

RESEARCH PAPER

## Synthesis, Characterization, and Anticancer Evaluation of a Novel Imine-Oxime Ligand Derived from 2-Hydrazinyl Benzothiazole and Its Palladium(II) Complex

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### ABSTRACT

The nano ligand, (2E,3E)-3-((4-((E)-1-(2-(benzo[d]thiazol-2-yl) hydrazineylidene) ethyl)phenyl) imino) butan-2-one oxime (BTHEPIBO), was synthesized in three steps. The first stage produced 2-hydrazineylbenzothiazole by reacting 2-mercaptobenzothiazole with hydrazine hydrate in ethanol. Second, a few drops of glacial acetic acid catalyzed the reaction of 4-aminoacetophenone with diacetyl monoxime in ethanol. In the last step, the two intermediate products formed nano BTHEPIBO ligand. The reaction with palladium (II) chloride produced a palladium (II) complex of this nano ligand. FT-IR, <sup>1</sup>H-NMR, UV-Vis, melting point determination, FESEM, XRD, and magnetic susceptibility measurements were used to determine the chemical structures of the nano ligand and its palladium complex. The synthesized nano ligand and palladium (II) complex were tested for their impact on MCF-7 breast cancer cell proliferation. The nano ligand and its combination inhibited cancer cell multiplication, suggesting possible use in cancer treatment. This paper shows the effective synthesis and complete characterization of a novel nano ligand and its complex, as well as their intriguing biological properties, paving the path for medicinal chemistry research.

### How to cite this article

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### INTRODUCTION

Heterocyclic benzothiazole has a benzene ring fused with a thiazole ring [1]. The thiazole ring has carbon, hydrogen, nitrogen, and sulphur atom [2, 3]. As a weak base, benzothiazole is a colorless liquid with low viscosity, 1.238 g/mL density, and 227-228°C boiling point [4]. It is a precursor of Schiff-bases, azo dyes, and other chemical molecules [1]. Biological, analytical, and organic fields use benzothiazole and its derivatives [5]. Benzothiazole's thioamide functional group makes it biologically effective. This chemical is anticancer,

antibacterial, antifungal, and antiviral, and its derivatives are anti-inflammatory and analgesic [6, 7]. Benzothiazole suppresses hunger and is found in tea leaves and blackberries [8]. Hofmann originally synthesized benzothiazole in 1887, and many methods have been developed for its derivatives [9]. Benzothiazole and its derivatives display considerable potential in coordination chemistry for synthesis of metal complex. Coordination compounds with nitrogen and sulphur donor atoms are fascinating due to their chemical, physical, biological, and pharmacological

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properties [10-13]. The metal ions involved in many biological processes are coordinated by nitrogen and sulphur atoms [14]. Coordinating the biologically active chemical with a transition metal ion usually boosts activity [15].  $R_1R_2C=NOH$  is the conventional formula for an imine oxime.  $R_1$  can be hydrogen for an aldoxime or ketoxime, and  $R_2$  can be an organic side chain [16-20]. A reductive amination reaction between hydroxylamine hydrochloride and an alcoholic carbonyl solution produces oximes [21]. This synthesis reaction is crucial to organic chemistry for several uses [22-25]. Ketones and primary amines generate imines ( $C=NR$ ) by removing water. The chemical process involves adding hydroxylamine to the carbonyl group to generate an unstable hemiacetal intermediate, which rapidly loses a water molecule to form an oxime with the  $C=N$  group [26]. Oximes have anticonvulsant, antibacterial, antioxidant, anticancer, depressive, and antiviral effects [27]. Oxime coordination chemistry can separate, purify, and remove metal ions [28]. These complexes produce the structural models for biological systems like vitamin  $B_{12}$  and have considerable anticancer activity [28]. Oximes coordinate via nitrogen, oxygen, and/or both [29].

In our study, the nano ligand containing the benzothiazole ring and its palladium(II) complex were synthesized. The techniques employed for characterization included FTIR,  $^1H$ -NMR, UV-Vis spectra, FESEM, and CHNS analysis. Additionally, the biological activity of the synthesized compounds was evaluated using the MTT assay to assess their potential as anticancer agents.

## MATERIALS AND METHODS

### *Materials and instruments*

This study used various high-purity chemical components from BDH, Aldrich, Merck, and HIMEDIA. A Shimadzu UV-165PCS spectrophotometer was used to acquire UV-Vis. spectra in the 200-1000 nm area with a concentration of  $1 \times 10^{-3}$  M, ethanol solvent, and a 1 cm absorption cell. A Varian Fourier Transform device was used to capture  $^1H$ -NMR spectra at 300 MHz using DMSO- $d_6$  as the solvent and tetramethyl silane as the internal standard reference. FTIR spectra were taken using a Shimadzu FTIR 8400S spectrophotometer in the 400-4000  $cm^{-1}$  range. Scientists used stuart melting point device to determine chemical melting points. Room-temperature susceptibility

was tested with the MSB-MKI magnetic susceptibility balance. A Shimadzu AA-6300 atomic absorption flame spectrophotometer measured the complexes' metal content, and an EA-300.mth elemental analyzer performed CHNS. The synthesized chemicals were investigated using a Czech MIRA3 TESCAN field emission scanning electron microscope (FESEM). X-ray diffraction (XRD) measurements were taken using the 20-80 degree D2 Phaser Bruker AXS GmbH. A Digital Conductivity Series Ino. Cond 3110 SET1 measured electrical conductivity at  $1 \times 10^{-3}$  M concentration in ethanol a solvent at room temperature.

