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Research Article

Benzimidazole-Platinum Complex and Its Cytotoxic activity on U87 Cell Lines

Aydan Arı[1], Salih Günnaz[1], Sevil İrişli[1*]

[1]Department of Chemistry, Ege University, Bornova, İzmir, Türkiye

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Abstract

The aim of the study is to evaluate the synthesis, characterization and cytotoxic activities of a novel benzimidazole-platinum(II) complex that may have strong cytotoxic activity and low side effects. A benzimidazole ligand, 2-(3-phenoxyphenyl)-1-phenyl-1H-benzimidazole (L) and its novel platinum complex (Pt-L) was synthesized and characterized by different spectroscopic methods. In the study, firstly the activity of the synthesized complex in the U87 cancer line was examined and then it has been studied on healthy Vero lines.

Keywords: Benzimidazole, Benzimidazole-Platinum complexes, glioblastoma, cytotoxic activity.

Benzimidazol-Platin Kompleksi ve U87 Hücre Hatları Üzerindeki Sitotoksik Aktivitesi

Öz

Çalışmanın amacı, güçlü sitotoksik aktiviteye ve düşük yan etkilere sahip olabilen yeni bir benzimidazol-platin(II) kompleksinin sentezi, karakterizasyonu ve sitotoksik aktivitelerinin değerlendirilmesidir. Bir benzimidazol ligandı, 2-(3-fenoksifenil)-1-fenil-1H-benzimidazol (L) ve bunun yeni platin kompleksi (Pt-L), sentezlenmiş yapıları farklı spektroskopik yöntemlerle karakterize edilmiştir. Çalışmada, sentezlenen kompleksin öncelikle U87 kanser hattındaki aktivitesi incelenmiş, daha sonra sağlıklı Vero hatları üzerinde çalışılmıştır.

Anahtar Kelimeler: Benzimidazol, Benzimidazol-Platin kompleksleri, glioblastoma,sitotoksik aktivite.

1. Introduction

The imidazole nucleus, especially benzimidazole, and related structures are molecules whose structure and function are very important from a biological perspective. Benzimidazoles may be considered as structural isosters of nucleotides owing to the fused heterocyclic nuclei in their structures and potential activity for chemotherapeutic applications [1]. The benzimidazole moiety itself is a crucial pharmacophore in modern drug discovery [2]. It is common in drugs that show various pharmacological activities such as anti-inflammatory [3], histamine-H3 antagonist [4], antioxidant [5], gastroprotective [6], antitumoral [7], antiparasitic [8],

*Corresponding Author: sevil.irisli@ege.edu.tr
Aydan ARI, https://orcid.org/0009-0004-0768-2232
Salih GÜNNAZ, https://orcid.org/0000-0002-7422-6593
Sevil İRİŞLİ, https://orcid.org/0000-0002-3727-2216

antimicrobial [9], anthelmintic [10]. They are also found in insecticidal and herbicidal [11] drugs.

The importance of Pt(II) complexes increased with the discovery of cis platinum by Rosenberg in 1965 and its clinical use in the treatment of tumors with cytotoxic effects. For this reason, researchers have focused on the synthesis of new analogous platinum(II) coordination compounds.

Platinum complexes containing imidazolic/benzimidazolic ligands [12, 13] have drawn attention of several research groups, since imidazole/benzimidazole and their derivatives are important class of organic compounds. In the last decade, a lof of benzimidazol – platinum complexes synthesized and reported their antineoplastic activities on different cancer cell lines [14, 15, 16]. Brain tumors are an aggressive type of cancer seen in all age groups, including infants. Commonly known brain tumors are neuroblastoma and glioma. Central nervous system tumors occupy a larger place among childhood cancers and constitute approximately 20% of all tumors. One of the therapeutic applications is drug therapy and it is inevitable to develop agents with antiproliferative effects. One of the main topics of these studies is that they focus on the effect of substituents attached to the C-2 carbon atom on cytotoxic activity [17, 18, 19]. The limited number of coordination compounds effective on neuroblastoma and glioblastoma [20, 21, 22] 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.

