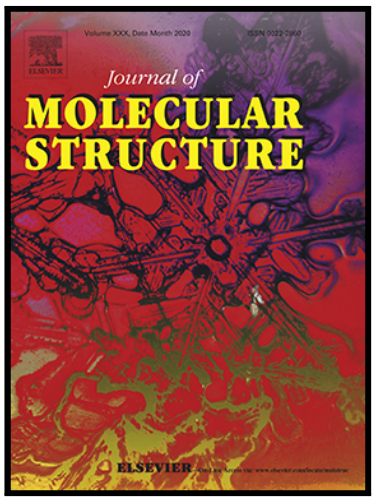

Novel Benzimidazole- Platinum(II) Complexes: Synthesis, Characterization, Antimicrobial and Anticancer Activity

Umut Doğan , Özge Özcan , Gizem Alaca , Aydan Arı , Salih Günnaz , H. Tansel Yalçın , Onur Şahin , Sevil Ürişli

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Novel Benzimidazole- Platinum(II) Complexes: Synthesis, Characterization, Antimicrobial and Anticancer Activity

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GRAPHİCAL ABSTRACT(Synopsis)

New three Platinum complexes **(1-3)** with 2,6-di-tert-butyl-4-(1-phenyl-1H-benzimidazol-2-yl) phenol **(L_1),** N, N-dimethyl-4-(1-phenyl-1H-benzimidazol-2-yl) aniline **(L_2)** and 4-(1H-benzimidazol-2-yl)-N, N-dimethylaniline **(L_3)** were synthesized. . The crystal structures of **L_1, 1** and geometrical isomer of 1 **(1a)** were determined by X-Ray crystallography. The cytotoxic activities of the compounds were tested on against SHSY-5Y and U-87 cell lines and antimicrobial effect on gram positive and gram negative bacteria .

Highlights

- New three Platinum complexes **(1-3)** with 2,6-di-tert-butyl-4-(1-phenyl-1H-benzimidazol-2-yl) phenol **(L_1),** N, N-dimethyl-4-(1-phenyl-1H-benzimidazol-2-yl) aniline **(L_2)** and 4-(1H-benzimidazol-2-yl)-N, N-dimethylaniline **(L_3)** were synthesized.
- The crystal structures of **L_1, 1** and geometrical isomer of 1 **(1a)** were determined by X-Ray crystallography.

- The cytotoxic activities of the compounds were tested on against SHSY-5Y and U-87 cell lines and antimicrobial effect on gram positive and gram negative bacteria .

Abstract

Three new Platinum complexes **(1-3)** with 2,6-di-tert-butyl-4-(1-phenyl-1H-benzimidazol-2-yl) phenol **(L_1),** N, N-dimethyl-4-(1-phenyl-1H-benzimidazol-2-yl) aniline **(L_2)** and 4-(1H-benzimidazol-2-yl)-N, N-dimethylaniline **(L_3)** were prepared and characterized by FT-IR, NMR and Elemental analyses. The crystal structures of **L_1, 1** and geometrical isomer of 1 **(1a)** were determined by X-Ray crystallography. The cytotoxic activities of the compounds were tested on against SHSY-5Y and U-87 cell lines. The antimicrobial evaluations of the compounds showed that they have a moderate antimicrobial effect on gram positive and gram negative bacteria.

Keywords

benzimidazole, benzimidazole platinum (II) complexes, cytotoxicity, antimicrobial activity

1.Introduction

The use of cisplatin as one of the most active anticancer drugs is widespread. Because of the side effects of cisplatin, such as dose-limiting toxicity, nephrotoxicity and a tendency for tumoral drug resistance, different approaches to design new drugs, especially active organic molecules with metal compounds, are observed **[1].** Chemical modification of platinum drugs may generate a new class of chemotherapeutic agents which have higher selectivity, limited drug resistance and increasing cell-membrane permeability.

Platinum complexes containing imidazolic/benzimidazolic ligands **[2-9]** have drawn the attention of several research groups, since imidazole/benzimidazole and their derivatives are an important class of organic compounds. They are capable of interacting with biomolecular targets. They also exhibit numerous activities such as antiproliferative **[10]** anti-inflammatory **[11,12]**, antiallergic **[13]**, antibacterial **[14]** and antiviral **[15]** properties. The capability of their scaffold is a useful structural motif for displaying chemical functionality **[16].** The substituent of the carbon atom between two nitrogen atoms in this system has effected the biological activities **[17,18].**

In the last decade, a lof of benzimidazole – platinum complexes synthesized and reported their antineoplastic activities on different cancer cell lines. But the reports, especially on brain cancer cells, are limited **[19,20]**. Glioblastoma is a highly malignant and aggressive primary brain tumor and can arise in children. The prognosis of glioblastoma remains very poor. Neuroblastoma is also the most common extracranial solid cancer in children **[21,22]**. Cisplatin based therapy is one of the most important chemotherapy treatments for neuroblastoma and glioblastoma, but the benefit of cisplatin is hampered by severe side effects, including neurotoxicity and nefrotoxicity **[23,24]**. The limited number of coordination compounds effective on neuroblastoma and glioblastoma **[25,26]** in the literature has guided us in the synthesis of new compounds that can be used for this purpose, elucidating their structures and determining their biological activities.

For this purpose, we report new Pt(II) – benzimidazole complexes that could prove to be potent antitumor agents. In the present work, we have synthesized three new platinum complexes with benzimidazole heterocycles which have different substituents at the C atom between the two nitrogen nuclei. In a series of investigations, we have studied the cytotoxicity of the ligands, cisplatin, $[PtCl_2(DMSO)(L_1)]$, $[PtCl_2(DMSO)(L_2)]$ and $[PtCl_2(DMSO)(L_3)]$ in U87 glioblastoma and SHSY-5Y neuroblastoma cell lines in *vitro*. We have also examined the antimicrobial activities of the ligands and complexes.