### *Preparation of the nano BTHEPIBO ligand*

The BTHEPIBO ligand was prepared by the following steps (See Fig. 1):

#### *Preparation of compound (A)*

2-Hydrazineylbenzothiazole (Compound-A) was synthesized by reacting 2-mercaptobenzothiazole (1.67 g, 0.01 mol) dissolved in 25 mL of absolute ethanol in a round-bottom flask. A solution containing 5 mL of hydrazine hydrate and 15 mL of absolute ethanol was added to the mixture with continuous stirring, and the reaction mixture was refluxed for 8 h at 78°C. The reaction was monitored by the blackening of lead acetate paper. The solution was then cooled at room temperature, filtered, and the resulting precipitate was collected. The precipitate was recrystallized from hot absolute ethanol to remove any unreacted residues, dried, and collected, yielding a 69% product with a melting point of 90-98°C.

#### *Preparation of compound (B)*

The synthesis of (E)-1-(4-((2-hydroxy-1,2-diphenylethylidene) amino) phenyl) ethan-1-one (Compound-B) was achieved by dissolving 4-aminoacetophenone (1.35 g, 0.01 mol) in 25 mL of absolute ethanol in a round-bottom flask. After that, diacetyl monoxime (1.01 g, 0.01 mol) dissolved in 25 mL of absolute ethanol and 5-6 drops of glacial acetic acid were added. The mixture was refluxed for 8 h. The precipitate was filtered, dried, and recrystallized from absolute ethanol, yielding an 82% product with a melting point of 98-100°C.

### *Preparation of the nano BTHEPIBO ligand*

The BTHEPIBO ligand was synthesized by dissolving 1.65 g (0.01 mol) of compound (A) in 25

mL of absolute ethanol under continuous stirring. A solution of 2.18 g (0.01 mol) of compound (B), dissolved in 15 mL of absolute ethanol, was added, along with 5-6 drops of glacial acetic acid. The mixture was refluxed for 8 h, cooled, and resulted in the formation of a precipitate. After filtration, drying, and recrystallization from absolute ethanol, the yield was 73%, with a melting point of 113-115°C.

#### Preparation of palladium (II) complex

The palladium (II) complex of the BTHEPIBO ligand was synthesized by dissolving 0.365 g (0.001 mol) of the BTHEPIBO ligand in 25 mL of absolute ethanol. A solution was separately prepared by dissolving 0.180 g (0.001 mol) of palladium (II) chloride in 25 mL of absolute ethanol, which was then added to the nano ligand solution. The mixture was refluxed with stirring for 2 h.

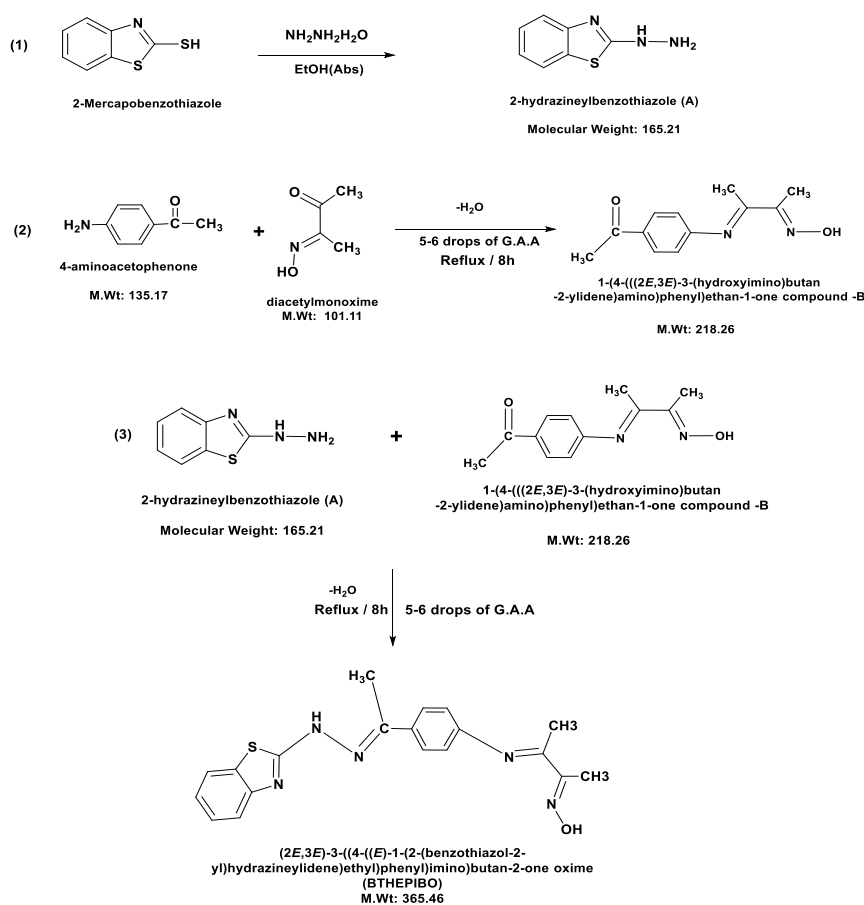


Fig. 1. Synthesis of the nano BTHEPIBO ligand.

