

RESEARCH PAPER

Synthesis, Nano-structural Characterization, and In Vitro Cytotoxic Potential of Nickel(II) and Copper(II) Nano-complexes Derived from a Tetrakis-azo Biphenyltetraamine-based Nano-ligand Bearing Propyl Ester Functionalities

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ABSTRACT

In the present investigation, a new tetrakis-azo nano-ligand designated PHBTA was synthesized through the diazotization of [1,1'-biphenyl]-3,3',4,4'-tetraamine followed by coupling with propyl 4-hydroxybenzoate under controlled alkaline conditions at 0–5 °C. Two transition metal nano-complexes, namely [Ni(PHBTA-2H)(H₂O)₂] and [Cu(PHBTA-2H)(H₂O)₂], were subsequently prepared and thoroughly characterized using elemental microanalysis (CHN), Fourier-transform infrared spectroscopy (FTIR), ultraviolet-visible (UV-Vis) spectrophotometry, proton nuclear magnetic resonance (¹H-NMR), field-emission scanning electron microscopy (FESEM), and X-ray diffraction (XRD). Spectroscopic evidence confirmed that the ligand coordinates through the phenolic oxygen atoms (after deprotonation) and the azo nitrogen atoms, functioning as a dibasic tetradentate chelating agent. The magnetic susceptibility measurements and electronic spectral data were consistent with a regular octahedral geometry around the Ni(II) center ($\mu_{\text{eff}} = 2.97$ B.M.) and a Jahn–Teller distorted octahedral arrangement for the Cu(II) ion ($\mu_{\text{eff}} = 1.71$ B.M.). FESEM micrographs disclosed marked changes in surface morphology upon complexation, with nanocrystalline aggregates forming in both nano-complexes. The in vitro cytotoxicity was evaluated via the MTT assay against human breast adenocarcinoma (MCF-7) and normal human foreskin fibroblast (HFF) cell lines. The Ni(II) complex displayed outstanding anticancer potency with an IC₅₀ of 7.153 µg/mL toward MCF-7, while the Cu(II) complex showed moderate activity (IC₅₀ = 232.4 µg/mL). These findings point to a promising role for Ni(II)-azo nano-chelates in cancer chemotherapy research.

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INTRODUCTION

Azo compounds constitute one of the most versatile families of organic molecules, finding widespread application in dye manufacturing, analytical chemistry, and, more recently,

medicinal research [1,2,4]. Their hallmark — N=N — chromophore endows them with intense coloration and a rich coordination chemistry, since the lone pairs on the nitrogen atoms can

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donate electron density to transition metal ions and form stable chelate rings [4]. When a phenolic hydroxyl group is positioned ortho to the azo linkage, the resulting ligand is capable of bidentate coordination through the deprotonated phenolate oxygen and one azo nitrogen, a motif that has been exploited extensively over the past two decades [5,6].

Polydentate azo ligands, in particular those carrying multiple azo groups within a single molecular framework, have attracted considerable attention because they can wrap around metal centers in geometrically diverse ways and give rise to multinuclear or polymeric architectures with interesting magnetic and optical properties [7]. Among the scaffolds investigated, the [1,1'-biphenyl]-3,3',4,4'-tetraamine platform is noteworthy: its four amino groups can be converted into four diazonium units, each of which may couple with an activated aromatic ring to yield a tetrakis-azo product [8]. Despite their potential, the coordination chemistry of these tetrakis-azo systems remains relatively underexplored, and very few studies have examined the nano-structural features or biological performance of their metal nano-complexes [11].

The biological relevance of metal-azo complexes stems from the well-documented enhancement of pharmacological activity upon chelation an effect commonly attributed to improved lipophilicity, which facilitates permeation across the cell membrane and interaction with intracellular targets such as DNA and regulatory enzymes [10,11]. Within this context, nickel and copper ions are of special interest: nickel(II) complexes have shown promising anticancer profiles in several reports [12,16], while copper(II) species are known to generate reactive oxygen species (ROS) that induce apoptosis in cancer cells [14]. Motivated by these observations, the present work reports on the synthesis of a new tetrakis-azo nano-ligand (PHBTA) derived from biphenyltetraamine and propyl 4-hydroxybenzoate, together with its Ni(II) and Cu(II) nano-complexes. The compounds were characterized at the molecular and nanoscale levels, and their *in vitro* cytotoxicity against MCF-7 breast cancer cells was evaluated for the first time.

MATERIALS AND METHODS

Chemicals and instrumentation

All reagents were of analytical grade and used without further purification. [1,1'-Biphenyl]-

3,3',4,4'-tetraamine (98 %, Aldrich), propyl 4-hydroxybenzoate (98 %, Pallav Chemicals), sodium nitrite (99 %, Merck), nickel(II) chloride hexahydrate (98 %, BDH), and copper(II) chloride dihydrate (98 %, Merck) were the principal starting materials. Hydrochloric acid (37 %, BDH), sodium hydroxide (98 %, BDH), ammonium acetate (99 %, Fluka), absolute ethanol (99.9 %, BDH), and DMSO (98 %, BDH) were employed as solvents or auxiliary reagents. All glassware was washed with distilled water and ethanol and then dried at 70 °C prior to use.