In this study; A new benzimidazole ligand containing a 3-phenoxy group at the C-2 position and its platinum(II) complex with and cytotoxic effect on glioblastoma cancer cell line, U87 cell, was evaluated.

2. Materials and Methods

2.1. Chemicals

The reagents 3-phenoxybenzaldehyde, N-phenyl-o-phenylene diamine, sodium metabisulfite ($Na_2S_2O_5$), dimethylformamide (DMF), acetone, chloroform, dichloromethane (DCM), diethylether used in the synthesis were commercially purchased from Sigma, Merck, and Aldrich. *cis*-dichlorobis(dimethyl sulfoxide)platinum(II) [$Pt(DMSO)_2Cl_2$], used as starting complex, was synthesized according to literature data [23]. However, the following synthesis methods were used to increase the yield and can be synthesized according to literature.

2.2. Instrumentations

Melting points were obtained using an Electrothermal Melting Point detection apparatus. Elemental analysis were performed by the Scientific Resarch and Analysis Laboratories of Inönü University. Infrared spectra were recorded on a Perkin Elmer FT-IR spectrophotometer in the range of 4000- 400 for ligand, 4000- 200 cm^{-1} for complex. ^1H and ^{13}C NMR spectra were measured at Varian AS 400 MHz spectrometer. $CDCl_3$ and DMSO-d_6, were used as the solvents and TMS was used as the internal standard. In thin layer chromatography (TLC) studies, ready-made plates coated with Kieselgel 60 F254 with a thickness of 0.2 mm were

used. Stains were detected using a UV lamp. The LC/Q-TOF/MS measurements in the compound were recorded using the Agilent 6550 QTOF LC/MS device at the Ege University MATAL center.

2.3. Synthesis of Ligand and its Platinum Complex

2.3.1. 2-(3-phenoxyphenyl)-1-phenyl-1H-benzimidazole (L)

3-phenoxybenzaldehyde (0.1075 g, 0.542 mmol) was kept in 2 ml DMF medium in a schlenk. Then, N-phenyl-ortho-phenylenediamine (0.998 g, 0.542 mmol) was added dropwise in 1 ml DMF medium and $Na_2S_2O_5$ was added. The reflux temperature was stirred for 6 hours. As a result of the reaction, cold water was added. The precipitated cream-colored solid was filtered with the aid of a nuclei funnel. This solid was crystallized from DCM/diethyl ether to give the pure product. Yield: 60%, Melting point: 141-142 ºC. FT-IR (KBr disk, cm^{-1}): υ Ar-H –C=N: 3398-2915. ^{1}H-NMR (400 MHz, $CDCl_3$) δ ppm:7.87 (dt, 1H, J_1=8.4Hz, J_2=0.8 Hz, Ar-*H*), 7.49-7.00 (m,15H, Ar-*H*), 6.86 (d, 2H, *J*=7.6 Hz, Ar-*H*). ^{13}C -NMR (100 MHz, $CDCl_3$) δ (ppm):157.2, 156.5, 151.8, 142.9, 137.2, 136.8, 131.6, 129.9, 129.8, 128.6, 127.3, 124.3, 123.5, 123.1, 119.9, 119.3, 119.1, 110.5. Elemental analysis (%) = ($C_{25}H_{18}N_2O$) (m.w.= 362,43); calculated; C, 82.85; H, 5.01; N, 7.73; found; C,81.80; H, 4.71; N,7.52.

2.3.2. Synthesis of Complex (Pt-L)

L ligand (0.03 g, 0.082 mmol) was dissolved in 2 ml chloroform medium in a schlenk. Then, the suspension of $Pt(DMSO)_2Cl_2$ (0.03 g, 0.082 mmol) prepared in 1ml chloroform medium in a test tube was added dropwise onto L. The mixture was then stirred at room conditions for 4 days. After the reaction, the solvent was dried under vacuum. The resulting solid was first washed with acetone. The insoluble portion was separated. The solvent was evaporated in vacuo and washed with diethyl ether. Yield: 72% Melting Point: 232^{0}C.