Table 1. Physical properties of the prepared BTHEPIBO ligand and its complexes.

Compound (Chemical formula)	Color	M.P (°C)	Yield %	Mol. Wt. (g/mol)	Calc. (Found)%				
					C	H	N	S	Pd
Nano ligand (BTHEPIBO) C <sub>19</sub> H <sub>19</sub> N <sub>5</sub> OS	Dark Yellow	113-115	73	365.46	62.45 (63.12)	5.24 (5.31)	19.16 (19.65)	8.76 (8.98)	-----
[Pd(BTHEPIBO)Cl]Cl C <sub>19</sub> H <sub>19</sub> Cl <sub>2</sub> N <sub>5</sub> OPdS	Reddish Brown	175-177	79	542.78	42.04 (42.63)	3.53 (3.59)	12.90 (13.34)	5.91 (6.17)	19.61 (19.94)

Precipitates was filtered, dried, and recrystallized from absolute ethanol. This process yielded a colored and pure precipitate of the metal complex with a yield of 79% and a melting point of 175-177°C. The physical properties of samples have been listed in Table 1.

#### MTT test

The cytotoxicity of the new BTHEPIBO ligand and its palladium (II) complex was evaluated over MCF-7 breast cancer cells and the normal HEK-293 cell line to assess their efficacy as anticancer agents. Firstly, the cancer cell lines were prepared, and cell suspensions were distributed into a 96-well flat-bottom plate, followed by incubation in a 5% CO<sub>2</sub> atmosphere at 37°C for 24 h. Subsequently, 100 µL of the cell suspension were added to each well, along with varying concentrations of the studied compounds (5, 10, 25, 50, 100, 250, 500 µg/mL), each in triplicate. The plate was then further incubated for 24 h at 37°C. After the incubation period, 10 mL of a 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium bromide solution at a concentration of 0.5 mg/mL was added to each well. The plate was incubated for an additional 4 h at 37°C. To dissolve the crystals, 100 µL of a solubilization solution were added, and the absorbance of the samples was measured at a wavelength of 570 nm using a microplate reader (DNM - 9602G). The absorbance values were used to determine cell viability, expressed as a percentage relative to untreated cells, indicating the number of viable cells in the medium after treatment with the compounds.

## RESULTS AND DISCUSSION

The BTHEPIBO ligand was introduced as dark yellow crystals, whereas its palladium (II) complex appears reddish-brown. Both the nano ligand and its complex demonstrated good solubility in various solvents such as ethanol, methanol, DMSO, and DMF, but they are insoluble in distilled water and ether. The molar ratio for preparing the palladium complex was determined using the molar ratio method, as described by June [30]. This method involves preparing the solutions of equal concentrations of the ligand and the metal ion, with employing different volumes of the nano ligand. Absorbance measurements are taken at appropriate wavelengths for each case. The relationship between the resulting absorbance and the molar ratio (VL/VM) is plotted, revealing a ratio of the complex as 1:1 (metal to nano ligand ratio). Additionally, the ionic nature of the prepared complex was assessed by measuring its molar conductivity using ethanol as the solvent at room temperature and a concentration of  $1 \times 10^{-3}$  M. The palladium (II) complex exhibited good molar conductivity, indicating an ionic character with a 1:1 metal to nano ligand ratio, with a measured conductivity value of  $38.18 \text{ ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$ .

#### <sup>1</sup>H-NMR results

The <sup>1</sup>H-NMR spectrum was studied using a Varian Fourier Transform instrument operating at a frequency of 300 MHz, as displayed in Fig. 2. Tetramethylsilane served as the internal standard,

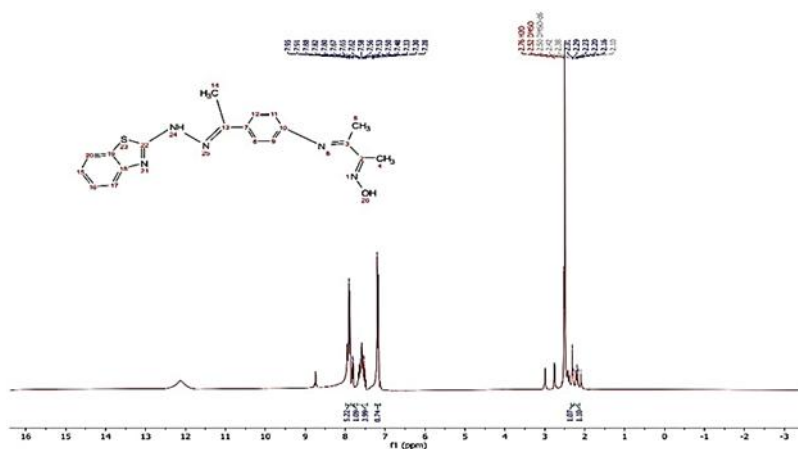


Fig. 2. <sup>1</sup>H-NMR spectrum of the prepared BTHEPIBO ligand.