FTIR spectra were recorded on a JASCO FT/IR-4200S spectrophotometer (Japan) as KBr pellets over the range 4000–400 cm⁻¹. UV-Vis spectra were obtained with a JASCO V-670 spectrophotometer using absolute ethanol as solvent at ambient temperature. ¹H-NMR spectra were acquired on a Bruker 500 MHz spectrometer in DMSO-d₆ with TMS as an internal standard. CHN elemental analyses were performed on an Elementar Analysensysteme (GMBH) analyzer. Melting points were determined on a Stuart SMP10 apparatus. FESEM imaging was conducted on an Oxford Instruments/ZEISS system operating at 10.00 kX magnification with a 1 µm cross-section distance. X-ray diffraction patterns were collected on a PANalytical X'Pert Pro diffractometer using Cu-Kα radiation (λ = 1.5406 Å) at a scan rate of 2°/min over the 2θ range of 10–80°. Magnetic susceptibility measurements were carried out by the Faraday method at room temperature.

Synthesis of the nano-ligand PHBTA

The target ligand was prepared in two consecutive steps following a modified protocol originally described by Al-Adilee and co-workers [8], with certain procedural refinements.

Step 1. Diazotization. A quantity of 0.535 g (0.0025 mol) of [1,1'-biphenyl]-3,3',4,4'-tetraamine was dissolved in a mixture of 5 mL concentrated HCl (37 %) and 40 mL deionized water. The solution was cooled in an ice-salt bath to below 5 °C. To this, a solution of 0.69 g (0.01 mol) of sodium nitrite dissolved in 25 mL distilled water was added drop-wise under vigorous stirring, maintaining the temperature strictly below 5 °C throughout. The resulting diazonium salt solution was kept under stirring for approximately 40 min to ensure complete tetra-diazotization.

Step 2. Azo-coupling. The freshly prepared diazonium solution was added drop-wise,

with continuous stirring and cooling, to a pre-cooled solution of 1.8 g (0.01 mol) propyl 4-hydroxybenzoate dissolved in a mixture of 17 mL NaOH (10 % w/v) and 30 mL cold ethanol at 0–5 °C. A light reddish-orange coloration developed progressively. After the addition was complete, the reaction mixture was stirred for 40 min and then left overnight at ambient temperature to allow full coupling. The precipitate was collected by filtration, washed several times with distilled water to remove inorganic salts, air-dried, and recrystallized from absolute ethanol. Final drying was carried out in an oven at 50 °C for several hours. The product was obtained in 83 % yield as a yellowish-red solid, m.p. 147–150 °C.

Preparation of the metal nano-complexes

Ni(II) complex. A solution of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (0.238 g, 0.001 mol) in 25 mL ammonium acetate buffer was added to a solution of PHBTA (0.979 g, 0.001 mol) in 25 mL absolute ethanol, maintaining a 1:1 molar ratio [M:L]. The mixture was heated under reflux for 3 hours. Upon cooling, a purple precipitate formed, which was filtered, washed with distilled water and ethanol, recrystallized

from absolute ethanol, and dried in air. Yield: 74 %, m.p. 330–332 °C.

Cu(II) complex. The same procedure was followed using $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ (0.171 g, 0.001 mol) in place of the nickel salt. A purple precipitate was isolated in 72 % yield, m.p. 344–346 °C.

In vitro cytotoxicity assay

The antiproliferative activity of PHBTA, $[\text{Ni}(\text{PHBTA}-2\text{H})(\text{H}_2\text{O})_2]$, and $[\text{Cu}(\text{PHBTA}-2\text{H})(\text{H}_2\text{O})_2]$ was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay on two cell lines: the human breast adenocarcinoma cell line MCF-7 and the normal human foreskin fibroblast cell line HFF. Cells were cultured in RPMI-1640 medium supplemented with 10 % fetal bovine serum and 1 % penicillin–streptomycin under a humidified atmosphere of 5 % CO_2 at 37 °C. Cells were seeded in 96-well plates at a density of approximately 1×10^4 cells/well and incubated for 24 h to allow attachment. The compounds were dissolved in DMSO and applied at concentrations ranging from 12.5 to 800 $\mu\text{g}/\text{mL}$. After 48 h incubation, 20 μL of MTT solution (5 mg/mL in PBS) was added to each well, and the plates