FT-IR (CsI disk, cm^{-1}): υ Ar-H = 3398-2915, υ S-O =1121, υ Pt-CI =319.13

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 8.41(d, 1H, *J*=8.4 Hz, Ar-*H*), 8.03 (d, 1H, *J*=7.6 Hz, Ar-*H*), 7.56-7.45 (m, 5H, Ar-*H*), 7.37 (t, 2H, *J*=7.8 Hz, Ar-*H*), 7.31-7.20 (m, 7H, Ar-*H*), 7.11(t, 1H, *J*=6.8 Hz, Ar-*H*), 6.83 (d, 2H, *J*=8.8 Hz, Ar-*H*), 3.34 (s, 3H, O-S-CH_3), 2.70 (s, 3H, O-S-CH_3). ^{13}C-NMR (100MHz, $CDCl_3$) δ (ppm): 157.0, 155.7,151.6, 139.5, 134.5, 130.2,130.1,130.0, 129.5,129.0, 127.2, 126.3, 125.6, 125.1, 124.0, 121.5, 120.7, 119.4,119.0, 111.3, 77.0, 76.6, 44.5, 43.8. Elemental analysis (%)= ($C_{27}H_{24}Cl_2N_2O_2PtS$) (706.53); calculated; C, 45.90; H, 3.42; N, 3.96; found; C, 45.73; H, 3.48; N, 4.02.

2.4. Cytotocxicity

Cytotocxicity tests were obtained by using U87 and Vero cells. Cells were purchased from ATCC, USA. Cells were 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 complex was added at different concentrations (0.1-0.5-1.0- 10- 100 µM) of this media and

incubated for 72 hours. Growth inhibitions of cells were measured spectrophotometrically using a standard method (MTT) at 570 nm.

3.Results and Discussion

3.1. Synthesis and spectroscopic studies

Benzimidazole derived from N-phenyl-ortho-phenylenediamine were synthesized. 2-(3-phenoxyphenyl)-1-phenyl-1H-benzo[d]imidazole(L)) ligand and Benzimidazole-Pt complex derived from this ligand were synthesized. The structures of the ligand and metal complex were elucidated using various spectroscopic methods (FT-IR, ^{1}H-NMR, ^{13}C-NMR) and elemental analysis. The general synthesis route of ligand is given in Figure 1.

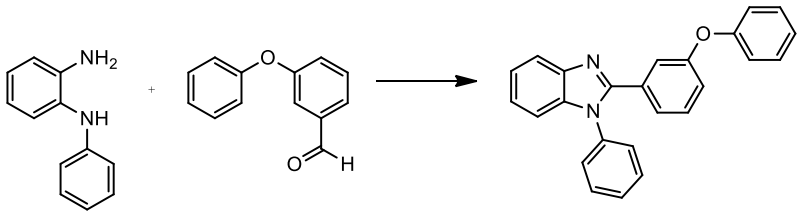

Figure 1. Synthetic pathway of Ligand L.

FT-IR spectrum of L (Figure 2) was obtained by preparing KBr pellets. Only aromatic groups are present in the L ligand, the ring bodies appear in the 700-800 cm^{-1} region. The disappearance of the peak of secondary N-H stretching vibrations, which are seen as a double peak in the 3300-3400 cm^{-1} region, indicates that the reaction has occurred to form a ligand L. Peaks related to aromatic C-H stretching vibrations appear in the spectrum above 3000 cm^{-1}. ^{1}H-NMR spectrum of L ligand was obtained by dissolving in $CDCl_3$ medium. In the ^{1}H-NMR spectrum (Figure 3), the peak of N-C=CH is observed at 7.88 ppm, while peaks corresponding to all other protons are observed at 7.49-6.85 ppm. Again, in the ^{13}C-NMR spectrum (Figure 4), there are peak groups depending on the structures of different C atoms. The C2 carbon (between two nitrogen atoms) of the imidazole ring is observed at 142.87 ppm. The carbons to which the oxygen atom of the functional group attached to the C2 carbon is attached were observed at 156.48 and 157.20 ppm.