and DMSO- $d_6$  was used as the solvent at room temperature. The  $^1\text{H}$ -NMR spectrum of the BTHEPIBO ligand displayed a singlet at a chemical shift (s, 3H,  $\delta$  = 2.31 ppm), corresponding to the protons of the methyl group ( $-\text{C}=\text{N}-\text{CH}_3$ ). A singlet at s, 3H,  $\delta$  = 2.10 ppm was observed, indicating the protons of the methyl group attached to the oxime group [31]. The protons of the methyl group linked to the imine group produced a singlet at s, 3H,  $\delta$

= 2.98 ppm [32]. Multiple signals were detected within the range of M, 8H,  $\delta$  = 7.28 - 8.82 ppm, representing the aromatic ring protons [33]. Additionally, singlet signals at S, 1H,  $\delta$  = 8.74 ppm and S, 1H,  $\delta$  = 12.16 ppm were attributed to the protons of the secondary amine ( $-\text{NH}$ ) group and the hydroxyl group of the oxime, respectively [34]. The singlet at 2.52 ppm was due to the protons of the DMSO- $d_6$  solvent.

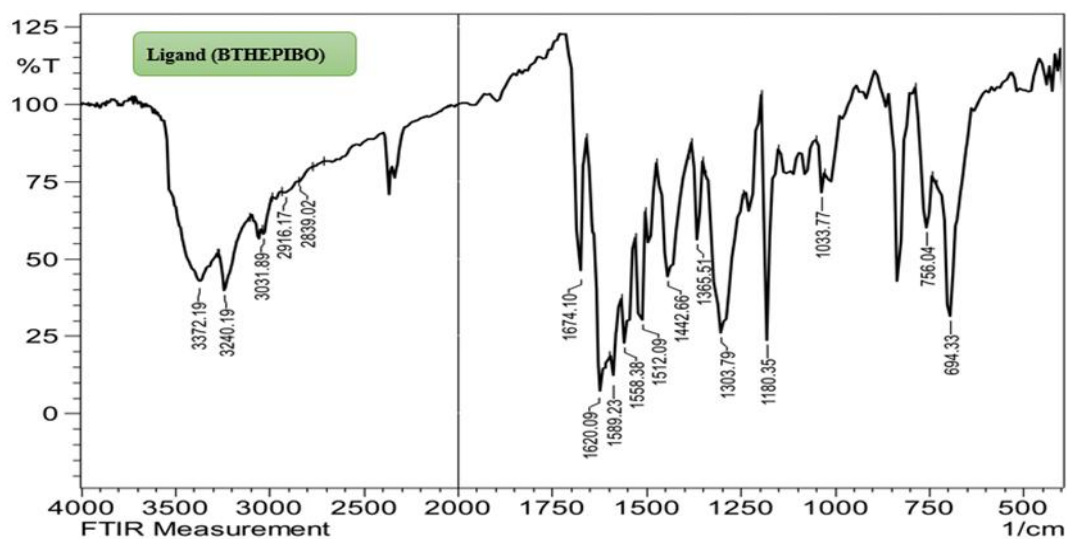


Fig. 3. FTIR spectrum of BTHEPIBO ligand.

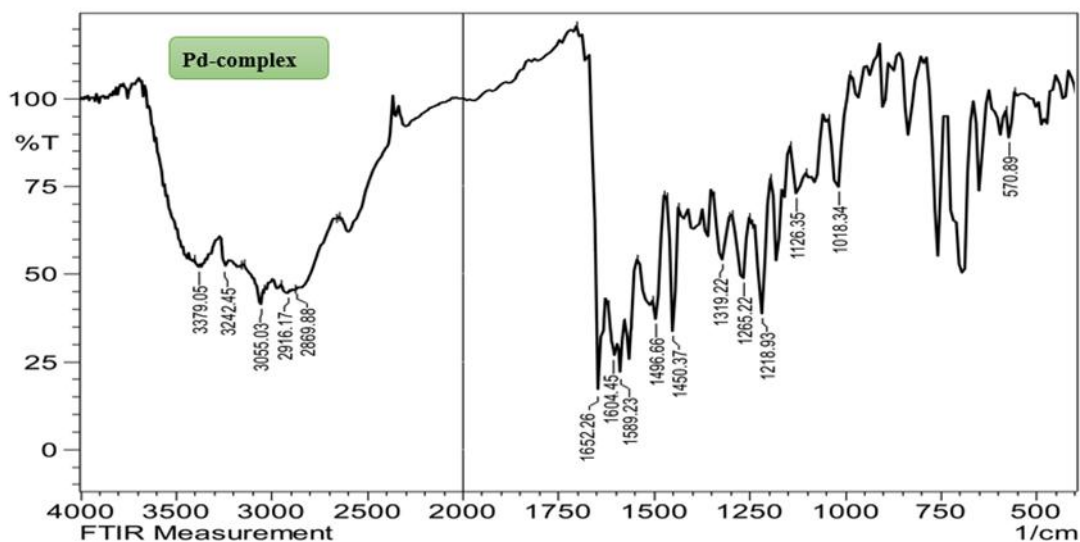


Fig. 4. FTIR spectrum of the palladium (II) complex.