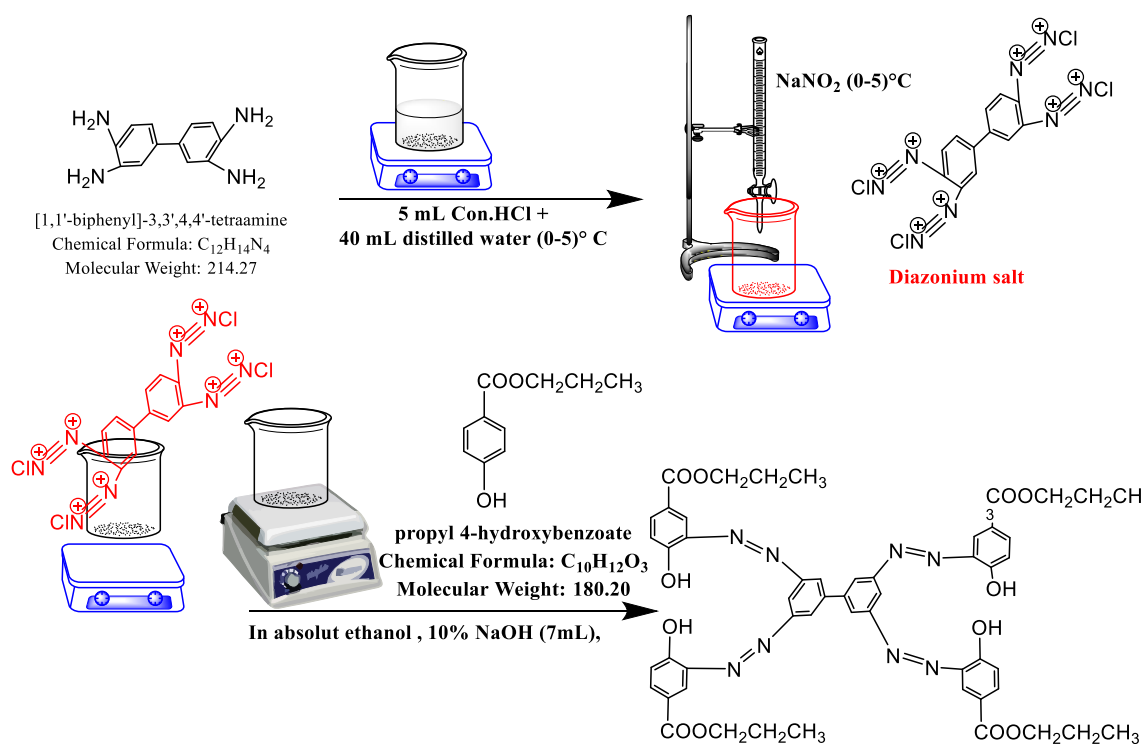


Fig. 1. Synthesis pathway of the tetrakis-azo ligand PHBTA via diazotization and azo-coupling reactions.

were incubated for a further 4 h. The formazan crystals were solubilized in 150 μ L DMSO, and the absorbance was read at 570 nm. Cell viability was expressed as a percentage relative to the untreated control, and IC_{50} values were extracted from dose–response curves.

RESULTS AND DISCUSSION

Elemental analysis (CHN)

The elemental microanalysis results for the free nano-ligand PHBTA and its two metal nano-complexes are compiled in Table 1. For PHBTA itself, the found percentages of C (63.87), H (5.18), and N (11.49) matched the calculated values for the molecular formula $C_{52}H_{50}N_8O_{12}$ (M.W. = 979.0 g/mol) within the accepted tolerance, thereby confirming the proposed structure. In the case of the Ni(II) complex, the data agreed with the formula $[Ni(PHBTA-2H)(H_2O)_2]$ ($C_{52}H_{52}N_8O_{14}Ni$, M.W. = 1071.7), where two protons from

phenolic OH groups are lost upon coordination and two water molecules occupy ancillary coordination sites. The nickel content was found to be 5.52 %, against a theoretical value of 5.48 %, which is consistent with a 1:1 metal-to-ligand stoichiometry. Similarly, for the Cu(II) complex $[Cu(PHBTA-2H)(H_2O)_2]$ ($C_{52}H_{52}N_8O_{14}Cu$, M.W. = 1076.6), the found Cu content of 5.96 % compared favorably to the theoretical 5.90 %. In both cases, the small deviations between experimental and calculated compositions lend strong support to the proposed formulae.

Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectra of the free nano-ligand and its two nano-complexes are presented comparatively in Fig. 2, and the principal absorption frequencies are gathered in Table 2. In the spectrum of PHBTA, a broad band centered at 3269 cm^{-1} is assignable to the O–H stretching vibration of the phenolic