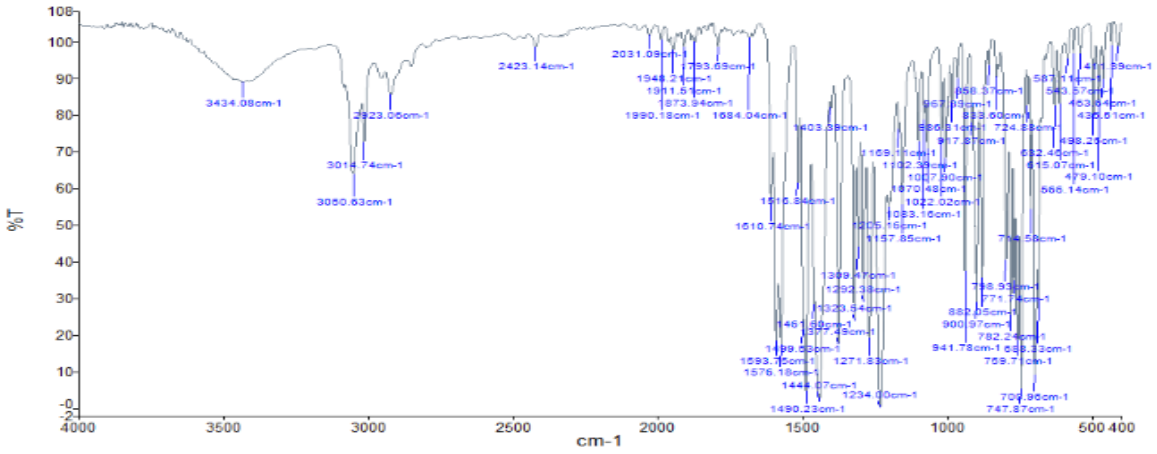

Figure 2. FT-IR Spectrum of the Ligand L.

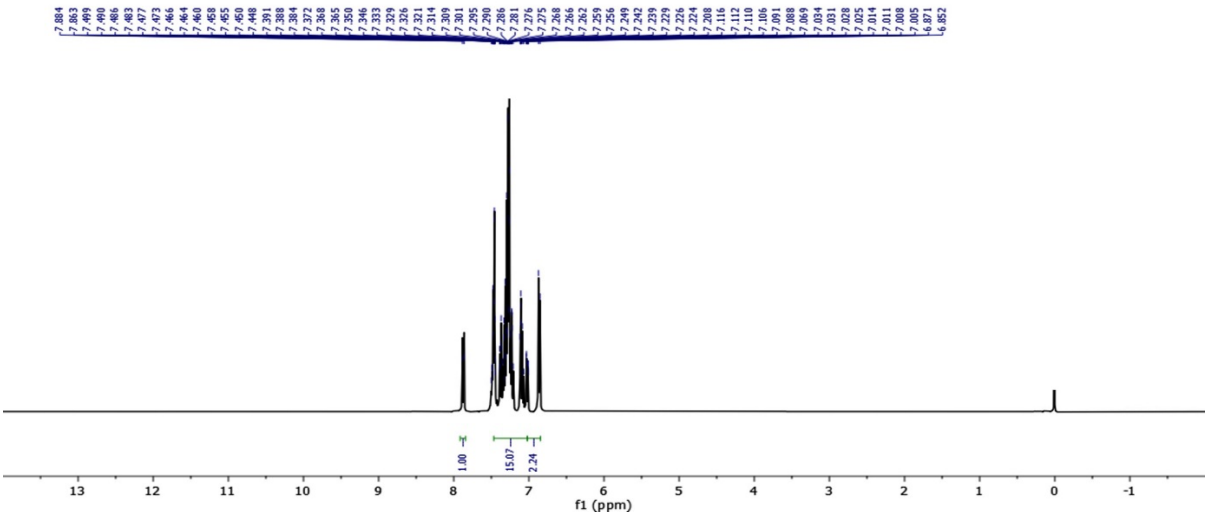

Figure 3. ^{1}H NMR Spectrum of the Ligand L.