### FT-IR results

To obtain the FTIR spectrum of the samples, potassium bromide was utilized. The spectrum of the free nano ligand in Fig. 3 exhibited two distinct absorption bands at 3372 and 3240  $\text{cm}^{-1}$ , corresponding to the hydroxyl group of the oxime and the secondary amine  $\nu(\text{N-H})$ , respectively [35-37]. An absorption band at 1674  $\text{cm}^{-1}$  was observed, indicating the azomethine group ( $\text{C=N}$ ) of the Schiff base, thereby confirming the formation of the nano ligand [38]. Distinct bands at 1620 and 1589  $\text{cm}^{-1}$  were attributed to the azomethine groups of the oxime and the benzothiazole ring, respectively

[39]. The  $\text{C=C}$  and  $\text{C-H}$  groups of the aromatic rings in the nano ligand are appeared at 1558, 1455, and 3031  $\text{cm}^{-1}$ , respectively. Additionally, a band at 1033  $\text{cm}^{-1}$  indicated the presence of the  $\nu(\text{C-S})$  functional group of the benzothiazole ring [40, 41]. The FTIR spectrum of the synthesized palladium (II) complex is shown in Fig. 4. The Schiff base's azomethine group shifted to a lower frequency than that of the free nano ligand spectrum. It was centered at 1674  $\text{cm}^{-1}$  in the free nano ligand and displaced as 1652  $\text{cm}^{-1}$  in the produced complex spectrum [42-46]. A decrease in the azomethine group's frequency of the oxime was noticed

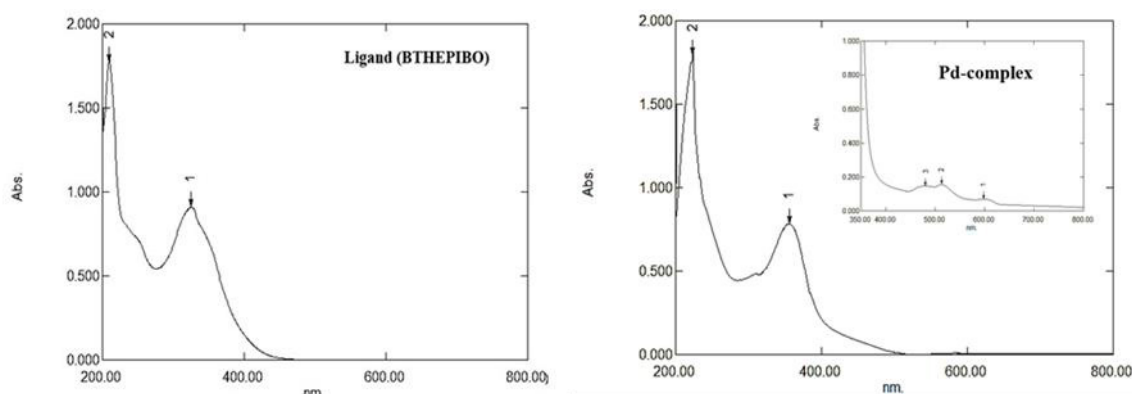


Fig. 5. UV-Vis spectra of BTHEPIBO ligand and its palladium (II) Complex.

Table 2. Spectral bands of BTHEPIBO ligand and palladium (II) complex.

Compound	$\nu$ (O-H) Oxime $\nu$ (N-H) 2°Amine	$\nu$ (C-H) Aromatic	$\nu(\text{C=N})$ Imine $\nu(\text{C=N})$ Oxime	$\nu(\text{C=N})$ Benzothiazole	$\nu(\text{C=C})$ aromatic	$\nu(\text{M-N})$
Nano ligand (BTHEPIBO)	3372 3240	3031	1674 1620	1589	1558 1442	-
[Pd(BTHEPIBO)Cl] <sub>2</sub> Cl	3379 3245	3055	1652 1604	1589	1566 1450	570

Table 3. UV-Visible absorption peaks, magnetic momentum and expected geometry of ligand (BTHEPIBO) and its palladium (II) Complex.

Compounds	$\lambda$ (nm)	$\nu$ ( $\text{cm}^{-1}$ )	Transitions	$\mu_{\text{eff}}$ (B.M)	Geometry
Nano ligand (BTHEPIBO)	212 324	47170 30864	$\pi-\pi^*$ $n-\pi^*$	-	-
[Pd(BTHEPIBO)Cl] <sub>2</sub> Cl	223 354 482 514 598	44843 28249 20747 19455 16722	Intra Nano ligand Intra Nano ligand $^1\text{A}_{1g} \rightarrow ^1\text{E}_g$ $^1\text{A}_{1g} \rightarrow ^1\text{B}_{1g}$ $^1\text{A}_{1g} \rightarrow ^1\text{A}_{2g}$	(Dia.)	Square planar $\text{dsp}^2$



around  $1604\text{ cm}^{-1}$  in the spectrum of the prepared compound. These changes strongly suggest that the nitrogen atoms of the azomethine groups coordinate the nano ligand with the palladium ion. The complex spectrum showed a new band at  $570\text{ cm}^{-1}$ , indicating the presence of a metal-nitrogen (M-N) group (See Table 2) [47].

