Synthesis and Biological Activity of a New Pt(II) Complex Involving 4-bromo-2,6-bis-hydroxymethyl-phenol and Nicotinamide

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ABSTRACT: *In the present study, a Pt(II) complex including 4-Bromo-2,6-bis-hydroxymethyl-phenol (BBHMP) and nicotinamide (NA) was synthesized and structurally analyzed by using spectral and thermal analysis methods. BBHMP and its Pt(II) complex in the presence of BBHMP and NA were investigated for their antimicrobial, cytotoxicity, gene expression, and antioxidant properties. The antimicrobial activity results showed that the platinum complex displayed a significant effect against Staphylococcus aureus and Candida albicans. The cytotoxicity of BBHMP and platinum complex were determined against human prostate adenocarcinoma (DU145) and breast (MCF7) cancer cell lines by applying the MTT assay. Cytotoxicity results suggested that the Pt(II) complex exhibited moderate cytotoxicity against the growth of these cancer cell lines when compared with the reference drug cisplatin is more effective than free BBHMP and NA. Gene expression results proved that the Pt(II) complex is a special bioactive chemical constituent and potential anticancer agent. The results obtained showed that the complex had highly inhibitory effects on gene expression. In addition, Pt(II) complex also displayed effective antioxidant activity.*

KEYWORDS: *Platinum(II); Bioactive compounds; Structural analysis; Bioactivity.*

INTRODUCTION

Multidentate ligands can easily form stable complexes with most transition metal ions [1-3]. These complexes have potential applications in inorganic, biological, medicinal and environmental chemistry. 4-Bromo-2,6-bis-hydroxymethyl-phenol (BBHMP) is a chemosensor for F^- ions in aqueous media [4] and a colorimetric method for the detection of F with BBHMP was described [5]. BBHMP, a multidentate ligand [6], is a halogenated phenol derivative that is obtained from 4-bromophenol, formaldehyde and sodium hydroxide and used for proteomic research. It has three oxygen atoms that can be coordinated to metal ions to form metal complexes [7-9]. In previous studies, copper(II), palladium(II), and gold(III) complexes were synthesized using BBHMP and characterized with various spectrophotometric techniques, and also thermodynamic parameters were determined. In these studies, the complexes were found to be active against *Staphylococcus faecalis* and *Escherichia coli* when their biological applications were investigated.

Nicotinamide (pyridine-3-carboxamide, NA) is a significant constituent of hydrogen-carrying co-enzymes such as nicotinamide-adenine dinucleotide. NA is a form

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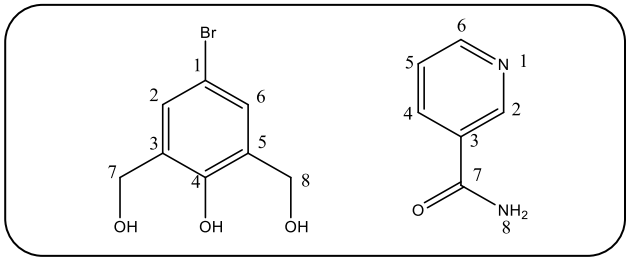


Scheme 1: Chemical structures of BBHMP and NA.

of vitamin B3 found in food and it plays a vital role in the metabolism of all living cells. It is used as a cream to treat acne and orally to prevent and treat pellagra. The presence of the pyridine ring, which is inherent in numerous nicotinamide compounds, is also of interest. Nicotinamide was used in many studies due to its chemical structure. Nicotinamide is a biologically active reagent that has significant antimicrobial, cytotoxicity, and anticancer activities [10-12]. So far, many studies reported the synthesis and biological properties of metal-based nicotinamide compounds [13-16]. For example, *Dilip et al.* [14] studied the synthesis, spectral analysis, biological and DNA cleavage properties of various transition metal complexes of nicotinamide. In another study by *Tella et al.* [15], Ni(II) nicotinamide complexes involving fumarate dianion and fumaric acid were synthesized to reveal their biological activities. Structurally, nicotinamide contains an aromatic ring and only the N1 atom interacts with the metal cation [16-20]. The chemical structures of BBHMP and NA are below:

Up to now, no references to the biological activity of a Pt(II) complex involving the multidentate ligand BBHMP and NA has been reported. Therefore, it is important to examine the biological activity that plays an important role in various chemical and biological fields. For these reasons, in the present study, the platinum(II) complex of BBHMP and NA was synthesized. The complex was characterized by using spectral and thermal analysis methods. The BBHMP and Pt(II) complex were evaluated for antimicrobial activities against *E. coli*, *S. typhimurium*, *L. monocytogenes*, *S. aureus*, *B. Cereus*, and *C. albicans*. A ampicillin and amphotericin B were used as antibiotics and the obtained results were compared with these antibiotics. The BBHMP and platinum(II) complex were also examined for cytotoxicity (with cisplatin used as a comparison) and gene expression against human prostate adenocarcinoma (DU145) and human breast (MCF7) cancer cell lines. In addition, the antioxidant activity of BBHMP, NA and the complex were determined.