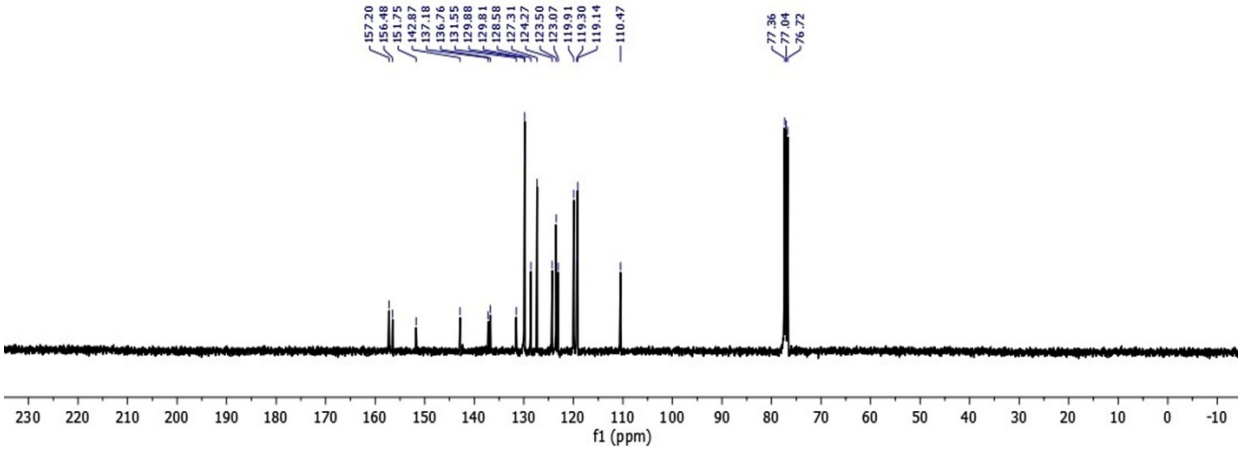

Figure 4. ^{13}C NMR Spectrum of the Ligand L.

[$PtCl_2$(DMSO)(L)] complex were prepared by treating [$PtCl_2(DMSO)_2$] with benzimidazole (L) in chloroform in a 1:1 molar ratio. Ligand and complex have been isolated as stable solids in air. Complex syntheses is given in Figure 5.

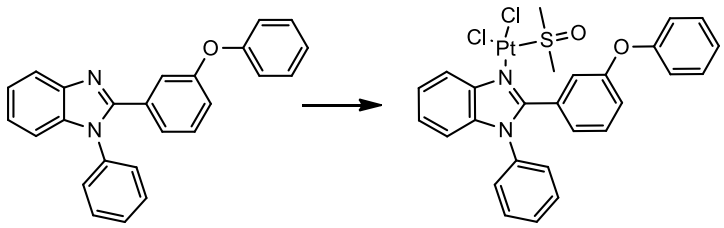

Figure 5. Synthetic pathway of complex (Pt-L).

