 1.45

(d, *J*= 6.8 Hz, 6H), 1.77-1.91 (m, 4H), 3.24-3.36 (m, 2H), 7.55 (dd, *J*= 6.0 and 3.2 Hz, 4H), 7.80 (dd, *J*= 6.0 and 3.2 Hz, 4H). Anal. calcd for $C_{22}H_{30}N_4Cl_4Pt.H_2O$: C, 37.46; H, 4.57; N, 7.94; found C, 37.09; H, 4.70; N, 7.95. mp >360 °C. Yield 17%. HRMS (m/z) calculated for **K2** [M+H] 175.1235, found 175.1234.

Cell Culture

HeLa cell line was obtained from Foot and Mouth Disease Institute (Ankara, Turkey). A549 and Beas-2B cell lines were purchased from American Type Culture Collection. Cells were seeded in 25 cm^2 flasks using either Dulbecco's modified Eagle's medium (HyClone Laboratories, Inc., Logan, UT) or RPMI 1640 Medium (Invitrogen Corporation, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (Cegrogen Biotech GmbH, Germany) and 1% penicillin-streptomycin mixture and then grown for 24 h at 37°C in 5% CO_2 in a humidified incubator.

Cytotoxic Activity

Cisplatin and carboplatin were used as reference drugs. *In vitro* cytotoxic effects of these compounds were performed according to MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. $1x10^5$ cells/well were seeded in 96-well plates for MTT assay. HeLa cells were incubated for 24 h at 37°C and 5% CO_2 in a humidified incubator. Cells were exposed to drugs for 24 h, then the medium was replaced with 5 mg/mL MTT and incubated for 4 h in 5% CO_2 incubator. After 48 h and 72 h incubation, the formazan crystals were dissolved in DMSO/ammonia and the optical density at 570 nm was measured with a microplate reader (BioTek Instruments Inc., Winooski, VT). For each compound, three independent experiments were performed. Results were expressed as the mean percentage of cell growth in the drug treatment group/control group. The mean of the control group was assumed as 100% survival.

Statistical Analysis

Statistical analysis was carried out using SPSS version 20 software for Windows (SPSS Inc., Chicago, IL, USA). Data were expressed as mean±standard deviation (SD), and statistical significance was assigned at the $p \leq 0.001$ and $p \leq 0.005$ levels. Results were analyzed using one-way ANOVA, Tukey's post-hoc test.

RESULTS AND DISCUSSION

The present paper investigates the *in vitro* cytotoxic effects of 2-isopropylbenzimidazole tetrachloroplatinate(II) (**K1**) and 2-(1-methylpropyl)benzimidazole tetrachloroplatinate (II) monohydrate (**K2**).

The first phase in the synthesis step to get the platinum compounds was the synthesis of the benzimidazole ligands (**L1** and **L2**). Ligand **L1** and **L2** were synthesized according to the procedure described previously (Gozelle et al., 2019), as shown in Figure 4. The synthesized yields of benzimidazole ligands were 68% and 70%, respectively. The platinum compounds **K1** and **K2** were synthesized, as shown in Figure 5. The synthesized yield of platinum compounds was 20% and 17%, respectively.

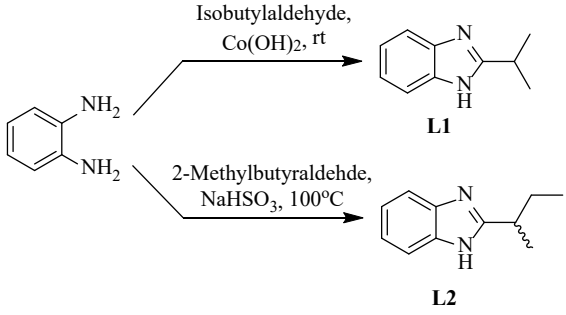

Figure 4. Synthesis of 2-substituted benzimidazole ligands.