2.Experimental

2.1. Chemicals

The reagents K_2PtCl_4, 3,5-Di-tert-butyl-4-hydroxybenzaldehyde, N-phenyl-o-phenylene diamine, ethanol, dichloromethane, hexane, 4-(Dimethylamino) benzaldehyde, used in the synthesis were commercially purchased from Merck. $Pt(DMSO)_2Cl_2$, used as starting complex, was synthesized according to literature data **[27]**. The synthesis of $\mathbf{L_2}$ and $\mathbf{L_3}$ exist in literature **[28].** However, the following synthesis methods were not use catalyst.

2.2. Instrumentations

Melting points were obtained using an Electrothermal Melting Point detection apparatus. Elemental analyzes were performed with the CHNS-932 Leco instrument. Infrared spectra were recorded on a Perkin Elmer Spectrum 100 FT-IR spectrophotometer in the range of 4000- 400 cm^{-1} for ligands, 4000- 200 cm^{-1} for complexes. ^{1}H and ^{13}C NMR spectra were measured at Varian AS 400 MHz spectrometer. $CDCl_3$ ve DMSO-d_6, were used as the solvents and TMS was used as the internal standard. X-Ray data were measured in Ondokuz Mayıs University, Turkey, by using the STOE IPDS and Sinop University, Turkey, by using Bruker D8 QUEST diffractometers.

2.3. X-Ray structure determination

A pale yellow, single crystal of compound $\mathbf{L_1}$ was obtained by a slow diffusion of a 1:3 dichloromethane/hexane system and yellow single crystals of compounds **1** and **1a** were obtained by a slow diffusion of 1:3 chloroform/hexane system at room temperature. X-Ray measurements were made in different centers. Intensity data for ligand $\mathbf{L_1}$ were collected on a STOE IPDS II diffractometer using graphite-monochromated Mo K_α radiation (λ= 0.71073 Å) at 296 K. All positional and thermal parameters were refined using the SHELXL-97 program **[29]**. The hydrogen atoms were identified in the difference Fourier map and were included in the refinement process as fixed contributions**.** Suitable crystals of complexes **1** and **1a** were selected for data collection, which was performed on a D8-QUEST diffractometer equipped with a graphite-monochromatic Mo-Kα radiation at 296 K. The structure was solved by direct methods using SHELXS-2013 **[30]** and refined by full-matrix least-squares methods on F^2 using SHELXL-2013 **[31]**. In **1a**, the large s.u. values and displacement parameters of Pt atom in the molecule are caused by the disorder. This disorder was modelled as two different orientations, as Pt1 and Pt2, with occupancy factors of 0.863(3) and 0.137(3), respectively. The figure shows the Pt1 atom, whose occupancy factor value is much higher than the other. The following procedures were implemented in our analysis: data collection: Bruker APEX2 **[32]**; program used for molecular graphics were as follows: MERCURY programs **[33];** software used to prepare material for publication: WinGX **[34].** Atomic coordinates, bond lengths and angles were deposited in the Cambridge Crystallographic Data Center, 12 Union Road, Cambridge CB2 1EZ, U.K. Crystal data and the structure refinement details of compounds are given in Table 1.

2.4. Biological Assesment

2.4.1. Antibacterial activity

The minimal inhibitory concentration (MIC) of compounds was determined by a micro-dilution method according to CLSI (2017) standards **[35].** The tested microbial strains included one yeast strain (*Candida albicans* ATCC 10239) as well as several gram positive and gram

negative bacterial strains. Gram-positive bacterial strains that were included in this study were: *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* DSM 13590, *Staphylococcus aureus* ATCC 25923 and *Staphylococcus epidermidis* ATCC 12228. On the other hand, Gram-negative bacterial strains included were: *Escherichia coli* ATCC 29998, *Pseudomonas aeruginosa* ATCC 27853 and *Salmonella typhimurium* CCM 5445. The lyophilized microorganisms were provided by Ege University, Faculty of Science, Department of Basic and Industrial Microbiology (Izmir, Turkey).

The microorganisms were grown in Mueller Hinton Broth until they reached exponential phase after 5h. The bacterial inocula were adjusted to 0.5 McFarland turbidity standard (A600=0.1), which corresponds to 1.5×10^6 colony-forming units (CFU /mL). 80 µL of stock concentration of each compound (500 µg/mL) were serially diluted with 80 µL MH to obtain concentrations within the range 0.9–500 µg/mL. After which, 20µL of each microbial inoculum (1.5×10^5CFU/mL) was added to each well. After incubation at 37°C for 24h, inhibition of bacterial growth was assessed visually. The MIC was defined as the lowest concentration of compound that is required to inhibit microbial growth. Minimum bactericidal concentrations (MBC) and minimum fungicidal concentrations (MFC) of compounds were evaluated by sub-culturing about 5–10 µL of wells with concentration equal or higher than MIC on Mueller-Hinton agar plate for bacteria or on sabouraud dextrose agar for yeast. The lowest concentration that didn't show bacterial growth was defined as the MBC value. The results are expressed in µg/mL. Positive control wells were supplemented with standard antimicrobial drugs, getamicin and flucytosine, against bacterial strains and yeast respectively. DMSO including MH broth in a well was employed as negative control. Control wells for sterility (MH broth) and confirmation of microbial growth (MH broth inoculated by each test microorganisms) were also included.