EXPERIMENTAL SECTION

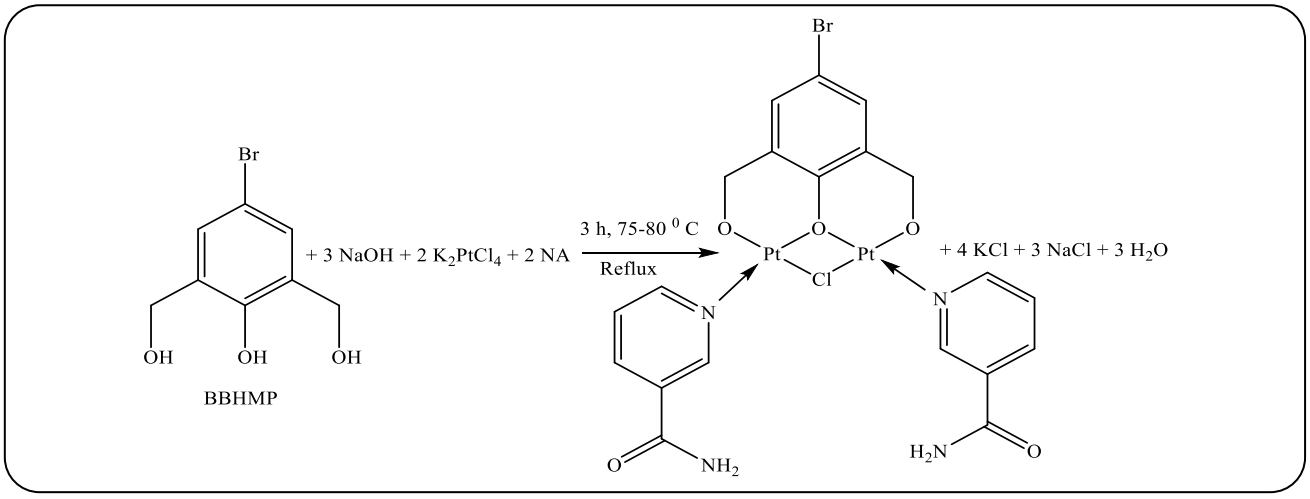
Material and measurements

All chemicals were purchased from various firms and used as supplied. Elemental analysis for carbon, hydrogen, oxygen, and nitrogen was measured by a Costech ECS 4010 CHNSO element analyzer, and an ICP-MS 7700X (Agilent) element analyzer was used for platinum. Conductivity analyses were calculated with an Inolab Thermal 740P in DMF. The magnetic moment experiments were obtained with a MK-1 Sherwood scientific magnetic susceptibility balance. Electrospray ionization mass spectra (ESI-MS) were determined on an Agilent 6400 Series Triple Quadrupole. Electronic spectra in the UV-Visible region were observed by using a Shimadzu UV-1700 Pharma spectrophotometer at room temperature. A Shimadzu FTIR-470 spectrometer was used for recording infrared spectra (4000-400 cm^{-1}) of solid samples in KBr pellets. ^1H NMR spectra were performed in $CDCl_3$ for the complex on a Bruker, DPX-400 spectrometer. The XRD powder pattern was recorded on a Shimadzu XRD-6000. The TG/DTA curves were created on a Seiko Exstar TG/DTA 6200 thermal analyzer at a temperature range of 25-1000 °C.

Synthesis of Pt(II) complex

Amounts of 0.116 g (1 mmol) BBHMP, 0.06 g (3 mmol) NaOH, and 0.121 g (2 mmol) NA were dissolved in 25 mL ethanol. An equivalent amount of solution of 0.83 g (2 mmol) K_2PtCl_4 was added and heated for 3 hours at ~80 °C under reflux conditions. A brown solid precipitate was obtained which dissolved in organic solvents such as DMF, DMSO, and $CHCl_3$.

$[Pt_2(BBHMP)(NA)_2Cl]$ or $[Pt_2(C_8H_6O_3Br)(C_6H_6N_2O)_2Cl]$: Yield (%): 78. Colour: Brown. MP. (°C): 203. Elemental Analysis (%): Calcd.: C 26.68, H 2.0, O 8.89, N 6.22, Pt: 43.37; Found: C 26.64, H 2.02, O 8.83, N 6.19, Pt: 43.34. UV-Vis. (nm): 278 (ε = 0.672 x 10^3 $M^{-1}cm^{-1}$), 362 (ε = 0.582 x 10^3 $M^{-1}cm^{-1}$), 417 (ε = 0.411 x 10^3 $M^{-1}cm^{-1}$). FTIR (cm^{-1}): 3352 $\nu(NH_2)$, 1698 ν(C=O), 1621 ν(C=N), 1525 ν(C=C), 1278 ν(C-N), 1231 ν(NHdef), 673 ν(Pt-O), 585 ν(Pt-N). ^1H NMR (300 MHz, $CDCl_3$) δ: 6.72 (s, 2H, C_2-H and C_6-H), 9.12 (s, 2H, C_2-H), 8.36 (m, 4H, C_4-H and C_5-H), 8.68 (d, J = 6 Hz, 2H, C_6-H), 4.37 (s, 4H, C_7-2H and



Scheme 2: Synthesis reaction for the Pt(II) complex in the presence of BBHMP and NA.

C_8-2H), 7.49-7.55 (m, 4H, NH_2). Conductivity ($\Omega^{-1}cm^2mol^{-1}$): 17.92. ESI-MS (*m/z*): For $[M_{BBHMP}]^+$, calcd.: 229.97, found: 230; for $[M_{NA}+H]^+$, calcd.: 122.98, found: 122 and for $[M]^+$, calcd.: 899.62, found: 899.66. According to the following synthesis reaction:

Antimicrobial activity

The antimicrobial activities of the BBHMP and platinum complex were tested against gram (-) *Escherichia coli* (*ATCC® 25922™*), *Salmonella typhimurium (ATCC®* 14028™), *Listeria monocytogenes (ATCC®* 19115™) gram (+) *Staphylococcus aureus* (*ATCC® 25923™*), *Bacillus cereus* (*ATCC®* 11778™), and fungal *Candida albicans* (*ATCC®* 1023™). The micro broth dilution method and Clinical and Laboratory Standards Institute (CLSI) [21-30] procedures were used to find the minimal inhibition concentration values (MIC). To measure the area for the zone of inhibition, ampicillin for bacterial strains and amphotericin B for fungal strains were chosen as antibiotics (positive control). DMSO, which has no activity, was used as a negative control. The tested micro-organisms were prepared in the concentration range 200 and 6.25 µg/mL and added into a sterile 96-well microplate. Then, the microplate was kept in incubation for the growth of testing micro-organisms for 24 hours at 37 °C. After incubation, the absorbance was recorded at 600 nm using a Thermo Multiscan GO Microplate Reader Spectrophotometer. Any color change from purple to pink determined visually was recorded as positive. The lowest concentration with a color change was considered to be the MIC value. Experiments were conducted four times.

Cell culture

Human prostate adenocarcinoma (DU145, ATCC®HTB-81™) and breast (MCF7-ATCC®HTB-22™) cancer cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) : F-12 medium and 10 % foetal bovine serum (FBS, Gibco™ 10270106), 2 mM glutamine (Multicell, USA) and 100 IU/mL penicillin-streptomycin (Invitrogen, Life Technologies). Cells were stored in a humidified incubator at 37 °C and 5 % CO_2.