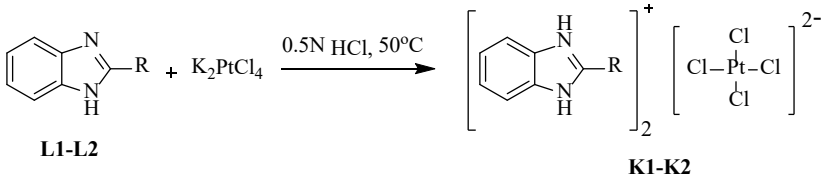

Figure 5. Synthesis of platinum compounds.

HRMS, IR, elemental analysis, ^{1}H-NMR, and melting point were used to characterize the synthesized compounds. X-ray structure of K1 was reported in our previous paper (Zeyrek et al. 2017). All characterized information proposed a 1:2 (platinum:ligand) stoichiometry for the platinum compounds. HRMS analysis of the platinum compounds **K1** and **K2** showed only m/z values of the benzimidazole ligands because **K1** and **K2** are salts that consist of a square planar tetrachloroplatinate(II) anion with hydrogen bonds to 2-substituted benzimidazoles.

Due to the platinum compounds' insolubility in the other NMR solvents, the ^{1}H-NMR spectra of the compounds were acquired by using dimethyl sulfoxide-d_6 (DMSO-d_6). It was observed that the peaks of the platinum compounds shifted towards the paramagnetic.

The cytotoxic activity of the synthesized compounds, cisplatin, and carboplatin was screened against HeLa, A549, and Beas-2B cells. Table 1. shows the inhibition values of compounds and reference drugs after 48h incubation. The inhibition percentage of cell viability on HeLa of **K1** and **K2** platinum compounds were determined as 51.43±0.80 (at 160 μM) and 53.42±2.21 (at 160 μM), respectively. The inhibition percentage of cell viability on A549 of **K1** and **K2** platinum compounds were determined as 54.96±0.47 (at 160 μM) and 88.16±0.22 (at 160 μM), respectively. Compound **K2** was determined more cytotoxic against A549 than carboplatin.

Table 1. Cytotoxic activity values expressed as inhibition % ± SD of compounds, cisplatin, and carboplatin after 48h incubation.

Comp.	μM	HeLa	A549	Beas-2B
L1	20	1.62±1.62	n.i.	n.i.
	40	2.19±1.72	18.75±5.42[a]	25.09±4.21[a]
	80	3.82±2.32	19.88±3.42[a]	27.23±3.81[a]
	160	4.93±2.67[b]	24.96±2.80[a]	29.13±4.18[a]
L2	20	n.i.	n.i.	n.i.
	40	0.65±0.28	6.68±5.48	16.20±3.75[a]
	80	1.57±0.57	16.02±2.30[a]	23.33±1.50[a]
	160	2.78±1.78[b]	19.97±6.35[a]	23.93±1.63[a]
K1	10	4.73±2.73	n.i.	4.65±1.72[a]
	20	5.44±1.50	n.i.	14.93±5.52[a]
	40	5.42±4.45	0.35±0.60	81.57±1.30[a]
	80	11.51±1.77[a]	10.01±1.59[a]	91.85±0.72[a]
	160	51.43±0.80[a]	54.96±0.47	92.11±0.39[a]
K2	10	3.67±1.81	n.i.	n.i.
	20	4.85±2.46	6.31±3.67[b]	3.14±0.63[a]
	40	4.89±2.97	17.30±3.43[a]	89.80±1.29[a]
	80	14.38±5.93[a]	37.98±0.87[a]	90.16±0.58[a]
	160	53.42±2.21[a]	88.16±0.22[a]	92.09±0.57[a]
Carboplatin	10	1.54±0.22[a]	10.13±8.53	17.03±3.74[a]
	20	2.03±0.20[a]	22.30±0.27[a]	20.43±4.18[a]
	40	4.07±0.22[a]	30.61±1.47[a]	33.97±0.32[a]
	80	8.33±0.70[a]	35.55±0.92[a]	47.28±0.26[a]
	160	14.07±0.2[a]	41.32±3.45[a]	51.87±0.85[a]
Cisplatin	5	16.1±0.52[a]	37.73±6.89[a]	39.65±5.37[a]
	10	18.81±1.10[a]	43.89±9.66[a]	62.18±3.59[a]
	20	51.33±0.58[a]	45.70±6.69[a]	84.61±0.22[a]
	40	53.68±1.15[a]	67.58±17.32[a]	89.76±0.25[a]