2.4.2. Cytotoxicity

Cytotoxicity tests were obtained by using U87, SHSY-5Y and Vero cells. Cells were purchased from ATCC, USA, and seeded 96-well plate $1x10^5$ cell/mL concentration. They were grown in culture media (MEM Earle's FG0325-BC, Merck, Germany,10% Fetal Bovine Serum FBS, A0500-3010, Cegrogen Biotech, Germany, 1% Gentamicine A2712, Merck, Germany). The test materials were added at different concentrations (0.1-0.5-1.0-10-100 µM, DMSO)culture media and incubated for 72 hours. Growth inhibitions of cells were measured spectrophotometrically using a standard method (MTT) at 570 nm. The results were repeated three times.

2.4.3. DNA Cleavage

The highly active complex **1** of the series was selected for DNA interaction, so DNA cleavage activity of the complex **1** was studied by an agarose gel electrophoresis method. The experiment involves incubating the plasmid DNA in Tris- HCl 1 mM EDTA (pH 7.5) at 37^oC for 24 hours. After incubation the 1 uL LD (6X) was added. The sample was loaded on

0.8 % agarose gel and subjected to electrophoresis at 100 V for 30 minutes. The gel was washed with the buffer (TBE X 0.5). After electrophoresis, agarose gel was stained with the SYBR GOLD (1:10.000) and washed TBE X 0.5 buffer. Then the gel was visualized on a blue led transilluminator.

2.5.Synthesis of Ligands

2.5.1. 2,6-di-tert-butyl-4-(1-phenyl-1H-benzimidazol-2-yl) phenol (L_1)

A mixture of 3,5-di-tert-butyl-4-hydroxybenzaldehyde (0.200 g, 0.821 mmol) and N- phenyl-o-phenylene diamine (0.151g, 0.821mmol) in ethanol (5 mL) were stirred and the reaction mixture was refluxed for 3 days. The solution was cooled to room temperature and the solid products were precipitated. Filtration was performed for the resulting solid, and then dried under vacuum. Final product was crystallized from the dichloromethane / hexane solution. The crystals formed have a pale yellow color. Yield= 68%, Melting points = 117 ^{0}C, ^{1}H (400 MHz, $CDCl_3$) δ (ppm) : 7.59 (d, 1H, *J*=8.0 Hz, Ar-*H*), 7.39-7.35 (m, 5H, Ar-*H*), 6.99 (t, 1H, *J*=7.6 Hz, Ar-*H*), 6.91-6.87 (m, 4H, Ar-*H*), 5.58 (s, 1H, O*H*), 1.38 (s, 18H, t-Bu*H*), ^{13}C (100 MHz, $CDCl_3$) δ (ppm) : 155.9 (N=*C*-N), 152.5 (*C*-OH), 140.2, 135.7, 135.6, 133.9, 129.6, 128.7, 124.1, 123.4, 119.5, 117.7, 109.5, 34.5 (t-Bu-*C*) , 30.2 (t-Bu-*C*H_3), Elemental analysis (%) = Calculated for ($C_{27}H_{30}N_2O$)(398.55); C ,81.40 ; H,7.59 ; N,7.03 ; Found ; C ,81.06 ; H,7.44 ; N,6.97.

2.5.2. N, N-dimethyl-4-(1-phenyl-1H-benzimidazol-2-yl)aniline (L_2)

The L_2 ligand was synthesized according to the L_1 ligand synthesis method. 4-(Dimethylamino) benzaldehyde (0.200 g, 1.34mmol) and N-phenyl-o-phenylene diamine (0.247g, 1.33 mmol) were used. The solid had a brownish-creamy color. , Yield= 66 %, Melting points = 222 ^{0}C , ^{1}H(400 MHz, $CDCl_3$) δ (ppm) : 7.84 (d, 1H, *J*=8.0 Hz, Ar-*H*), 7.51-7.35 (m, 5H, Ar-*H*), 7.33-7.26 (m, 3H, Ar-*H*), 7.22-7.18 (m, 2H, Ar-*H*), 6.57(d, 2H, *J*=8.8 Hz, Ar-*H*), 2.95 (s, 6H,N ($C*H*_3$)$_2$),^{13}C (100 MHz, $CDCl_3$) δ (ppm) : 153.2 (N=*C*-N), 150.8 (*C*N(CH_3)$_2$), 143.2, 137.6, 137.3, 130.5, 129.8, 128.3, 127.6, 122.6, 122.4, 119.1, 117.0, 111.3, 110.0, 40.1, Elemental analysis(%) = Calculated for ($C_{21}H_{19}N_3$) (313,40); C , 80.48 ; H,6.11 ; N,13.41 ; Found ; C ,80.21 ; H,5.99 ; N,13.17.

2.5.3. 4-(1H-benzimidazol-2-yl)-N, N-dimethylaniline (L_3)

The L_3 ligand was synthesized according to the L_1 ligand synthesis method.4-(Dimethylamino) benzaldehyde (0.500 g, 3.35 mmol) and o-phenylenediamine (0.181 g, 1.67 mmol) were used. Yield = 68 %, Melting points= 180^{0}C, ^{1}H (400 MHz, DMSO) δ (ppm): 7.98

(d, 2H, *J*=9.2 Hz, Ar-*H*), 7.49-7.47 (m, 2H, Ar-*H*), 7.13-7.09 (m, 2H, Ar-*H*), 6.82 (d, 2H, *J*=8.8 Hz, Ar-*H*), 2.98 (s, 6H, N-C*H*$_3$). ^{13}C (100 MHz, DMSO) δ (ppm): 152.8 (N=*C*-N), 151.7 (*C*N(CH$_3$)$_2$), 139.9,128.0, 121.8, 117.8, 114.8, 112.3, 40.2, Elemental analysis (%) = Calculated for ($C_{15}H_{15}N_3$) (237,13); C, 75.92; H,6.37; N,17.71; Found, C, 75.64; H,6.23; N,17.36.