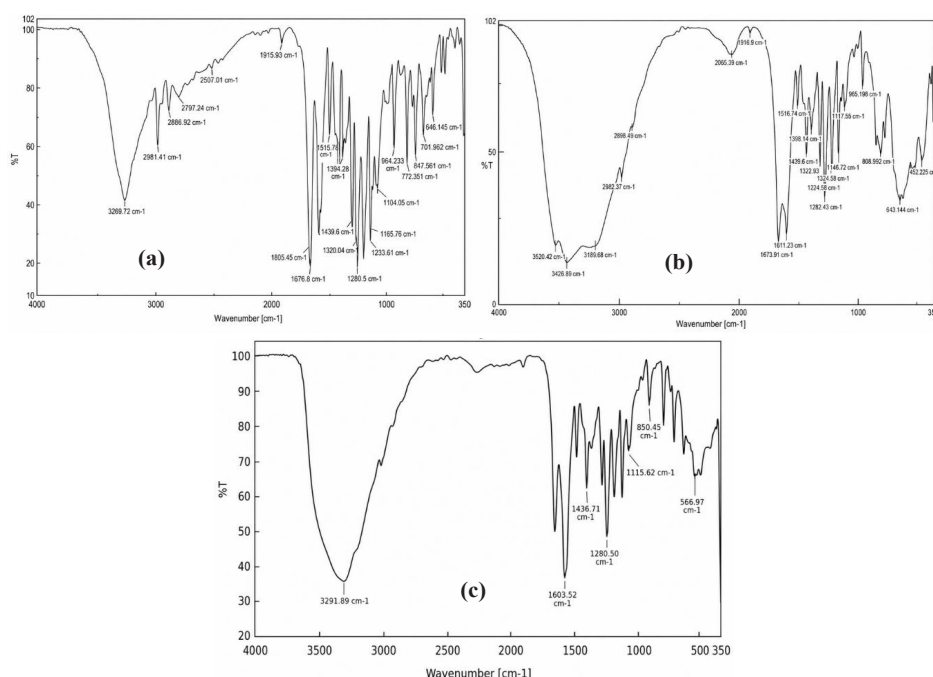


Fig. 2. FTIR spectra of (a) the free nano-ligand PHBTA, (b) $[Ni(PHBTA-2H)(H_2O)_2]$, and (c) $[Cu(PHBTA-2H)(H_2O)_2]$.

Table 1. Elemental analysis data (CHN) for PHBTA and its Ni(II) and Cu(II) nano-complexes.

Compound	C calc. (found)	H calc. (found)	N calc. (found)	M calc. (found)	Yield %	M.P. (°C)
PHBTA ($C_{52}H_{50}N_8O_{12}$)	63.80 (63.87)	5.15 (5.18)	11.45 (11.49)	—	83	147–150
$[Ni(PHBTA-2H)(H_2O)_2]$ ($C_{52}H_{52}N_8O_{14}Ni$)	58.28 (58.31)	4.89 (4.94)	10.46 (10.51)	5.48 (5.52)	74	330–332
$[Cu(PHBTA-2H)(H_2O)_2]$ ($C_{52}H_{52}N_8O_{14}Cu$)	58.01 (58.08)	4.87 (4.93)	10.41 (10.46)	5.90 (5.96)	72	344–346

hydroxyl groups. The fact that this band is broad rather than sharp suggests the involvement of intermolecular and perhaps intramolecular hydrogen bonding. Two bands at 2981 and 2886 cm^{-1} correspond to the aliphatic C–H stretching modes of the propyl ester chains. A strong absorption at 1676 cm^{-1} is characteristic of the C=O stretching of the ester carbonyl group, while the peaks at 1515 and 1439 cm^{-1} are attributed to aromatic C=C and N=N (azo) stretching vibrations, respectively. Additional bands at 1280 and 1165 cm^{-1} arise from C–O stretching of the phenolic and ester functionalities.

Upon coordination with the Ni(II) ion, several notable changes were observed. The O–H band

shifted from 3269 to 3426 cm^{-1} , accompanied by alteration of its intensity and breadth. This displacement suggests modification of the hydrogen-bonding network as the phenolic oxygen engages in metal–ligand bonding. The C=O band remained essentially unchanged at 1673 cm^{-1} , indicating that the ester carbonyl is not directly involved in the coordination sphere a finding consistent with the ^1H -NMR evidence discussed later. The azo band appeared at 1439 cm^{-1} without significant shift, though a very slight broadening was noticed. More importantly, new weak bands emerged at 643 and 452 cm^{-1} , assigned to the $\nu(\text{M–O})$ and $\nu(\text{M–N})$ stretching vibrations, respectively, providing unambiguous confirmation