#### UV-Vis spectra

Solutions of metal complex compounds have distinctive colors due to their absorption in the visible region of the spectrum, in addition to other absorptions in the ultraviolet region because they contain partially filled secondary (d) levels with electrons [48]. The UV-visible spectrum of the BTHEPIBO ligand in Fig. 5 shows two distinct absorption peaks at  $212\text{ nm}$  ( $47170\text{ cm}^{-1}$ ) and  $324\text{ nm}$  ( $30864\text{ cm}^{-1}$ ). The first peak indicates the  $\pi\text{-}\pi^*$  transition, while the second peak indicates the  $n\text{-}\pi^*$  transition of the azomethine ( $\text{C}=\text{N}$ ) group [49]. The spectrum of the palladium (II) complex in Fig. 5 shows new absorption peaks that were not present in the free Nano ligand's spectrum. In addition to the peaks at  $223\text{ nm}$  ( $44843\text{ cm}^{-1}$ )

and  $354\text{ nm}$  ( $28249\text{ cm}^{-1}$ ), which all indicate intra ligand transitions, new absorption peaks are appeared at  $482\text{ nm}$  ( $20747\text{ cm}^{-1}$ ),  $514\text{ nm}$  ( $19455\text{ cm}^{-1}$ ), and  $598\text{ nm}$  ( $16722\text{ cm}^{-1}$ ), corresponding to the electronic transitions  $^1\text{A}_1\text{g} \rightarrow ^1\text{E}_\text{g}$ ,  $^1\text{A}_1\text{g} \rightarrow ^1\text{B}_1\text{g}$ , and  $^1\text{A}_1\text{g} \rightarrow ^1\text{A}_2\text{g}$ , respectively. The presence of these transitions confirms that the complex has a square planar geometry. Magnetic susceptibility measurements performed on the complex, which gave values close to zero, proved that all electrons in the secondary (d) shell are paired (See Table 3) [50].

#### XRD analysis

A study of the crystal structures of both the nano BTHEPIBO ligand and its palladium (II) complex was conducted using XRD measurement in Fig. 6. The XRD data provided valuable information about the crystal structure, crystal volume, and purity of the prepared compounds [51-53]. Analysis of the XRD patterns revealed that the presence of sharp peaks indicates the formation of a crystalline lattice, confirming that the compound exhibits a crystalline or semi-crystalline nature. However, if

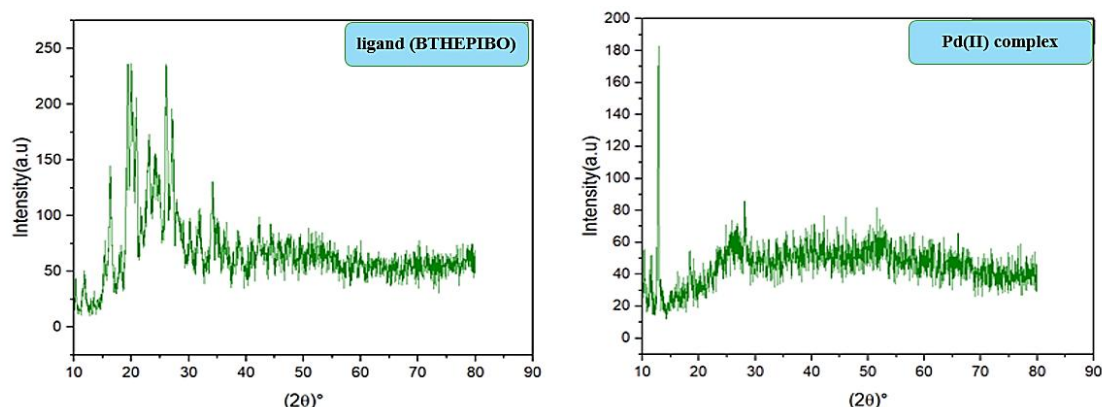


Fig. 6. XRD patterns of the nano BTHEPIBO ligand and its palladium (II) complex.

Table 4. Summary of the information obtained from the XRD analysis i.e., diffraction angles, d-spacing, and relative intensities for nano BTHEPIBO ligand and its palladium (II) complex.

Compound	No .	Peak Position °2θ	Peak Width (FWHM)	Crystallite size(nm)	Rel. Int [%]	Lattice Strain
Nano ligand (BTHEPIBO)	1	26.106	0.514	16.58	100	0.0097
	2	19.274	0.409	20.59	92	0.0100
	3	27.070	0.539	15.84	80	0.0098
[Pd(BTHEPIBO)Cl]Cl	1	12.798	0.430	19.43	100	0.0167
	2	26.464	4.639	1.83	64.46	0.0861
	3	42.888	0.183	48.73	52.81	0.0029

the peaks appear broad and not sharp, it indicates that the compound is non-crystalline [54]. The Debye-Scherrer equation was used to calculate the crystallite sizes for the BTHEPIBO ligand and the palladium complex.

Analysis of the XRD patterns revealed distinct differences in crystallite size, dislocation density, and interplanar spacing ( $d$ ) between the nano ligand and the prepared palladium (II) complex. These differences confirm the coordination process between the nano ligand and the palladium (II) ion, as detailed in Table 4. The data obtained from the XRD measurements indicate that the prepared materials possess a nanoscopic nature [32].

#### FESEM analysis

Using SEM analysis, detailed information

about the surface morphology, particle size, and shape of both samples was obtained [55]. FESEM measurement was employed with a scale of 200 nm and a magnification power of (Mag = 135KX). As depicted in Fig. 7, analysis of the FESEM images of the nano ligand (BTHEPIBO) revealed small, agglomerated, non-homogeneous spherical particles with an average particle size of 36.65 nm. In contrast, the FESEM image of the palladium (II) complex showed very small, unevenly distributed spherical granules with an average particle size of 29.88 nm [56].