2.6. Synthesis of Complexes

2.6.1. Synthesis of [$PtCl_2$(DMSO)(L_1)] (1)

In complex synthesis, **L_1** (0.0300 g, 0.0753 mmol) and cis-$PtCl_2$(DMSO)$_2$(0.0318 g, 0.0753 mmol) were mixed in a 1:1 molar ratio of chloroform at room temperature conditions for 24 h. After evaporation of the solvent, the crude product was crystallized from dichloromethane / hexane. The crystals formed are a white-yellow color. Yield= 63%, Melting points =275 ^{0}C decomposition, FT-IR (CsI disk , cm^{-1}) υ_{S-O} =1111-1143, υ_{Pt-Cl} =315-330, ^{1}H (400 MHz, $CDCl_3$) δ (ppm) : 8.35 (d, 1H, *J*=8.8 Hz Ar-*H*), 7.95 (s, 2H, Ar-*H*), 7.50-7.45 (m, 5H, Ar-*H*), 7.34 (t, 2H, *J*=7.6 Hz, Ar-*H*), 7.22 (d, 1H, *J*=7.6 Hz ,Ar-*H*), 5.68 (s, 1H, O*H*), 3.29 (s, 3H, Pt-S-C*H*$_3$), 2.39 (s, 3H, Pt-S-C*H*$_3$), 1.38 (s, 18H, t-Bu*H*). ^{13}C (100 MHz, $CDCl_3$) δ (ppm) :156.2 (N=*C*-N), 153.3 (Ar*C*-OH), 136.0, 135.2, 129.9, 129.3, 127.5, 125.0, 124.7, 119.0, 118.3, 111.1, 44.4 (Pt-S-*C*H$_3$), 43.1 (Pt-S-*C*H$_3$), 34.7 (t-Bu-*C*), 30.1 (t-Bu-*C*H$_3$) , Elemental Analysis (%) = Calculated ($C_{29}H_{36}N_2SO_2PtCl_2$) (742.66); C, 46.90 ; H, 4.89 ; N, 3.77 ; S, 4.32 ; Found ; C ,47.43 ; H, 5.18 ; N, 3.47 ; S, 4.26.

2.6.2. Synthesis of [$PtCl_2$(DMSO)(L_2)] (2)

The synthesis of the [$PtCl_2$(DMSO)(L_2)] complex was carried out in the same way as the synthesis step of the [$PtCl_2$(DMSO)(L_1)] complex. Yield 65 %, Melting points = 251 ^{0}C decomposition, FT-IR (CsI disk , cm^{-1}) υ_{S-O} =1137-1140, υ_{Pt-Cl} =298-319 , ^{1}H (400 MHz, $CDCl_3$) δ (ppm) : 8.38 (d, 1H, *J*=8.0 Hz, Ar-*H*), 7.88 (d, 2H, *J*=8.8 Hz, Ar-*H*), 7.48-7.45 (m, 5H, Ar-*H*), 7.32 (t, 2H, *J*=7.6, Ar-*H*), 7.18 (d, 1H, *J*=8.8 Hz, Ar-*H*), 6.70 (d, 2H, *J*=9.2 Hz, Ar-*H*), 3.44 (s, 3H, Pt-S-C*H*$_3$), 3.02 (s, 6H, N(C*H*$_3$)$_2$), 2.58 (s, 3H, Pt-S-C*H*$_3$). ^{13}C (100 MHz, $CDCl_3$) δ (ppm) : 152.8 (N=*C*-N), 151.6 (*C*N(CH$_3$)$_2$), 145.0, 135.3, 132.6, 129.2, 128.4, 127.7, 127.4, 124.8, 124.6, 118.9, 110.9, 44.4 (Pt-S-*C*H$_3$), 43.8 (Pt-S-*C*H$_3$), 40.0 (N(*C*H$_3$)$_2$, Elemental Analysis (%) = Calculated ($C_{23}H_{25}N_3SOPtCl_2$) (656.07); C , 42.01 ; H, 3.83 ; N, 6.39 ; S, 4.88 Found ; C , 41.62 ; H , 4.01 ; N, 6.28 ; S,4.70.

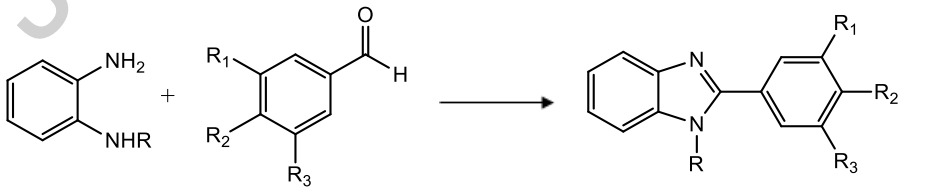
2.6.3. Synthesis of Complex [$PtCl_2$(DMSO)(L_3)] (3)