MTT analysis for cytotoxicity

Cytotoxicity was determined by MTT assay [27, 31-35]. The MTT method is a widely used cell viability test for cytotoxicity and can be used with all cancer cell types because it metabolizes all living cells. In this method, both DU145 and MCF7 cancer cell lines were added to a sterile 96-well microplate by using a culture medium (7500 cells/well in 200 µL) for 24 hours. A hemocytometer with trypan blue dye was used to determine cell viability and 95 % cell viability was confirmed. To adhere cells to the wells, the microplate was incubated in a 5 % CO_2 incubator for 24 hours at 37 °C. Then, the BBHMP or platinum(II) complex prepared at the concentrations of 25, 50, 100, 200, 400, and 800 µM was transferred to the wells and incubated for 24 hours. After 24 hours, 20 µL / 200 µL per well was transferred from 5 mg/mL MTT (3- (4,5-dimethyl-thiazol-2-yl) -2,5-diphenyltetrazolium bromide) solution and kept in incubation at 37 °C for an additional 4 hours. During incubation, MTT was converted to a colored formazan salt that is insoluble in water by the metabolic activity of living cells. The formazan blue occurring

in the cell lines was dissolved in DMSO (200 µL/well). A Thermo Multiscan GO Microplate Reader Spectrophotometer (492 nm) was used to measure the optical density. The measured absorbance value for each concentration of BBHMP or platinum complex was compared with the DMSO-treated control. All experiments were repeated six times. The percentage growth inhibition was found with the following formula [36]:

$$\text{Growth inhibition\%} = \frac{\text{OD control} - \text{OD treatedsample}}{\text{OD control}} \times 100 \quad (1)$$

IC_{50} levels were obtained by probit analysis using SPSS 20 software and compared with the reference drug cisplatin. As a result of probit analysis, there were sufficient IC_{50} values in the BBHMP and Pt(II) complex for anticancer activity. Therefore, gene expression analysis of BBHMP and Pt(II) complex was performed for anticancer activity.

Gene expression analysis

Total RNA was isolated from three wells in six cell culture well plates from each group treated with IC_{50} dose of BBHMP or Pt(II) complex and vehicle-treated control using the PureLink® RNA mini kit (Life Technologies, USA) according to the manufacturer's instructions. The Qubit® Fluorometer (Thermo Scientific, USA) was used to record extracted RNA concentrations. The total RNA concentration was adjusted to 100 ng/µL with a high-capacity complementary DNA (cDNA) reverse transcription kit (Life Technologies, USA) for the synthesis of the first strand of cDNA. cDNA synthesis was completed with the thermal cycler Applied Biosystems® Veriti® (Thermo Scientific, USA) at the three steps which were Step 1: 25 °C, 10 min; Step 2: 37 °C, 120 min and Step 3: 85 °C, 5 min, respectively. The synthesized cDNA was kept at -20 °C for later analysis steps [27, 33, 37-41]. Expression levels of the mitochondrial apoptosis genes in both cancer cells of vehicle-treated control and treated with BBHMP or Pt(II) complex. IC_{50} doses for 24 hours were determined with quantitative real-time-polymerase chain reaction (qRT-PCR) by using the PowerSYBR® Select Master Mix (Life Technologies, USA) with an ABI 7500 Real-Time PCR system (Thermo Scientific, USA). Genes, primer sequences, and PCR conditions are presented in Table 1**.** The gene expressions were found as relative fold change compared to vehicle-treated control and normalized with glyceraldehyde-3-phosphate dehydrogenase messenger RNA (GAPDH mRNA) expressions. The expression levels of mRNAs were examined by using the comparative cycle threshold (2-ΔΔCt) method (User Bulletin 2, Applied Biosystems, CA). All experiments were performed four times.

Antioxidant activity

Antioxidants are free radical scavengers and the DPPH (2,2-Diphenyl-1-picrylhydrazyl) assay is a simple and rapid method widely used to determine the free radical scavenging capacity of different antioxidants [27, 42-47]. The radical in DPPH gives a purple color with maximum absorption at 517 nm. When the radical reacts with an antioxidant, the absorbance of DPPH decreases and the color disappears [48]. In this study, different concentrations of BBHMP, NA, and platinum(II) complex were prepared by serial dilution in $CHCl_3$ at a concentration range of 0.5 to 2 µg/ml. The reaction medium composition was DPPH radical (0.5 µg/mL, 100 µL), and test components dissolved in 100 µL $CHCl_3$. BHT (2,6-di-tert-butyl-4-methylphenol) in MeOH at a concentration range of 0.5-2 µg / mL was used as a positive control. $CHCl_3$ and MeOH were chosen for the blanks. These were then incubated in the dark for 1 hour, and the IC_{50} (50 % inhibition of DPPH color) values were calculated by measuring the absorbance against a blank at 517 nm with a Tecan-PC endless M200 Pro Plate reader. Experiments were performed in duplicate. Antioxidant activity % was found using the following formula:

$$\text{Antioxidant\%} = \frac{\text{Ablank} - \text{Asample}}{\text{Ablank}} \times 100 \quad (2)$$

Where *Ablank* is the absorbance of the blank and *Asample* is the absorbance of the sample.

RESULTS AND DISCUSSION

Structural analysis

Elemental analysis of C, H, O, N, and Pt complied with the proposed molecular formula complex. The molar conductance of the platinum(II) complex can be found by using the formula [49]:

$$\text{Am} = \frac{\text{K}}{\text{C}} \quad (3)$$

Table 1: Genes, primer sequences and PCR conditions for qRT-PCR.