The results (mean±SD) of three independent experiments. a: statistically significant from the control group ($p \leq 0.01$), b: statistically significant from the control group ($p \leq 0.05$), n.i.: no inhibition.

The cytotoxic activity of compounds and reference drugs after 72h incubation was shown in Table 2.

The cytotoxic activity results showed that **K1** displayed 29.46±5.50 (at 160 μM) on HeLa, 53.47±1.2 at 160 μM) on A549. The inhibition percentage of cell viability of **K2** was 53.47±1.20 (at 160 μM) on HeLa and 93.95±0.53 (at 160 μM) on A549. Compound **K2** was more cytotoxic against human lung carcinoma cells than carboplatin.

Table 2. Cytotoxic activity values expressed as inhibition % ± SD of compounds, cisplatin, and carboplatin after 72h incubation.

Comp.	μM	HeLa	A549	Beas-2B
L1	20	4.98±0.42[a]	n.i.	n.i.
	40	3.55±0.13[b]	16.23±2.14[a]	19.32±2.44[a]
	80	2.88±0.92[b]	16.51±2.08[a]	24.82±3.77[a]
	160	3.00±2.32[b]	19.59±0.29[a]	26.23±2.49[a]
L2	20	n.i.	n.i.	n.i.
	40	4.16±3.31	14.65±1.18[a]	n.i.
	80	4.64±3.79	15.30±1.45[a]	n.i.
	160	15.52±4.7[a]	20.34±2.32[a]	n.i.
K1	10	13.54±6.79[a]	1.87±1.68	15.45±2.60[a]
	20	10.54±1.04[b]	5.39±2.34	19.36±3.25[a]
	40	15.83±0.35[a]	9.17±3.88	87.71±1.73[a]
	80	13.67±4.66[a]	26.07±3.62[a]	95.06±0.16[a]
	160	29.46±5.50[a]	53.47±1.20[a]	95.15±0.13[a]
K2	10	n.i.	1.95±0.59	n.i.
	20	n.i.	6.97±0.87[a]	n.i.
	40	n.i.	14.34±1.49[a]	90.41±0.24[a]
	80	0.86±1.06	36.57±1.17[a]	91.38±0.57[a]
	160	27.42±2.03[a]	93.95±0.53[a]	91.99±0.22[a]
Carboplatin	10	1.08±0.53	19.63±1.73[a]	22.03±8.41[a]
	20	1.30±0.50[b]	25.61±0.17[a]	33.96±7.79[a]
	40	18.79±0.61[a]	40.49±0.95[a]	36.24±6.63[a]
	80	26.4±0.80[a]	46.68±1.22[a]	62.17±2.85[a]
	160	48.17±0.50[a]	46.73±0.10[a]	86.05±4.21[a]
Cisplatin	5	21.67±0.70[a]	42.88±2.20[a]	60.99±9.51[a]
	10	30.00±1.78[a]	48.00±0.03[a]	82.61±3.44[a]
	20	63.99±0.44[a]	58.74±1.36[a]	91.67±2.11[a]
	40	67.67±1.72[a]	87.96±1.78[a]	93.44±1.63[a]

The results (mean±SD) of three independent experiments. a: statistically significant from the control group ($p \leq 0.01$), b: statistically significant from the control group ($p \leq 0.05$), n.i.: no inhibition.