FT-IR spectrum (Figure 6) of the complex was taken by creating a CsI pellet. Pt-Cl stretching vibrations appear to be shifted to the weak area compared to the initial complex. The S-O stretching vibration of the DMSO group bonded via S is observed at 1121 cm^{-1} [23]. The *cis* Pt-Cl stretching vibrations shifted according to the initial complex and, as expected, a peak containing a shoulder was observed at 319.13 cm^{-1}. The ^{1}H-NMR spectrum of the complex is given in Figure 7. In the spectrum, the defining peak is the methyl protons of the dmso group. These peaks are located at 2.70 ppm and 3.34 ppm as supporting the cis structure of complex. Again, in the ^{13}C-NMR spectrum (Figure 8), carbons of this group are observed in the range of 44.6-43.8 ppm. No significant shift is observed in the peaks of the carbon atoms of the ligand in the complex, as expected. The result of the elemental analyses is suitable with the theoretical value [24].

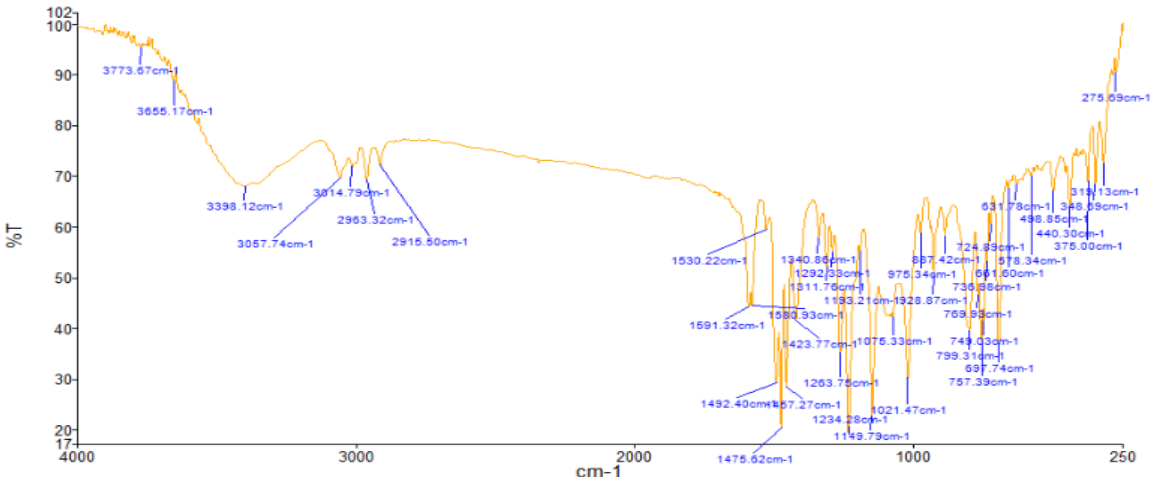

Figure 6. FT-IR Spectrum of the complex (Pt-L)