	Mitochondrial apoptosis signalling	
Genes	Primer sequences	PCR conditions
P53 F 5’	CACGAGCGCTGCTCAGATAGC 3’	1cycle of 2 min at 50 ℃
R 5’	ACAGGCACAAACACGCACAAA 3’	and 10min at 95°C for 15s,
BCL2 F 5’	ATGTGTGTGGAGAGCGTCAA 3’	annealing and extension
R 5’	ACAGTTCCACAAAGGCATCC 3’	at 60 °C for 1 min
BAX F 5’	TTCATCCAGGATCGAGCAGA 3’	
R 5’	GCAAAGTAGAAGGCAACG 3’	
APAF1 F 5’	GATATGGAATGTCTCAGATGGCC 3’	
R 5’	GGTCTGTGAGGACTCCCCA 3’	
Cyt-C F 5’	AGTGGCTAGAGTGGTCATTCATTTACA 3’	
R 5’	TCATGATCTGAATTCTGGTGTATGAGA 3’	
Caspase 3 F 5’	GGTATTGAGACAGACAGTGG 3’	
R 5’	CATGGGATCTGTTTCTTTGC 3’	
GADPH F 5’	TTGGTATCGTGGAAGGACTCA 3’	
R 5’	TGTCATCATATTTGGCAGGTTT 3’	

where C is the molar concentration and K is the specific conductance. For the molar conductance, the complex was dissolved in 1 x 10^{-3} M DMF solvent at room temperature. It was measured and found to have a molar conductance value of 17.92 $ohm^{-1}mol^{-1}cm^2$. As a result, the obtained complex is a non-electrolyte complex [50, 51]. According to the magnetic moment measurement, the synthesized Pt(II) complex is diamagnetic as expected in low-spin platinum(II) complexes, and has a square planar geometry.

ElectroSpray İonization Mass Spectrometry (ESI-MS) was recorded to specify molecular weight and fragments of the complex. In the ESI-MS spectra of the platinum complex (Fig. 1), the peaks at *m/z* = 229.97 (found: 230) and 122.98 (found: 122) were compatible with $[M_{BBHMP}]^+$ and $[M_{NA}]^+$, respectively. The peak at *m/z* = 899.62 (found: 899.66) corresponds to the molecular weight ($[M]^+$) of the molecular formula of the complex among several molecular weights recorded based on the isotope distribution of platinum. In addition, the spectrum shows isotope peaks at *m/z* 654 and 656, which correspond to the cation resulting from the removal of NA molecules from the complex.

In the electronic spectra of the Pt(II) complex in $CHCl_3$ between 200 and 450 nm, there is a wide range of bands resulting from both π-π* (-C=N, -C=O) and n-π* transitions (long pair electrons on the O and N atoms). The complex exhibits three absorption bands in the range 417 (ε = 411 l mol^{-1} cm^{-1}), 362 (ε = 582 l mol^{-1} cm^{-1}) and

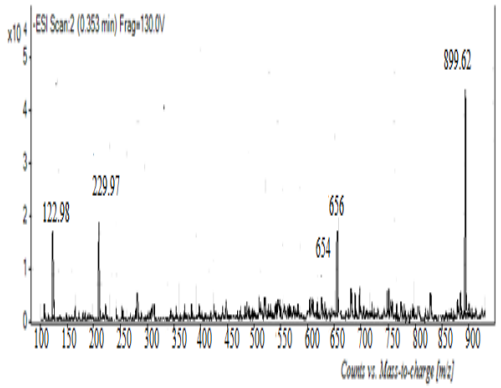


Fig. 1: ESI-MS spectra of the Pt(II) complex in CH_3OH.

278 nm (ε = 672 l mol^{-1} cm^{-1}) which were attributed to $^1A_{1g} \rightarrow {}^1A_{2g}$, $^1A_{1g} \rightarrow {}^1B_{1g}$, and $^1A_{1g} \rightarrow {}^1E_g$ transitions, respectively. These results indicate that the synthesized platinum complex has a low spin square-planar coordination of the central Pt^{2+} ion with the surrounding BBHMP and NA [52-55].

The FTIR spectra for BBHMP, NA [56-58], and Pt(II) complex are given in Table 2. The ν(NH_2) stretching vibration seen at 3351 cm^{-1} in the free NA is also seen at 3352 cm^{-1} in the complex. According to this result, there is no significant shift in the spectrum of the complex, which indicates that the NH_2 group is not involved in coordination. The band observed at 1231 cm^{-1} also

Table 2: Characteristic FT-IR values (cm^{-1}) for BBHMP, NA and Pt(II) complex.

Compound	ν(NH_2)	ν(C=O)	ν(C=N)	ν(C=C)	ν(C-N)	ν(NHdef)	ν(PtO)	ν(PtN)
BBHMP	-	-	-	1618	-	-	-	-
NA	3351	1680	1612	1542	1255	1163	-	-
Complex	3352	1698	1621	1525	1278	1231	673	585

supports that there is no coordination at this point due to the N-H deformation vibration position in metal complexes [59]. The ν(C=N) and ν(C-N) vibrations which occur at 1612 and 1255 cm^{-1} in NA are shifted in the complex due to coordination *via* the N of the pyridine ring [60]. The vibrations of ν(C=O) and ν(C=C) at 1680 and 1542cm^{-1} in the free NA shift to higher frequencies in the complex. The Pt^{2+} bonded oxygen atoms in the BBHMP structure attract electrons from neighboring atoms, making the bonds between neighboring atoms more polar. Therefore, functional groups occur at higher wave numbers. In the FTIR spectrum for the complex, the presence of peaks at 673 and 585 cm^{-1} which may be attributed to ν(Pt-O) and ν(Pt-N) confirms coordination by the oxygen and nitrogen atoms in the ν(C-O) and ν(C-N) groups, respectively [61].

^{1}H NMR (300 MHz, $CDCl_3$) spectra for BBHMP, NA and their Pt(II) complex support the coordination of the Pt^{2+} ion with BBHMP and NA molecules. According to ^{1}H NMR results for BBHMP, the characteristic ^{1}H NMR signals at δ = 4.62 and 5.8 ppm are assigned to aliphatic CH_2 (s, 1H, C_7-H and s, 1H, C_8-H) and OH (s, 1H, OH) protons, respectively. The peak at δ = 7.41 ppm (s, 1H, C_2-H and s, 1H, C_6-H) is attributed to the protons of the aromatic ring. The ^{1}H NMR spectra for NA [62] have proton signals at δ = 7.46 (s, 1H, C_2-H), 8.48 (d, 1H, C_4-H), 7.55 (t, 1H, C_5-H), 8.36 (d, 1H, C_6-H) and 5.14 ppm (s, 2H, NH_2). In the ^{1}H NMR spectra of the synthesized complex, the location of the CH_2 moieties bound to O atoms in the Pt(II) complex shifted compared to BBHMP, suggesting deshielding in the protons of the CH_2 moieties because of binding to the Pt^{2+} ion via the O atoms of BBHMP. In addition, OH peaks disappeared and small shifts were observed in other values.