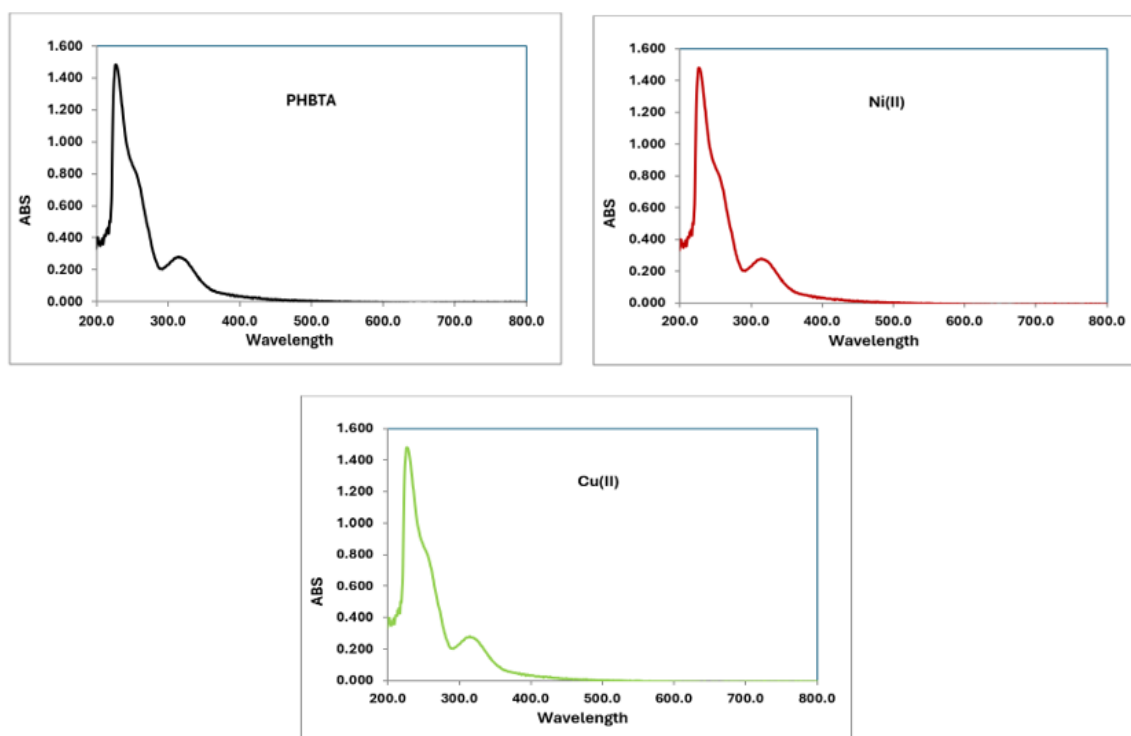


Fig. 3. UV-Vis electronic spectra of the free nano-ligand PHBTA and its metal nano-complexes in absolute ethanol.

Table 2. Selected FTIR absorption bands (cm^{-1}) for PHBTA and its Ni(II) and Cu(II) nano-complexes.

Assignment	PHBTA	Ni(II) complex	Cu(II) complex
$\nu(\text{O–H})$	3269 (br)	3426 (br)	3291 (br)
$\nu(\text{C–H})$ aliph.	2981, 2886	2982, 2898	2983
$\nu(\text{C=O})$	1676 (s)	1673	1678
$\nu(\text{N=N})$	1439	1439	1436
$\nu(\text{C=C})$ Ar	1515	1516	1513
$\nu(\text{C–O})$	1280	1282	1280
$\nu(\text{M–O})$	—	643 (w)	566 (w)
$\nu(\text{M–N})$	—	452 (w)	455 (w)

s = strong, w = weak, br = broad

that the nickel ion is bound through the phenolate oxygen and azo nitrogen donor sites.

In the Cu(II) complex, a similar pattern was observed, albeit with some differences. The O–H stretch moved to 3291 cm^{-1} , while the C=O band stayed at 1678 cm^{-1} and the azo band shifted slightly to 1436 cm^{-1} . The new M–O and M–N bands appeared at 566 and 455 cm^{-1} , respectively. The different frequencies of these metal–ligand vibrations relative to the Ni(II) complex reflect the differences in ionic radii and bonding strengths of the two metal centers. Taken together, the FTIR data provide compelling support for a coordination mode in which the ligand acts as a dibasic tetradentate chelate, binding through two phenolate oxygens and two azo nitrogens, while the ester groups remain uninvolved.

Electronic spectra and magnetic measurements

The electronic spectrum of the free nano-ligand PHBTA, recorded in absolute ethanol, displayed two absorption maxima. The first, at 228 nm , is attributed to the $\pi \rightarrow \pi^*$ transition arising from the conjugated aromatic C=C system, while the second, appearing at 325 nm , corresponds to the $n \rightarrow \pi^*$ transition involving the lone pair electrons on the azo nitrogen atoms ($-\text{N}=\text{N}-$). These assignments are in good agreement with previously reported values for structurally analogous azo dyes [15].

The electronic spectrum of the Ni(II) complex exhibited four absorption bands at 710 , 394 , 360 , and 240 nm . The first three are characteristic of d–d transitions expected for a d^8 ion in a regular octahedral ligand field and are assigned as ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{2g}(\text{F})$ (ν_1 , 710 nm), ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{F})$ (ν_2 , 394 nm), and ${}^3\text{A}_{2g} \rightarrow {}^3\text{T}_{1g}(\text{P})$ (ν_3 , 360 nm), respectively. The band at 240 nm is attributed to intra-ligand transitions. The effective magnetic moment of 2.97 B.M. falls within the range expected for a high-spin octahedral Ni(II) complex (2.83 – 3.50 B.M.) with sp^3d^2 hybridization, further corroborating the proposed geometry [16].

For the Cu(II) complex, a broad d–d absorption band of moderate intensity was observed at 544 nm . The breadth of this band is indicative of three closely spaced electronic transitions: ${}^2\text{B}_{1g} \rightarrow {}^2\text{A}_{1g}$ ($\text{dx}^2\text{-y}^2 \rightarrow \text{dz}^2$), ${}^2\text{B}_{1g} \rightarrow {}^2\text{B}_{2g}$ ($\text{dx}^2\text{-y}^2 \rightarrow \text{dyz}$), and a charge-transfer component, which overlap due to the Jahn–Teller distortion characteristic of hexacoordinated Cu(II) systems. An additional band at 234 nm was attributed to intra-ligand transitions. The measured μ_{eff} of 1.71 B.M. is

consistent with a single unpaired electron in a distorted octahedral environment with sp^3d^2 (low-spin) hybridization [17]. The Jahn–Teller effect causes either axial elongation (Z-out) or compression (Z-in), leading to the observed spectral broadening (Fig. 3).