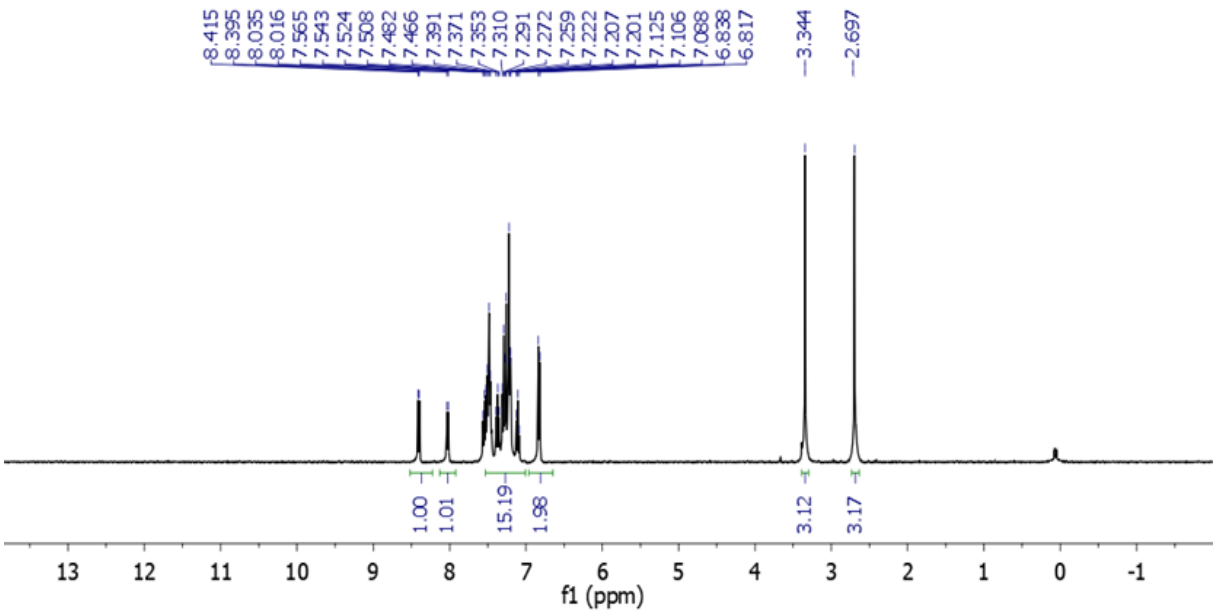

Figure 7. ^{1}H NMR Spectrum of the complex (Pt-L)

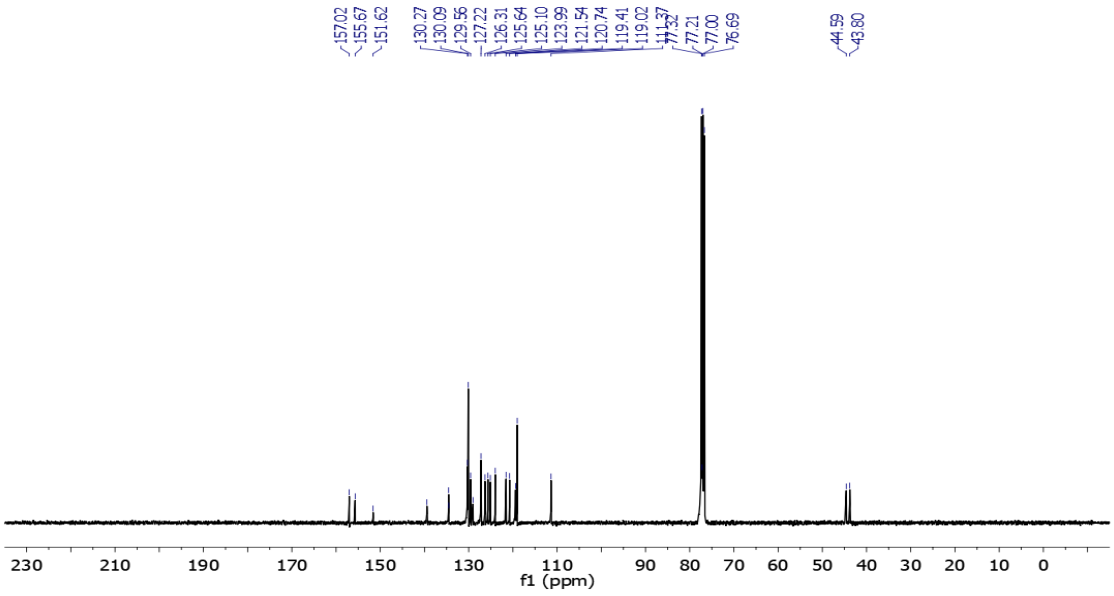

Figure 8. ^{13}C NMR Spectrum of the complex (Pt-L).

3.3 Biological Activity

The results of the cytotoxicity of complex Pt-L on U87 and healthy Vero cells are given in Figure 9. Five different concentrations were tried for all measurements. Toxicity measurements of the complex (Pt-L) on Cancer cell U87 show an IC_{50} value of 31.45 µM. For healthy Vero cells, this value is 9.43 µM (Table 1). Considering the cytotoxic activity of the complex synthesized in the U87 cell line, it is important to calculate selectivity index (SI) values to determine its selectivity and potential inducing anticancer agent compared to the complexes and the cancer drug cis-platinum. These two results show that the complex has no effect on the

cancerous cell. For cis-Pt, which is also widely used in cancer chemotherapy, the IC_{50} values for its effect on these two cell lines are 5.70 and 5.11 µM respectively [25, 26, 27].