Although various single-crystal methods were studied for Pt(II) complex [63-65], the single crystal could not be obtained. Thus, the XRD-powder pattern was used in order to test the degree of crystallinity of the complex [14, 66]. The XRD powder diffraction patterns of the platinum(II) complex were determined in the range 2θ = 5-80° (Fig. 2). The XRD patterns of the Pt(II) complex show the sharp crystalline peaks indicating their crystalline phase. The average crystallite size (D) of the complex was calculated using the Scherrer formula [67, 68]. The complex has an average crystallite size of 42.6 nm. As seen from Fig. 2, the diffraction peaks at the 2θ of 39.5°, 45.9°, and 67.2° may be attributed to the characteristic (111), (200), and (220) crystalline planes of Platinum, suggesting that the complex is in a nanocrystalline phase.

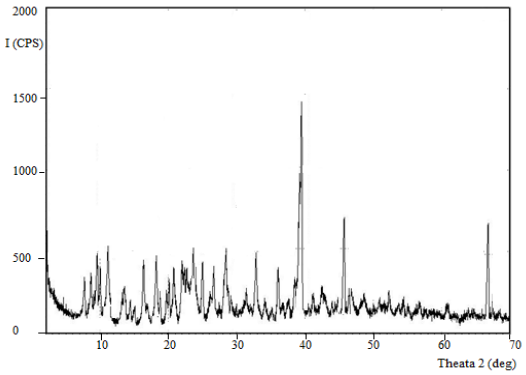


Fig. 2: XRD patterns of the Pt(II) complex.

The thermal analysis results for the obtained complex are summarized in Table 3. In the thermal behavior of the complex, the 1st step with a weight loss of 0.3 % is in the range of 25-200 °C due to the removal of possible water molecules. The 2nd step between 200-600 °C is the decomposition of the organic compound + Cl (56.6 %). The range 600-800˚C indicates the formation of 2 mol PtO (47.2 %) and the compound remaining over 800 °C was determined to be 2 mol Pt (43.1 %). The molecular weight ratio for the amount of PtO and Pt shows very good agreement with the proposed structure according to TGA data [49, 69].

Solution study results

The stability of Pt(II) complex in a physiological buffer with time was studied by UV-Visible

Table 3: Thermal analysis decompositions of the Pt(II) complex.

Compound	Steps	Tb-Tc (°C)	Weight loss (%)	Assignments
Pt (II) complex	1st	25-200	0.3	Possible water molecule
	2nd	200-600	56.6	Organic Compound + Cl
	3rd	600-800	47.2	2 PtO
	4th	800-1000	43.1	2 Pt

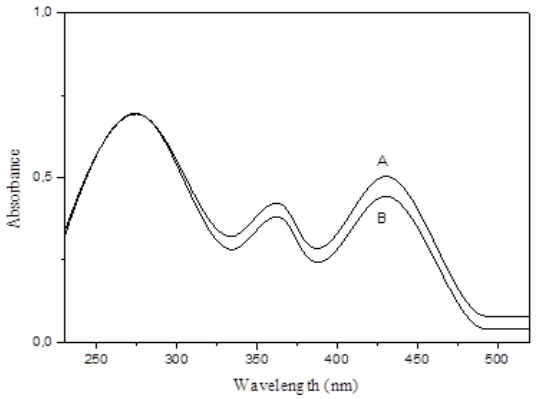


Fig. 3: UV-Vis. spectra of the Pt(II) complex in DMSO and physiological buffer (0.1 M phosphate, 4 mM NaCl, pH 7.4) at t = 0 (A) and t = 18 h (B).

spectrophotometer [70-72]. The complex was dissolved in DMSO (5 mM) and physiological buffer (0.1 M phosphate, 4 mM NaCl, pH 7.4). The stability of the Pt(II) complex was determined spectrophotometrically. The electronic spectra for the complex were determined to evolve in time over 18 h (Fig. 3). According to Fig. 3, the transitions between 250 and 450 nm are stable after 18 h, confirming that the synthesized complex is stable when dissolved in a physiological buffer.

Antimicrobial activity results

As the antimicrobial activity of NA was reported in the literature [13-15, 73, 74], the antimicrobial activities of the BBHMP and platinum(II) complex were investigated in the present study by using the micro broth dilution method. The compounds were tested against bacterial and fungal strains that can ensure resistance to antibiotics through biochemical changes and Minimal İnhibition Concentration (MIC) values were calculated. The antimicrobial activities of BBHMP and its platinum(II) complex were investigated against bacterial and fungal strains. The antimicrobial activity results are presented in Fig. 4 and Table 4. In previous studies, the antibacterial and antifungal activities of various Pt(II) complexes exhibited a broad spectrum of antimicrobial activity. In the previous studies [75-77], the antibacterial and antifungal activities of various Pt(II) complexes had significant antimicrobial activity. When compared with the antimicrobial activity values in these studies, the synthesized Pt(II) complex has important activity, especially against *Staphylococcus Aureus* strain. According to the obtained results, while free BBHMP shows moderate antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli* at 6.25 µg/mL concentrations, the complex has a significant effect against *Staphylococcus aureus* and *Candida albicans* at 25 and 50 µg/mL concentrations. These results show that the complex is more active against fungi. As a result, the complex has increased antimicrobial activity compared with free BBHMP and NA. This important activity of the complex may be because of the polarity of the metal and the change in the structure of the complex. This can be explained by partially sharing the positive charge on Pt(II) with the donor groups, such as N and O on BBHMP and NA.

Cytotoxicity results

Anticancer treatments cause cytotoxicity against cancer cell proliferation [78, 79]. The cancer cells are cultured. After applying the procedure, MTT assay is applied to check the proliferation or viability of the cells. The Pt(II) complexes stimulate cytotoxicity [49, 80-83]. In a previous study, four Pt(II) complexes with 2-substituted benzimidazole ligands were synthesized and their cytotoxicity was investigated against the human breast cancer (MCF7) cell line by *Gümüş et al.* [80]. Based on the reported test results, the synthesized platinum complexes were determined to be less active than cisplatin and exhibited moderate cytotoxicity on the MCF 7 cell line. Due to the appreciable stability with time of the complex in DMSO under physiological-like conditions, the complex could be evaluated as a potential cytotoxic agent.