The synthesis of the [$PtCl_2$(DMSO)(L_3)] complexes were carried out in the same way as the synthesis step of the [$PtCl_2$(DMSO)(L_1)] complex. Yield= 67%, Melting points = 207 ^{0}C decomposition, FT-IR (CsI disk , cm^{-1}) υ_{S-O} =1120-1134 , υ_{Pt-Cl} =318-321 , ^{1}H (400 MHz,DMSO) δ (ppm) : 13.53 (s, 1H, N*H*), 8.58 (d,2H, *J*=9.2Hz, Ar-*H*), 8.11 (d,1H, *J*=*7.2* Hz Ar-*H*), 7.52 (d, 1H, *J*=7.2 Hz, Ar-*H*), 7.39-7.32 (m, 2H, Ar-*H*), 6.99 (d, 2H, *J*=9.2 Hz, Ar-*H*), 3.25 (s, 3H, Pt-S-CH_3), 3.06 (s, 6H, N(CH_3)$_2$), 2.86 (s, 3H, Pt-S-CH_3). ^{13}C (100 MHz,DMSO) δ (ppm) : 152.9 (N=*C*-N), 152.5 (*C*N(CH$_3$)$_2$), 141.0, 132.8, 130.3, 124.4, 123.5, 118.3, 114.1, 112.4, 111.8, 44.1 (Pt-S-*C*H$_3$), 43.2 (Pt-S-*C*H$_3$), 40.9 (N(*C*H$_3$)$_2$) , Elemantal Analysis (%) = Calculated ($C_{17}H_{21}N_3SOPtCl_2$)(580.04); C ,35.12 ; H,3.64 ; N,7.23 ; S, 5.51 Found; C ,34.89 ; H,3.77 ; N,6.98 ; S,5.40 .

3.Results and Discussion

3.1. Synthesis and spectroscopic studies

L_1 ligand was synthesized with the reaction of 3,5-Di-tert-butyl-4-hydroxybenzaldehyde and N- phenyl-o-phenylenediamine in ethanol. Although the syntheses of **L2** and **L3** ligands are given in literature **[28]** here we resent a reaction pathway that omits the use of a catalyst. In this study **L_2** and **L_3** ligands were isolated in the absence of a catalyst according to the reaction pathway of **L_1** ligand. General synthetic route of the ligands is given in Scheme 1.



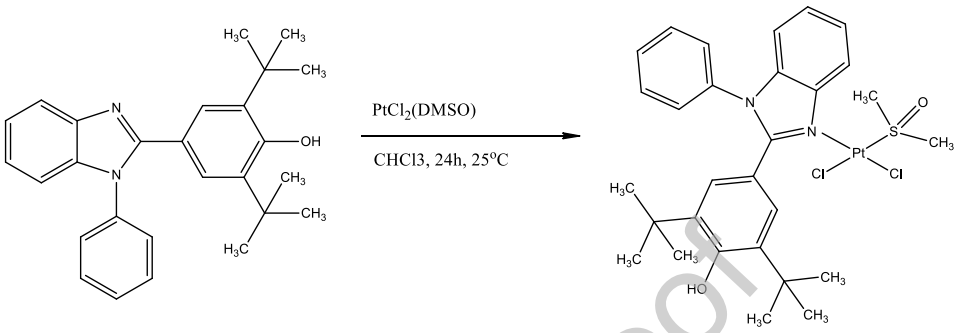
L_1:R=Ph, R_1=R_3= -tert-butyl;R_2=-OH

L_2:R=Ph;R_1=R_3=H;R_2=-NMe_2

L_3:R=H , R_1=R_3=H;R_2=-NMe_2

Scheme 1 : Synthesis pathway of ligands

The $[PtCl_2(DMSO)(L)]$ **(1-3)** complexes were prepared by treating $[PtCl_2(DMSO)_2]$ with benzimidazoles (L) in 1:1 molar ratio in methanol. All ligands and complexes were isolated as air stable solids. The synthesis of complex **1** is given in the Scheme 2.



Scheme 2 : The synthesis of complex 1

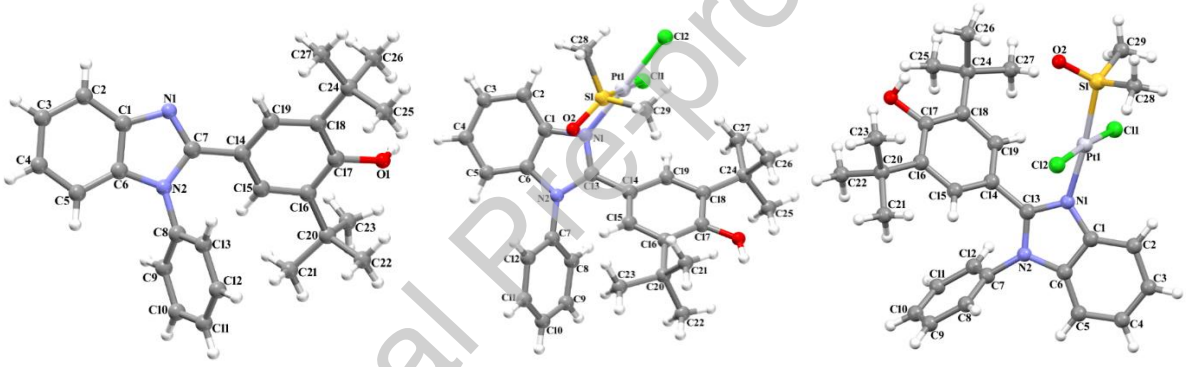
^1H NMR and ^{13}C NMR spectra of the ligands and their complexes were recorded in dmso-d_6 or $CDCl_3$ according to the solubilities. The solubility of complex **2** is very poor. Although the ^{13}C NMR spectrum of **2** was recorded overnight. The signals of the benzimidazoles in ^1H NMR spectra of the complexes have shifted downfield compared to the free ligands **[36].** This means that the charge in the benzimidazole is changing as a result of the coordination. Similar data results were found in the ^{13}C NMR spectra of the complexes. The ^{13}C data shows that the ligands have bound to the metal via imine nitrogen atoms. These results are in agreement with the similar complexes data **[36,37].** FT-IR spectra show that the υ(C=N) modes increased upon complexation as expected. $\upsilon_{\text{Pt-Cl}}$ values are shifted to the low frequency region according to the starting complex [$PtCl_2(DMSO)_2$] **[27]**.