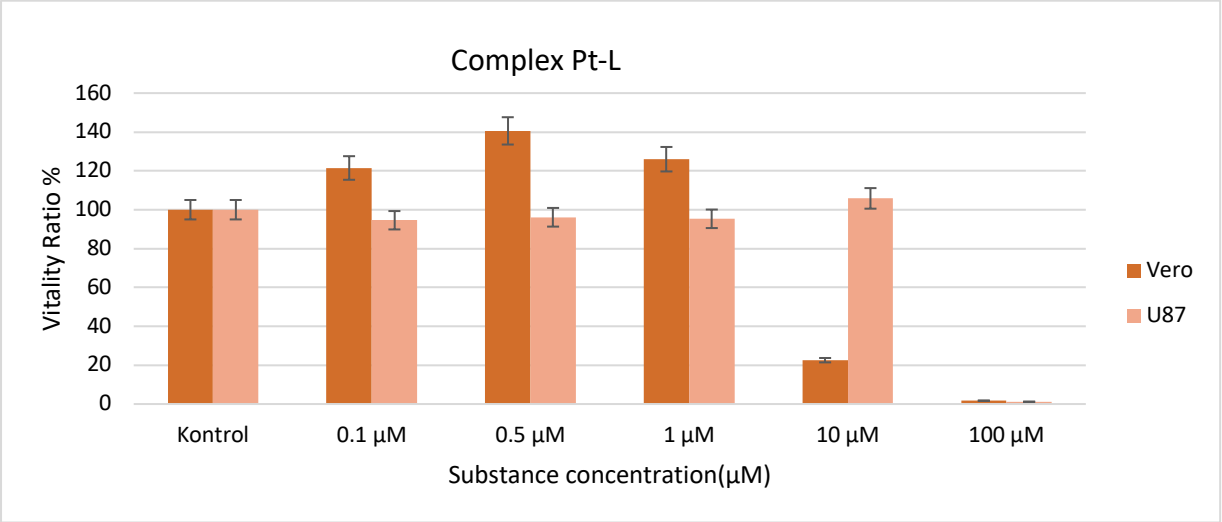

Figure 9. Cytotoxicity control of complex Pt-L on U87 and healthy Vero cells

Table 1. IC_{50} (µM) and Selectivity (SI) Values of Complex (Pt-L)

	IC_{50} (µM) (SI)	
	Complex (Pt-L)	**Cis platin**
U87	31.45**(0.29)**	5.70 (0.90) [25, 26]
VERO	9.43	5.11[27]

(SI = Healthy cell IC_{50} / Diseased cell IC_{50})

The fact that the complex had no activity on the cancer cell showed that the presence of the aromatic group at the C2 carbon did not make a special contribution.

4. Conclusion

In this study, a new benzimidazole ligand and metal complex were synthesized, and their structures were elucidated using spectroscopic methods. The aim of the study was to determine the cytotoxic activity of the synthesized complex on the U87 cell line, a type of glioblastoma cell. This is particularly important as there are limited studies on the activity of complexes against glioblastomas, which are aggressive brain tumor cells, especially prevalent in children. In this context, the activity of the complex on healthy Vero cells was also compared. The cytotoxicity of the synthesized Pt-L complex was found to be low for U87 cells and high for Vero cells. Therefore, the complex was not found to be promising for brain tumor Glioblastoma cancer chemotherapy.

Ethics in Publishing

There are no ethical issues regarding the publication of this study.

Author Contributions

Aydan Arı: Conducted the experiment.

Salih Günnaz: Interpreting spectroscopic studies, writing publication.

Sevil İrişli: Determining the topic, managing the study, interpreting, and writing publication.

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