Table 4: Antimicrobial activity values for BBHMP and Pt(II) complex

Name of organisms	Inhibition % BBHMP/Antibiotic (Complex/Antibiotic)	MIC (µg/mL) BBHMP/Antibiotic (Complex/Antibiotic)
E. coli	36/28 (69/11)	6.25/6.25 (6.25/6.25)
S. typhimurium	66/31 (72/18)	12.5/6.25 (200/6.25)
S. aureus	32/21 (32/16)	6.25/6.25 (25/6.25)
L. monocytogenes	71/13 (68/8)	6.25/6.25 (6.25/6.25)
B. cereus	70/34 (72/10)	6.25/6.25 (12.5/6.25)
C. albicans	69/49 (31/26)	50/6.25 (50/6.25)

** Ampicillin and amphotericin B were antibiotics for bacterial and fungal strains.*

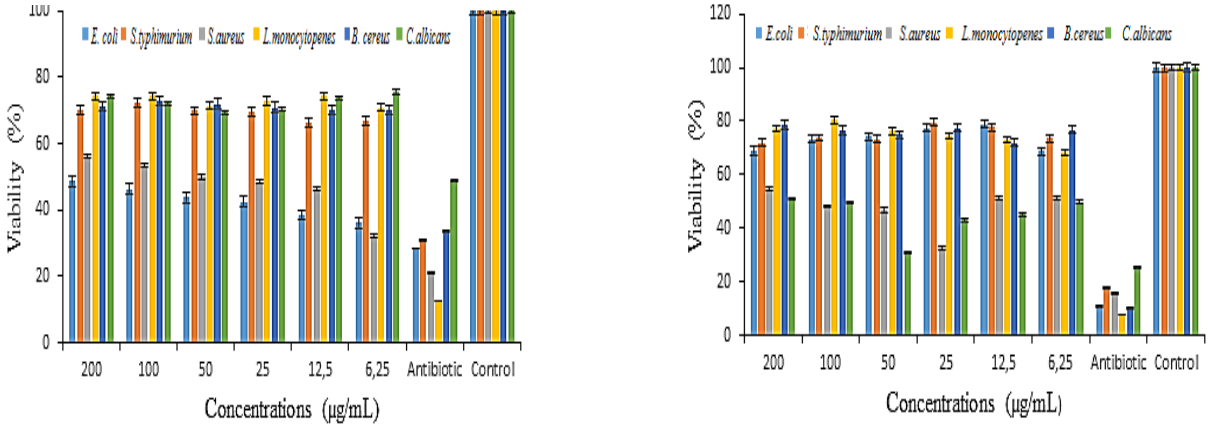


Fig. 4: Viability (%) values of E. coli, S. typhimurium, S. aureus, L. monocytogenes, B. cereus and C. Albicans with the BBHMP (a) and Pt(II) complex (b) at different concentrations (200-6.25 µg/ml). (n = 4) ± S.E.

Previously, many studies reported the cytotoxicity properties of NA [84-87]. Hence, the cytotoxicity of BBHMP and its platinum(II) complex was examined for human prostate adenocarcinoma (DU145) and breast (MCF7) cancer cell lines with an MTT assay. For comparison purposes, cisplatin activity was evaluated under the same experimental conditions. The results are presented in Fig. 5. The cell viability (%) of DU145 and MCF7 cancer cells treated with BBHMP and Pt(II) complex was effective at 100, 200, 400, and 800 µM concentrations after 24 hours compared to their respective control groups. The IC_{50} values within the micromolar range (50 % inhibition) were found from the dose-response curves (Table 5). As a result of probit analysis, the IC_{50} data for BBHMP and platinum complex were 94.72 and 81.96 µM for DU145 and 74.42 and 67.27 µM for MCF7, respectively. Although the IC_{50} values of BBHMP and the complex were higher than cisplatin for DU145 and MCF7 cancer cells, they showed moderate cytotoxicity against the same cancer cells. As a result, the cytotoxicity of the complex is more effective than free BBHMP and NA. In comparison with the previously reported IC_{50} data [80, 88-90], the synthesized complex was evaluated to be less effective against DU145 and MCF7.

Gene expression results

Some anticancer agents, in particular phenolic compound-inducible molecules, were linked to the activation of apoptosis signaling pathways in cancer cells. Recently, it was reported that Pt(II) complexes are an anticancer drug candidate. In one of the studies for this purpose [91], a series of tetrazole-bridged dinuclear Pt(II) complexes were synthesized and their gene expressions were investigated. The results indicated that the complex had highly inhibitory effects on gene expression. Hence, in this study, the apoptotic effect of BBHMP and the Pt(II) complex was investigated against DU145 and MCF7 cancer cells using gene expression profiles. For NA, the gene

Table 5: IC_{50} (μM) values for cytotoxicty activity of BBHMP and the Pt(II) complex.

	IC_{50} (μM)	
Compound	Du145	MCF7
BBHMP	94.72 ± 2.73	74.42 ± 2.19
Complex	81.96 ± 2.89	67.27 ± 1.36
cisplatin	20.92 ± 0.49	18.87 ± 0.55