#### Effect of the BTHEPIBO ligand on the growth of breast cancer (MCF-7) and normal (HEK) cell lines

The study on the effect of the BTHEPIBO ligand on the growth of the MCF-7 breast cancer cell

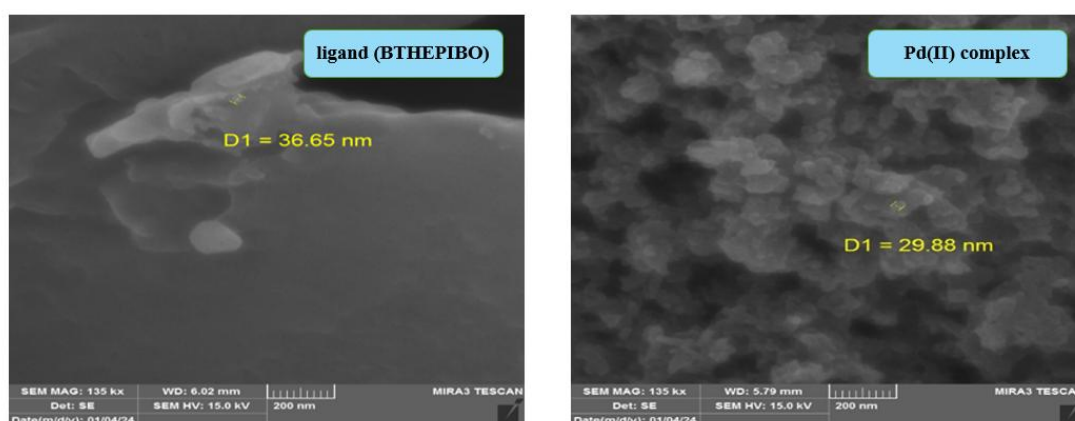


Fig. 7. FE-SEM images of BTHEPIBO ligand and its palladium (II) complex.

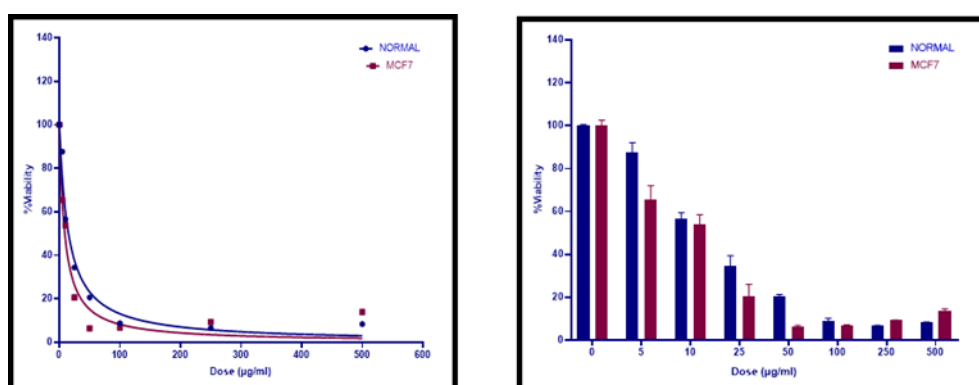


Fig. 8. Comparison of viable cells at selected concentrations for the breast cancer cell line MCF-7 and the normal cell line HEK by employing the nano BTHEPIBO ligand.



line revealed a low inhibition rate at the lowest concentration of 5  $\mu\text{g/mL}$ , where the inhibition rate of breast cancer cell growth was 34.10%. In contrast, the lowest inhibition rate for the growth

of normal HEK cells at the same concentration was 12.34%. However, at a concentration of 50  $\mu\text{g/mL}$ , the ligand exhibited a significantly higher inhibition rate, with the growth inhibition of breast cancer

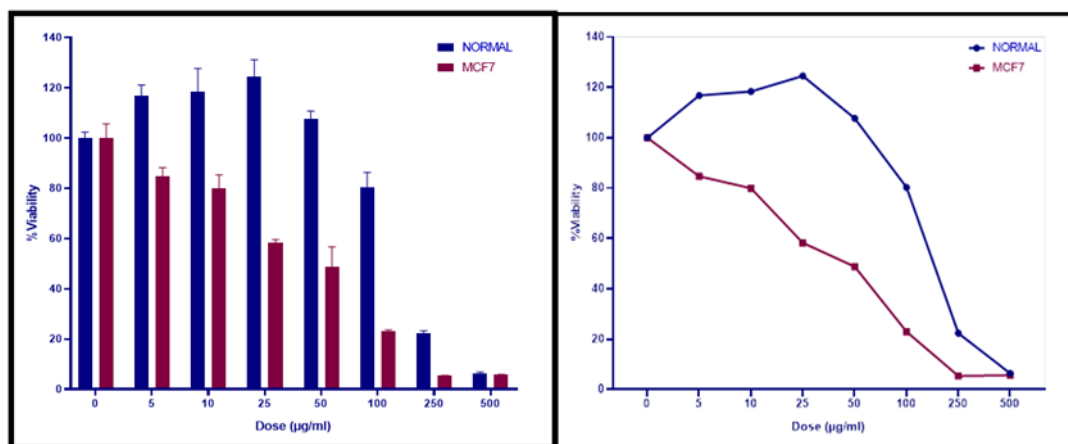


Fig. 9. Comparison of viable cells at selected concentrations for the breast cancer cell line MCF-7 and the normal cell line HEK by employing the palladium (II) complex.