¹H-NMR spectroscopy

The ${}^1\text{H}$ -NMR spectrum of PHBTA was recorded at 400 MHz in DMSO-d_6 . A singlet at $\delta\ 9.03\text{ ppm}$, integrating for approximately 4H , was assigned to the phenolic hydroxyl protons. The relatively low-field position of this signal reflects the deshielding influence of the adjacent electron-withdrawing azo ($-\text{N}=\text{N}-$) and ester ($-\text{COOPr}$) groups, together with possible intramolecular hydrogen bonding with the azo nitrogen atoms. The aromatic protons resonated as a cluster of multiplets between $\delta\ 7.00$ and 7.95 ppm , which can be sub-divided into several groups: signals at $\delta\ 7.89$ – 7.95 ppm were attributed to protons adjacent to the azo and ester functions, those at $\delta\ 7.60$ – 7.80 ppm to the biphenyl core and terminal ring protons, and those at $\delta\ 7.52$ – 7.56 ppm to protons in relatively electron-rich environments. A set of signals at $\delta\ 6.72$ – 6.78 ppm (ca. 2H) were ascribed to aromatic protons experiencing the resonance donating effect of the phenolic OH group.

Regarding the propyl ester portion, a strong signal at $\delta\ 3.89\text{ ppm}$ (ca. 12H) was assigned to the $-\text{OCH}_2-$ methylene protons directly attached to the ester oxygen. A multiplet near $\delta\ 1.90\text{ ppm}$ corresponded to the middle $-\text{CH}_2-$ methylene protons, and a signal around $\delta\ 1.00\text{ ppm}$ was attributed to the terminal $-\text{CH}_3$ protons of the propyl chain. The residual solvent peak appeared at $\delta\ 2.50\text{ ppm}$. Overall, the spectral pattern is entirely consistent with the proposed tetrakis-azo structure of PHBTA.

X-ray diffraction (XRD)

The powder XRD patterns of the free nano-ligand and the two nano-complexes were recorded over the 2θ range of 10 – 80° (Fig. 4). The diffractogram of PHBTA showed a series of well-defined sharp peaks at $2\theta = 25.72^\circ$ (100%), 20.10° (70.38%), 45.58° (32.14%), and several minor reflections, indicating a predominantly crystalline nature with d-spacings ranging from 8.65 to $3.46\text{ \AA}$. Upon formation of the Ni(II) complex, the diffraction pattern changed substantially the main reflection shifted to $2\theta = 16.14^\circ$ (100%) with additional peaks

at 18.79° (92.43%), 20.27° (75.13%), and 57.33° (68.80%). A notable broadening of the peaks was observed, with the FWHM increasing from 0.1476° for the free nano-ligand to 0.1968–0.5904° for the complex. The peak broadening is attributable to the reduction in crystallite size that accompanies metal coordination and the incorporation of water molecules into the lattice. The mean crystallite size was estimated using the Scherrer equation ($D = K\lambda / \beta \cos \theta$), where $K = 0.9$ and β is the full width at half maximum of the most intense reflection.

The calculated average crystallite size for the most intense reflection of PHBTA was approximately 33.12 nm ($2\theta = 25.72^\circ$, $d = 3.461 \text{ \AA}$), whereas for the Ni(II) complex the average crystallite size decreased to approximately 40.77 nm ($2\theta = 16.14^\circ$, $d = 5.489 \text{ \AA}$), and the Cu(II) complex exhibited a crystallite size of about 51.30 nm ($2\theta = 17.10^\circ$, $d = 5.181 \text{ \AA}$), all confirming the nanocrystalline nature of the prepared nano-compounds [18]. For the Cu(II) complex, the XRD pattern exhibited sharp intense peaks at $2\theta = 17.10^\circ$ (100%), 21.52°