Figure 6. and Figure 7. show the inhibition percentage of compounds and reference drugs. The benzimidazole ligands (**L1** and **L2**) show no significant inhibition activity on all cell lines after 48h and 72h incubation. The cytotoxic activity of **K1** and **K2** against human cervical carcinoma after 48h incubation is higher than 72h incubation. **K1** and **K2** have no significant inhibition for HeLa cell line after 72h incubation. The cytotoxic activity of **K2** against human lung carcinoma after 72h incubation is higher than 48h incubation.

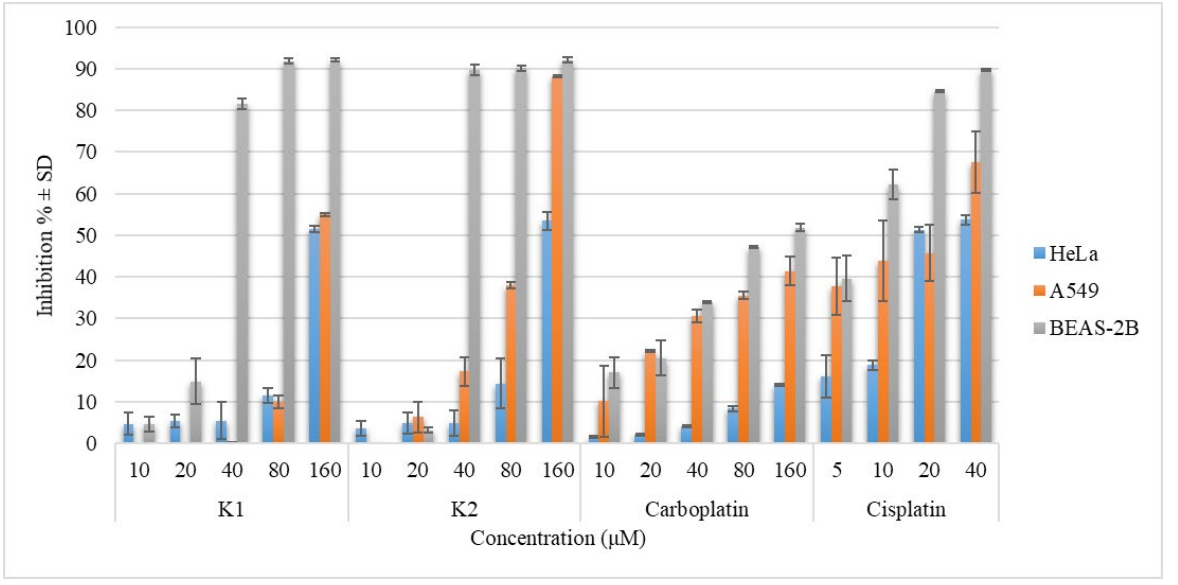

Figure 6. Inhibition percentage of cell viability on HeLa, A549, and Beas-2B cell lines treated with different concentrations of the compounds and positive controls after 48h incubation.