Table 5. Effect of the nano BTHEPIBO ligand on the cancer cell line of breast cancer MCF-7 and its comparison with the normal cell line Hek-293 at the same concentration using the MTT assay for 24 h.

Conc. $\mu\text{g/mL}$	Cancer cell MCF-7			Normal cell Hek-293		
	Cell Viability		Cell Inhibition %	Cell Viability		Cell Inhibition %
	Mean	SD		Mean	SD	
0	100	0	0	100	0	0
5	65.90	4.09	34.1	87.66	4.35	12.34
10	18.83	3.46	81.17	56.53	2.89	43.47
25	5.21	0.11	94.79	34.37	4.99	65.63
50	5.86	0.20	94.14	20.61	0.70	79.39
100	6.48	0.30	93.52	8.70	1.45	91.3
250	7.33	0.76	92.67	6.57	0.28	93.43
500	8.44	0.69	91.56	8.30	0.11	91.7
IC50	8.09			28.68		

Table 6. Effect of the palladium (II) complex of the BTHEPIBO ligand on the MCF-7 breast cancer cell line and comparison with the normal Hek-293 cell line at the same concentration using the MTT assay for 24 h.

Conc. $\mu\text{g/mL}$	Cancer cell MCF-7			Normal cell Hek-293		
	Cell Viability		Cell Inhibition %	Cell Viability		Cell Inhibition %
	Mean	SD		Mean	SD	
0	100	0	0	100	2.439	0
5	84.67	3.620	15.33	116.78	4.294	16.78
10	79.94	5.423	20.06	118.36	9.407	18.36
25	58.26	1.369	41.74	124.55	6.737	24.55
50	48.85	7.905	51.15	107.73	3.049	7.73
100	23.02	0.672	76.98	80.31	5.995	19.69
250	5.39	0.146	94.61	22.40	0.821	77.6
500	5.68	0.221	94.32	6.45	0.410	99.59
IC50	35.91			218.4		

cells reaching 94.14%. The highest inhibition rate for the growth of normal HEK cells was observed at a concentration of 250  $\mu\text{g/mL}$ , peaking at 93.43%. The best inhibition rate for breast cancer cells at 50  $\mu\text{g/mL}$  was 94.14%, while the inhibition rate for the normal cell line at the same concentration was 79.39%. The inhibition rate of normal cell growth can be reduced by modifying the ligand. Based on the obtained results, the half-maximal inhibitory concentration ( $\text{IC}_{50}$ ) of the ligand against the breast cancer cell line was 8.09  $\mu\text{g/mL}$ , while the  $\text{IC}_{50}$  for normal HEK cells was 28.68  $\mu\text{g/mL}$ . These findings suggest that the BTHEPIBO ligand is a promising candidate for breast cancer treatment [57], as shown in Table 5 and Fig. 8.

*Effect of the palladium (II) complex on the growth of breast cancer (MCF-7) and normal (HEK) cell lines*

When studying the effect of the palladium (II) complex of the BTHEPIBO ligand on the growth of the MCF-7 breast cancer cell line, the complex showed a low inhibition rate at a concentration of 5  $\mu\text{g/mL}$ , where the inhibition rate of the growth of the breast cancer cell line was 15.33%. Meanwhile, the lowest inhibition rate for the growth of normal (HEK) cells was observed at a concentration of 50  $\mu\text{g/mL}$ , showing an inhibition rate of 7.73%. However, it showed a higher inhibition rate at a concentration of 250  $\mu\text{g/mL}$ , where the inhibition rate of the growth of breast cancer cells was 94.61%. The inhibition rate for the growth of normal (HEK) cells at the same concentration was 77.60%. Based on the obtained results, the half-maximal inhibitory concentration ( $\text{IC}_{50}$ ) of the complex against the breast cancer cell line was 35.91  $\mu\text{g/mL}$ , while the ( $\text{IC}_{50}$ ) for normal (HEK) cells was 218.4  $\mu\text{g/mL}$ , as shown in Table 6 and Fig. 9. By comparing the results of the study of both BTHEPIBO ligand and its palladium(II) complex, a similarity in the results was found, which suggests a wide potential for treatment [58].

## CONCLUSION

This study involved the synthesis of the novel imine-oxime ligand derived from 2-hydrazinylbenzothiazole, along with its palladium (II) complex. Various spectroscopic and physical methods were employed to elucidate the structures of both the ligand and its palladium (II) complex. FTIR data confirmed the formation of azomethine groups, which shifted to lower

frequencies upon coordination, indicating the formation of metal complexes. This technique also demonstrated that the nano ligand coordinates with the palladium (II) ion through the nitrogen atoms of the azomethine groups of the Schiff base and the oxime azomethine group, confirming the tridentate nature of the nano ligand. Additionally, the UV-visible spectrum of the palladium (II) complex revealed a square planar geometry. Molar conductivity measurements indicated that the palladium (II) compound possesses an ionic nature. Furthermore, FESEM and XRD measurements showed that the palladium (II) complex has a nanoscale size. The MTT assay was utilized to investigate the potential use of the nano ligand and its palladium (II) complex as anticancer agents against the MCF-7 breast cancer cell line. Both compounds demonstrated significant activity against the cell lines used in the study.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

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