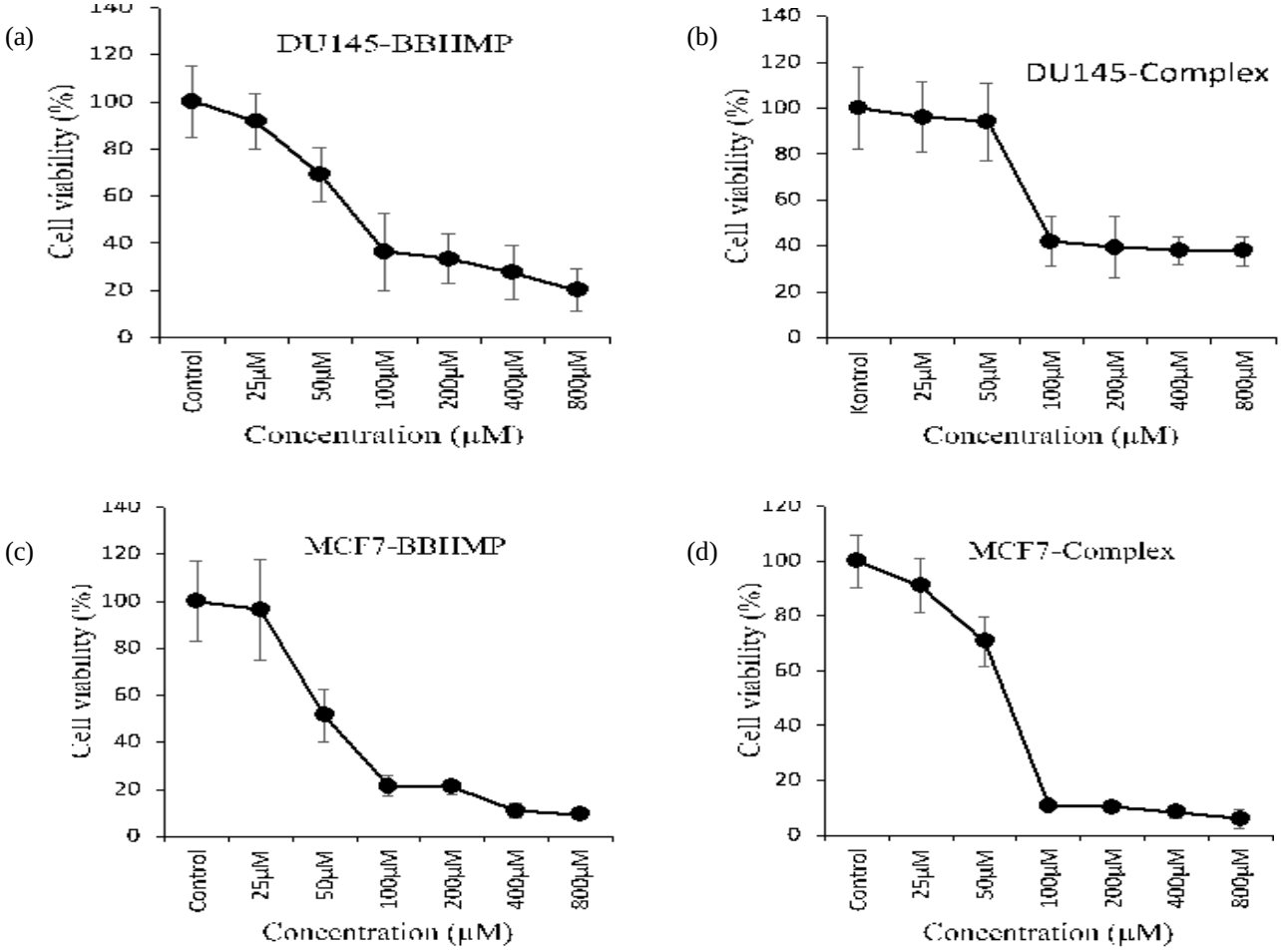


Fig. 5: Cell viability (%) in DU145 and MCF7 cells treated with six different doses of BBHMP or Pt(II) complex for 24 hours. All values are presented as mean values of percentages for each group (n = 6) ± SE. Probit analysis was performed to calculate IC_{50} values and MTT values at 24 hours were used for this..

expression results in the literature were used [92]. Gene expression results are given in Fig. 6. According to Fig. 6, selected genes related to the apoptosis inhibitor, apoptosis activator and cell cycle control groups were determined using the qRT-PCR method. In all cancer cells, while tumour suppressor P53 increased, BCL-2 gene expression is suppressed and pro-apoptotic BAX gene expression increased. Increasing BAX expression triggers the release of Cyt-C from mitochondrial membranes. This gene is linked to APAF 1 and activates caspase 3. As a result of the increase in the expression of caspase 3, death is observed in cancer cells.

Antioxidant activity results

The DPPH radical scavenging method [93-97], which is widely used in antioxidant activity studies and