The complex **1** was isolated as a cis complex from the reaction medium. The studies on growth of single crystals from chloroform / hexane have shown that the cis compound occurs in trans compound **(1a)** as well for two days. Yield of this compound was little, and solubility is higher than in the cis compound. But crystals **(1a)** were suitable for Single Crystal X-Ray determination.

3.2. Crystal Structures

The pale-yellow single crystals of $\mathbf{L_1}$ and yellow crystals of **1** and **1a** were obtained by the dichloromethane / hexane (1:3) and chloroform / hexane (1:3), respectively. Particular bond lengths and angels are shown in Table 2. Crystal structure of $\mathbf{L_1}$, **1** and **1a** are given in

Figure 1. Crystal structure of $\mathbf{L_1}$ is deviated from the planarity which C7-C14-N1 angle between benzimidazole moiety and 3,5-ditersiyerbutyl-4-hidroxyphenyl is 125^0. This deviation may be caused by the inter molecular hydrogen bonding. O- H-----N with D-A distance 2.746 A^0. The difference between the N1-C7 and N2-C7 bond lengths is significant. This is attributable to multiple bonds. In both complexes 1 and 1a, the geometries of platinum (II) ions are slightly distorted square planar geometry with N1-Pt1-CI2 angle (179.8^0(2)) and N1-Pt1-S1 angle 174.7^0(2)), respectively. N1-C13 multiple bonds in complexes **1** and **1a** are longer than the free ligand $\mathbf{L_1}$**.**This indicates that imidazole nitrogen bound to the Platinum**.** Pt-N=C moeities are bent with an average bond angles of 126.2^0(5) and 122.8^0(6), respectively. Pt-N bond lengths are nearly equavalent in these complexes, but they are shorter than Pt-N bonds in platinum –amine complexes [36].This shows electron donating ability of imines are greater than amines. DMSO ligand is bonded via S atom as expected. The Pt-N, Pt-CI and C-N bond lengths in **1** and **1a** are comparable to the literature values **[36,37,38] .**



(a) (b) (c)

Figure 1: Molecular structures of compounds $\mathbf{L_1}$ (a) , Complex **1(b)** and **1a (c)**

3.3 Biological Activity

3.3.1. Antimicrobial Activity

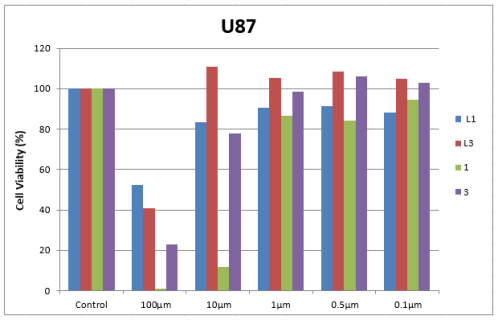
The antimicrobial activities were evaluated against several gram positive and gram negative bacteria as well as one yeast strain. The MIC values of the ligands and complexes are given

shown in Table 3 as well as MBC and MFC values in Table 4. All the ligands and complexes evaluated in this study showed antimicrobial effect against test microorganisms with MIC ranging from 62.5 µg/mL to 250 µg/mL (Table 3). MBC and MFC results of positive controls were found to be the same as MIC results. Looking at the values, it is seen that the ligands have moderate antimicrobial activity **[39,40,41]**. The activities of these compounds may be attributable to the benzimidazole ring. In particular, the $\mathbf{L_3}$ ligand has higher activity than others. This can be explained by the formation of a hydrogen bond through the pyridine – type nitrogen atom with the active centers of the cell **[4,37,38]**. The complexes have lower activity than the ligands. This result may have arised from the low lipophilicities of the complexes, meaning the penetration of the complexes and lipid membranes of the cells **[37,4].**

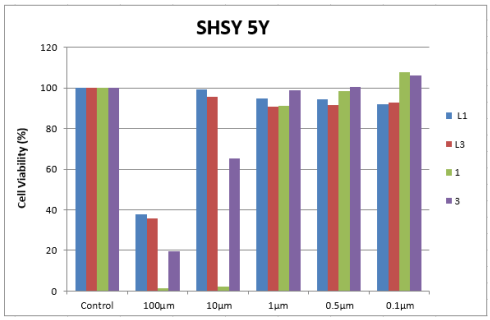
3.3.2. Cytotoxicity

To evaluate the potential usefulness of the synthesized compounds - ligands and complexes- as antitumor agents, two cell lines, U87 (Glioblastoma) and SHSY-5Y(neuroblastoma) were treated. Stock solutions of the ligand and complexes solutions were prepared with DMSO. The cytotoxic activity was evaluated after 72 h incubation with different concentrations of the ligand $\mathbf{L_1}$,$\mathbf{L_2}$, $\mathbf{L_3}$ and complexes **1, 2 , 3** and expressed as cell viability (% of control cells).The $\mathbf{L_1}$ and $\mathbf{L_3}$ ligands and their complexes have a higher toxicity against these cell lines than $\mathbf{L_2}$ and its complex. So we focused on ligands $\mathbf{L_1}$, $\mathbf{L_3}$ and complexes **1** and **3**. The measurements were performed at 0.1-0.5-1.0-10-100 µM and compared with cisplatin as a positive control (100µM). Antitumor activities of the compounds are given in Figure 2 . The complexes **1** and **3** have higher activities than the ligands against the studied cell lines. However, $\mathbf{L_1}$ and $\mathbf{L_3}$ ligands showed higher activity at lower concentrations than their counterparts in the literature **[42].**