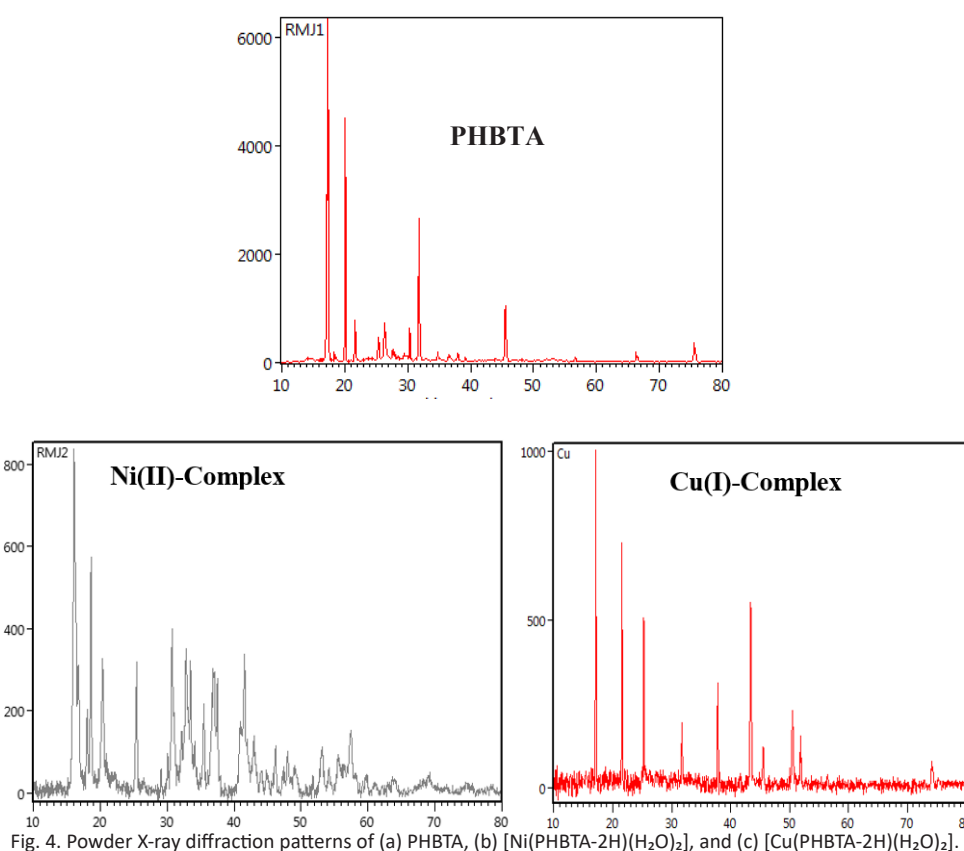


Fig. 4. Powder X-ray diffraction patterns of (a) PHBTA, (b) $[\text{Ni}(\text{PHBTA-2H})(\text{H}_2\text{O})_2]$, and (c) $[\text{Cu}(\text{PHBTA-2H})(\text{H}_2\text{O})_2]$.

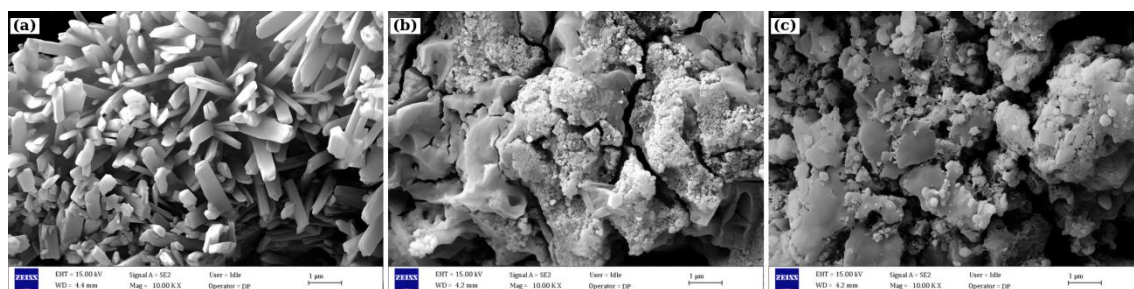


Fig. 5. FESEM micrographs of (a) PHBTA (10.00 kV), (b) $[\text{Ni}(\text{PHBTA-2H})(\text{H}_2\text{O})_2]$, and (c) $[\text{Cu}(\text{PHBTA-2H})(\text{H}_2\text{O})_2]$.

(97.54%), 25.18° (92.30%), 31.74° (82.89%), and 37.69° (82.57%), indicating a relatively high degree of crystallinity. The crystallite sizes ranged from 29.79 to 59.16 nm, and the presence of numerous well-resolved peaks is attributable to the ordered lattice arrangement of this complex. The clear differences between the XRD patterns of the free nano-ligand and the nano-complexes constitute further evidence for successful nano-complex formation and structural reorganization upon coordination.

Field-emission scanning electron microscopy (FESEM)

The surface morphology of PHBTA and its Ni(II) and Cu(II) nano-complexes was examined by FESEM at a magnification of 10.00 kX, and the resulting micrographs are displayed in Fig. 5. The free nano-ligand PHBTA exhibited a relatively ordered morphology consisting of small, regular crystalline platelets with low agglomeration

compared to typical organic dyes. This relatively uniform particle distribution reflects the good crystallinity of the compound, which is consistent with the sharp peaks observed in its XRD pattern.

After complexation with Ni(II), the morphology underwent a marked transformation. The Ni(II) complex presented a fairly homogeneous surface composed of fine granular aggregates that were more compact and ordered than those of the parent ligand. The particles appeared to be interconnected in a relatively uniform manner, suggesting an improved degree of crystalline ordering driven by the chelate effect. This observation aligns well with the FTIR and elemental data, which confirmed successful chelation.