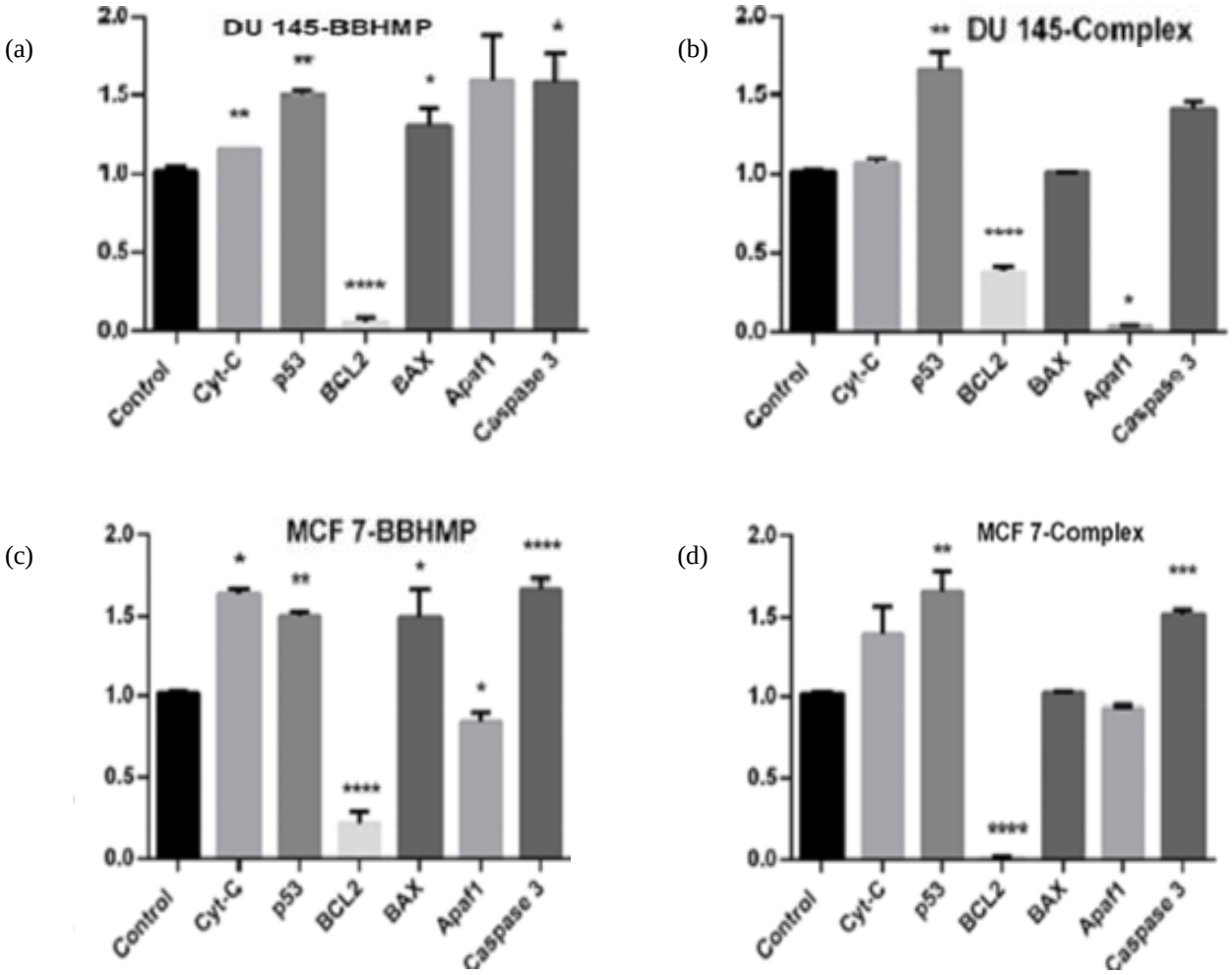


Fig. 6: Relative fold change recorded with quantitative real-time PCR (qRT-PCR) analysis of apoptosis pathway genes in vehicle-treated control and BBHMP or Pt(II) complex-exposed DU145 and MCF7 cell lines. All values were normalized to GAPDH expression and presented relative to control; (n = 4) ± SE. *(p < 0.05), ** (p < 0.01), *** (p < 0.001), * (p < 0.0001), analyzed by one-way ANOVA, post hoc Tukey test.***

determined rapidly with a UV-Visible spectrophotometer, was studied at different concentrations for the antioxidant activities of BBHMP, NA, and Pt (II) complex. The IC_{50} value is the concentration of the sample causing a 50 % reduction in DPPH concentration and is obtained from linear regression of the concentration plots of the tested compounds against the mean percentage of antioxidant activity [98]. The lower the IC50 value, the higher the antioxidant activity of the samples tested. The antioxidant results and IC_{50} values of antioxidant activity for BBHMP, NA, and the complex are given in Fig. 7 and Table 6. According to the antioxidant results, antioxidant activity was effectively increased as a result of the electron-withdrawing effect of the platinum(II) ion. The complex also displayed important free radical scavenging when studied with DPPH. Finally, the antioxidant activity of BBHMP and NA increased after complexation with the Pt^{2+} ion. In comparison with previously reported antioxidant data [75, 99, 100], the complex had significant antioxidant activity.

CONCLUSIONS

In this work, a platinum(II) complex was synthesized with BBHMP (4-Bromo-2,6-bis-hydroxymethyl-phenol) and NA (nicotinamide). The obtained complex was characterized by using spectral and thermal analysis. According to the results, BBHMP and NA molecules coordinated Pt^{2+} ions through oxygen and nitrogen atoms

Table 6: IC_{50} (µM) values for antioxidant activity of BBHMP, NA, and the Pt(II) complex

Compound	IC_{50} (µM)
BBHMP	0.40 ± 1.20
NA	0.48 ± 1.23
Complex	0.34 ± 1.17
BHT	0.28 ± 1.32

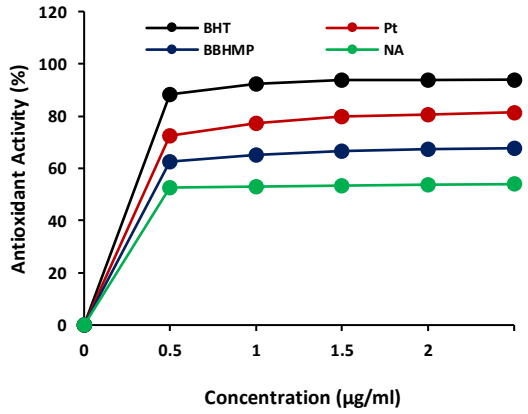


Fig. 7: Percent antioxidant activity for BBHMP, NA, and the Pt(II) complex at room temperature. (n=2)± S.E.

with a square-planar structure. The reaction of BBHMP and NA with Pt(II) is a complexation reaction. One molecule of BBHMP and two molecules of NA react with two molecules of the Pt(II) ion. The two Pt atoms are connected to each other via phenolic oxygen and Cl bridge. Load balance was achieved with 3 oxygen atoms from BBHMP and one Cl atom. Elemental analysis and ESI-MS results support the proposed form and the closed formula. From antimicrobial activity results, BBHMP and the complex are active against some bacterial and fungal strains. Whereas the BBHMP indicates moderate antimicrobial activity against *Staphylococcus aureus* and *Escherichia coli*, the complex has a significant effect against *Staphylococcus aureus* and *Candida albicans*. Consequently, the complex shows increased antimicrobial activity compared with BBHMP. Additionally, cytotoxicity results suggest that the Pt(II) complex and free ligands have reasonable toxic activity. BBHMP and the Pt(II) complex indicate significant cell-growth inhibition against human prostate adenocarcinoma (DU145) and breast (MCF7) cancer cell lines using the MTT assay. There is complex-induced apoptosis in these cancer cells with IC_{50}: 94.72 and 74.42 µM for BBHMP and 81.96 and 67.27 µM for the Pt(II) complex, respectively. Several cellular parameters were found using qRT-PCR assay. The experiments revealed that BBHMP, NA, and the Pt(II) complex may cause cell death in DU145 and MCF7 cancer cells by inducing mitochondrial membrane permeability changes which lead to cytochrome c release resulting in apoptotic cell death. In conclusion, the antioxidant activity results indicate that the complex is effective in preventing the formation of DPPH radicals. In addition, the IC_{50} values observed in cytotoxicity and antioxidant activities show that the Pt(II) complex is different and selective.

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