Complex **1** has a IC_{50} value of 2.123 µM and 2.686 µM against the U87 and SHSY-5Y respectively (Table 5). These values for cisplatin are 5.7 µM and 18.6 µM, respectively **[43,44].** This shows that the activity of the complex 1 is comparable with the cisplatin. The values of complex **3** are 11.2 µM and 13.09 µM respectively. Our results showed that complex 1 has higher cytotoxic activity than complex 3 after 72 h incubation. When compared with the existing complexes in the literature, it has been observed that the complexes 1 and 3 are effective at much lower concentrations on the studied cell lines. **[19,20].**



(a)



(b)

Figure 2 : Effects of L_1 , L_3 , 1 ,3 on the progression of the cells of U87 and SHSY 5Y

Literature data suggesting that benzimidazoles containing substituents at the C2 position have activity on various cancer cell types are available [**45,46].** The cytotoxic activity of complex **3** should be due to the presence of a branched functional group in the C2 position. The solubility-enhancing effect of the branched functional group may be an important factor.

The cytotoxic activities of the complex **1** and complex **3** were determined on Vero cells in order to calculate the selectivity index (SI) values. The measurement results of cell viability are given in Figure 3 .The IC_{50} value of the complex **1** on the Vero cell line is 4.66 µM. Literature value for the reference drug cisplatin on Vero cells is 5.11 µM. According to this result, the SI value of the complex **1** for the U87 cell line is 1.73 , for SHSY-5Y is 2.19. Especially 2.19 value is approximately 8 times larger than that of cisplatin(SI = 0.27) (47). Although the SI values of complex 3 (IC_{50}13.9 = µM ,SI=0.57 for U87, IC_{50} =11.2 µM, SI=0.66 for SHSY-5Y) were better than that of cisplatin (SI=0.90 and 0.27 ,respectively) , the results have shown that the compound 3 could also damage healthy cells.

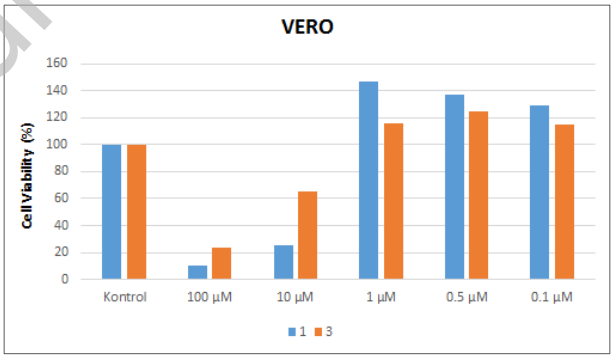


Figure 3 : Effects of complexes 1 and 3 on the progression of the cells of Vero.

An Investigation of the molecular mechanism of the cytotoxic effect of **1** on DNA was performed using the agarose gel electrophoresis. According to the result (Figure 4).Complex **1** has created little change in the mobility or resolution of plasmid DNA. It prevents

permanent dissolution of DNA, possibly due to the instability of complex **1** intercalation. As a result, it was understood that Complex **1** could not bind to DNA.This may suggest that the cytotoxicity of **1** is independent of the DNA damage and a possible mechanism should have a different path **[48].**

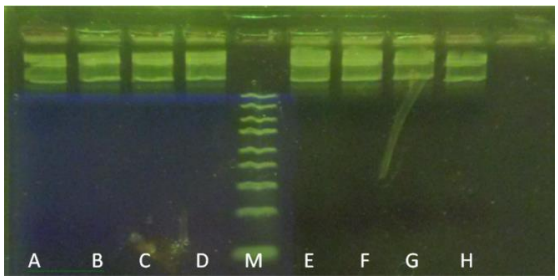


Figure 4. Complex- plasmid DNA interaction agarose gelelectrophoresis image in Tris-EDTA buffer. 1 mg complex was dissolved in 1mL DMSO. A: control (complex concentration zero); B-1:5, C-1:10, D-1:25, E-1:100, F-1:500, G-1:1000 diluted complex.

4.Conclusion

In the present study, three new benzimidazole - platinum(II) complexes were synthesized and their cytotoxic activities were evaluated. 2-substituted benzimidazoles and their platinum complexes have potencial as pro-drugs for glioblastoma and neuroblastoma cells. [In particular, complex 1 was found to have a much higher selectivity for U87 and SHSY-5Y cell lines than the reference drug cisplatin. High antitumor activity can be explained by the fact that it contains a bulky and pi-conjugated heterocyclic ligand[47].Our compounds are not bonding directly to the DNA, interaction mechanism should be different cis-Platin **[49]**. All the ligands and complexes are active in both types of bacteria. However, complexes were found to have lower activity than ligands.

Declaration of competing interests

None.

Supplementary Data

CCDC number for **L1**:1952965 and **1**: 1953224, **1a**: 1953223 contains the supplementary crystallographic data for compounds L1 , 1 and 1a respectively. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or by e-mail: deposit@ccdc.cam.ac.uk.