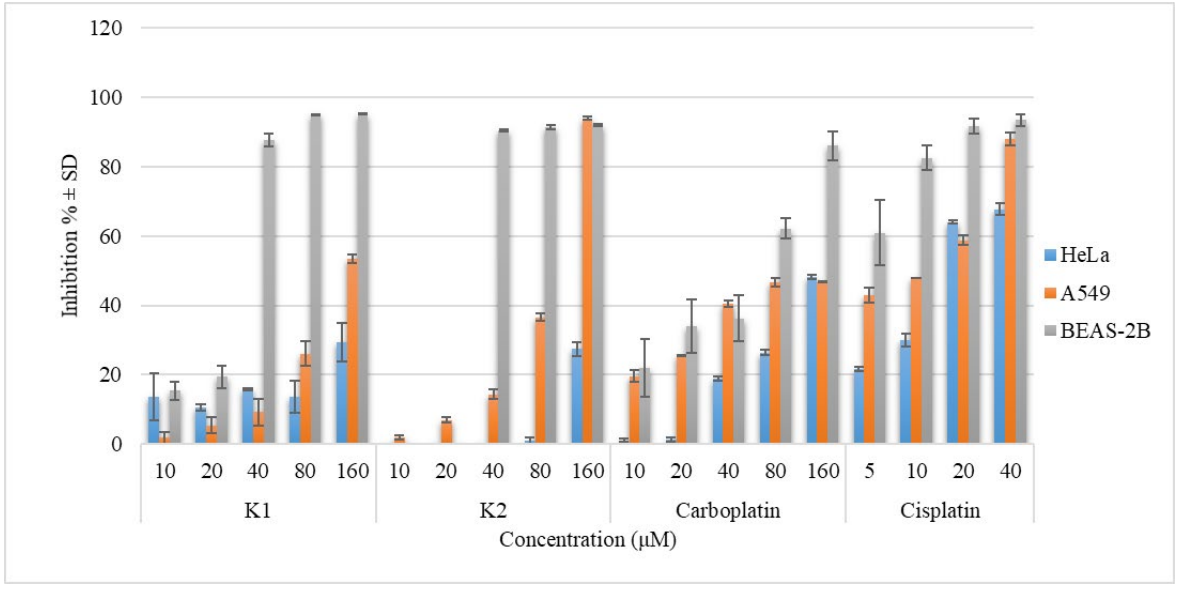

Figure 7. Inhibition percentage of cell viability on HeLa, A549, and Beas-2B cell lines treated with different concentrations of the compounds and positive controls after 72h incubation.

As shown in Table 1, the compounds being tested are arranged in an order to decrease cytotoxic effect cisplatin>**K1**≈**K2**>carboplatin on HeLa cells for 48h incubation. **K1** and **K2** have a moderate cytotoxic effect against HeLa cells for 48h incubation. The compounds being tested are arranged in an order to decrease cytotoxic effect cisplatin>**K2**>**K1**>carboplatin on A549 cells for 48h incubation. Compounds **K1** and **K2** showed similar selectivity to cisplatin against HeLa. Moreover, **K2** showed more selectivity than the other compounds against A549.

As shown in Table 2., the compounds being tested are arranged in an order to decrease cytotoxic effect

cisplatin >carboplatin>**K1**≈**K2** on human cervical carcinoma HeLa cells for 72h incubation. The compounds being tested are arranged in an order to decrease cytotoxic effect cisplatin>**K2**>**K1**>carboplatin on human lung carcinoma A549 cells for 48h incubation. Compounds **K1** and **K2** showed no selectivity against HeLa.

CONCLUSION

The main aim of this paper was to investigate the cytotoxic activities on human cervical carcinoma HeLa cells after 48h and 72h incubation of the platinum compounds. With this purpose, 2-Isopropylbenzimidazole tetrachloroplatinate(II) (**K1**) and 2-(1-methylpropyl)benzimidazole tetrachloroplatinate (II) monohydrate (**K2**) were synthesized, and elemental analysis, IR, and ^{1}H-NMR were used to characterize these compounds. Besides, the effects of these compounds on HeLa, A549, and Beas-2B cells were examined. The primary data obtained in this study lead us to conclude that the platinum compounds (**K1** and **K2**) showed higher inhibition values than carboplatin against HeLa and A549 cells after 48h incubation. Moreover, it is observed that the inhibition values of **K1** and **K2** against HeLa at 160 μM were similar to the inhibition value of cisplatin at 40 μM. The inhibition values of **K2** against A549 at 160 μM were similar to the inhibition value of cisplatin at 40 μM. Despite the fact that **K2** showed more selective than the other compounds against A549. This work highlights the cytotoxic potential on human cervical carcinoma and human lung carcinoma of platinum compounds derived from benzimidazole ligands. Those results must be taken into consideration for further studies.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR CONTRIBUTION STATEMENT

Design, synthesis, and analysis of compounds, writing the manuscript and statistical analysis: M.G., Cytotoxic activity: A.K.S., critical review: M.G., A.K.S. All authors gave final approval for publication.

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