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Table 1. Refinement details of compounds $\mathbf{L_1}$, **1** and **1a**

	L_1	1	1a
Empirical formula	$C_{27}H_{30}N_2O$	$C_{29}H_{36}Cl_2N_2O_2PtS$	$C_{29}H_{36}Cl_2N_2O_2PtS$
Formula weight	398.53	742.65	742.65
Crystal colour	Pale , yellow	Yellow	Yellow
Crystal system	Tetragonal	Monoclinic	Triclinic
Space group	*P42$_1$c*	*P* 2$_1$/*n*	*P-1*
a,b,c (Å)	20.9960 (8) , 20.9960(8), 13.2999(5)	14.2007 (8) , 12.5822(8), 18.6309(12)	10.3779(9) , 12.4574(12), 13.4157(13)
α , β, γ (^{o})	90, 90, 90	108.246(2)	65.159(4) , 82.410(5) ,76.077(4)
Volume, Å^3	5863.0 (5)	3161,5 (3)	1526.7 (3)
Z	8	4	2
D_{calc} (Mg/m^3)	0.903	1.560	1.615
μ (Mo Kα) mm^{-1}	0.06	4.70	4.87
F (000)	1712	1472	736
Crystal size, mm	0.63 × 0.54 × 0.50	0.05 × 0.04 × 0.03	0.06 × 0.03 × 0.02
T(K)	296	296	296
λ (Mo Kα) Å	0.71073	0.71073	0.71073
θ angles,(^{0})	1.8 to 28	3.2 to 26.4	2.9 to 28.2
h, k, l limits	-27:26, -27:27, -12:17	-16:17, -15:15, -23:23	-13:13, -16:16, -17:17
Reflns : Total , uniq , R_{int}	34670 ,3645, 0.059	49356, 5131, 0.056	50088 ,6737, 0.059
Observed data [*I* > 2σ(*I*)]	3645	5131	6737
$T_{minimum}$, $T_{maximum}$	0.968 , 0.978	0.515 , 0.745	0.419 , 0.746
Data / restraints / parameters	6763, 39, 282	6432,2, 344	7548 , 2 ,353
Goodness-of-fit on F^2	0.824	1.197	1.288
Final R indices [I>2σ(I)]	R_1=0.0467 ,WR_2= 0.1002	R_1=0.0514 , WR_2= 0.0957	R_1=0.0797, WR_2= 0.1640
R indices (all data)	R_1= 0.0906 ,WR_2=0.1128	R_1=0.0707, WR_2= 0.1066	R_1=0.0905 WR_2= 0.1690
Largest diff. , peak hole (e Å^{-3})	0.21, -0.19	2.86 , -1.21	2.84 , -2.37

Table 2 Selected bond lengths and angles

BOND LENGTHS (Å)			BOND ANGLES (^{0})		
L1	1	1a	L1	1	1a

N1-C7	1.312(3)	N1-C13	1.322(9)	N1-C13	1.330(11)	N1-C7-C14	125.0(2)	C13-N1-Pt1	126.2(5)	C13-N1-Pt1	122.8(6)
N2-C7	1.375(3)	N2-C13	1.365(9)	N2-C13	1.360(12)	N2-C7-C14	123.1(2)	N1-Pt1-Cl1	89.14(18)	N1-Pt1-Cl1	87.2(2)
D—H···A (O1-H1···N1)		Pt1-Cl1	2,322(19)	Pt1-Cl1	2.316(3)	C7-C14-C19	120.7(2)	Cl1-Pt1-Cl2	90.67(9)	Cl1-Pt1-Cl2	175.39(14)
H···A	1.77(5)	Pt1-Cl2	2.305(2)	Pt1-Cl2	2.274(3)	N1-C7-N2	111.9(2)	N1-Pt1-S1	89.51(18	N1-Pt1-S1	174.7(2)
D···A	2.746(3)	Pt1-N1	2.031(6)	Pt1-N1	2.025(7)			S1-Pt1-Cl1	178.62(8)	S1-Pt1-Cl1	95.89(16)
D—H···*A*	168(5)	Pt1-S1	2.197(2)	Pt1-S1	2.198(3)			N1-Pt1-Cl2	179.8(2)	N1-Pt1-Cl2	88.9(2)

Table 3 MIC values (µg/mL) of the compounds.

Microorganisms	**MIC values (µg/mL) for the compounds and reference antimicrobial agents**							
Compound	**L_1**	**L_2**	**L_3**	**1**	**2**	**3**	**Gent.**	**FC**
E. coli ATCC 29998	125	62,5	62,5	250	125	125	15.6	-
S. aureus ATCC 25923	125	125	125	250	125	250	7.8	-
S. epidermidis ATCC 12228	125	125	62,5	250	125	125	7.8	-
E. faecium DSM 13590	125	125	125	250	125	125	15.6	-
E. faecalis ATCC 29212	125	125	125	250	125	125	15.6	-
S.typhimurium CCM 5445	125	125	62,5	250	125	125	7.8	-
P. aeruginosa ATCC 27853	250	250	250	250	250	250	15.6	-
C. albicans ATCC 10231	125	125	62,5	125	125	125	-	15.6

Gent: Gentamicin **; FC:**Flucytosine

Table 4 MBC and MFC values (µg/mL) of the compounds

Microorganisms	MIC values (µg/mL) for the compounds and reference antimicrobial agents							
Compound	**L_1**	**L_2**	**L_3**	**1**	**2**	**3**	**Gent.**	**FC**
E. coli ATCC 29998	125	125	62,5	250	250	250	15.6	-
S. aureus ATCC 25923	250	250	250	500	500	500	7.8	-
S. epidermidis ATCC 12228	125	125	62,5	250	250	250	7.8	-
E. faecium DSM 13590	125	250	125	250	125	250	15.6	-
E. faecalis ATCC 29212	250	125	125	500	125	250	15.6	-
S. typhimurium CCM 5445	125	250	125	250	250	250	7.8	-
P. aeruginosa ATCC 27853	500	500	250	500	500	500	15.6	-
C. albicans ATCC 10231	125	125	62,5	250	125	250	-	15.6

Gent: Gentamicin ; **FC:**Flucytosine

Table 5. IC_{50} (µM) Values of Compounds **L_1**, **L_3**, **1** and **3**.

	IC_{50} (µM)			
	L_1	**L_3**	**1**	**3**
U87	9,406	64,24	2,686	13,09
SHSY 5Y	19,07	18,62	2,123	11,2