The Cu(II) complex, on the other hand, displayed closely packed granular agglomerates with limited interstitial voids. The surface roughness increased perceptibly compared to both the free nano-ligand and the Ni(II) nano-complex, and the primary particles appeared somewhat larger

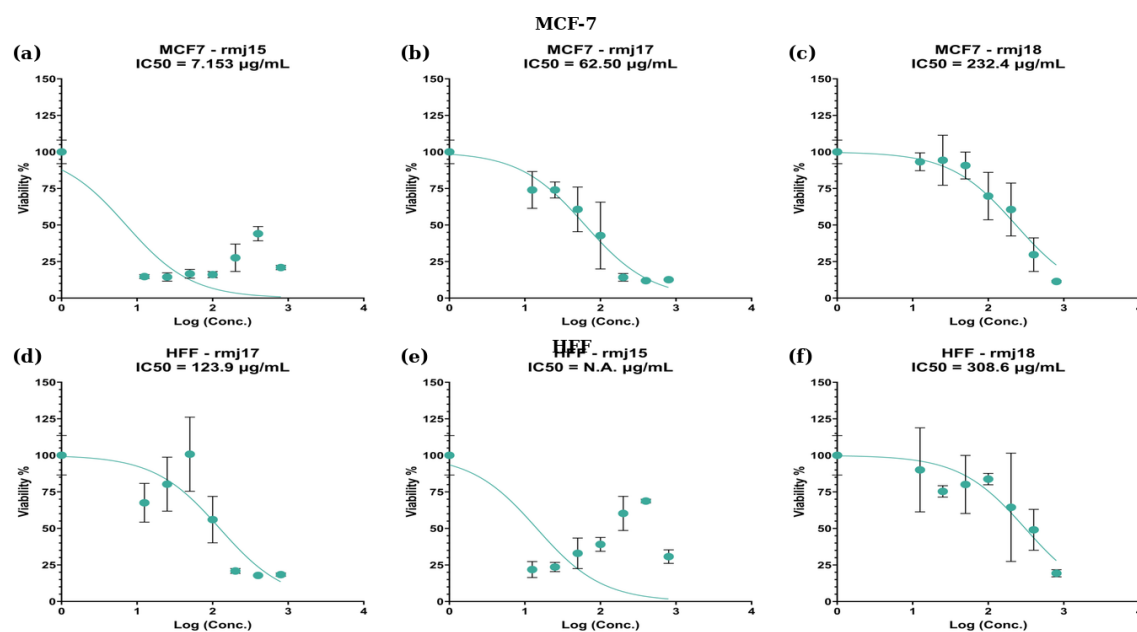


Fig. 6. Dose-response curves showing the effect of PHBTA and its Ni(II) and Cu(II) nano-complexes on the viability of MCF-7 (a-c) and HFF (d-f) cell lines.

Table 3. IC₅₀ values (µg/mL) for PHBTA and its Ni(II) and Cu(II) nano-complexes against MCF-7 and HFF cell lines.

Compound	IC ₅₀ MCF-7	IC ₅₀ HFF
PHBTA	62.50	123.9
[Ni(PHBTA-2H)(H ₂ O) ₂]	7.153	N/A
[Cu(PHBTA-2H)(H ₂ O) ₂]	232.4	308.6

and more densely aggregated. This difference in morphology can be attributed to the Jahn–Teller distortion in the Cu(II) coordination sphere, which introduces structural strain and alters the crystal packing mode. In both nano-complexes, the obvious morphological changes serve as additional visual evidence of nano-complex formation and structural reorganization at the nanoscale.

In vitro cytotoxicity

The cytotoxic potentials of the free nano-ligand PHBTA and its Ni(II) and Cu(II) nano-complexes were assessed via the MTT assay at concentrations ranging from 12.5 to 800 µg/mL against the MCF-7 breast cancer cell line and the normal HFF fibroblast cell line. The resulting IC₅₀ values are summarized in Table 3 and the dose–response curves are shown in Fig. 6.

The free nano-ligand PHBTA exhibited moderate antiproliferative activity against MCF-7 cells, with an IC₅₀ of 62.50 µg/mL and a dose-dependent decline in cell viability. More strikingly, the Ni(II) nano-complex showed outstanding cytotoxicity, recording an IC₅₀ as low as 7.153 µg/mL roughly nine-fold more potent than the uncomplexed ligand. This dramatic enhancement is attributed to the chelation effect, whereby coordination to the nickel ion increases the overall lipophilicity of the molecular assembly by delocalizing the metal's positive charge over the chelate ring, thereby facilitating permeation through the lipid bilayer of the cell membrane [19]. Once inside the cell, the complex can interact with DNA via intercalation or groove binding and may also interfere with key metalloenzymes that regulate the cell cycle, triggering apoptotic pathways [20].

In contrast, the Cu(II) nano-complex displayed a comparatively modest IC₅₀ of 232.4 µg/mL against MCF-7, which is actually higher (i.e., less potent) than the free nano-ligand. This somewhat counterintuitive result may be explained by the Jahn–Teller distorted geometry of the Cu(II) center: the elongated axial bonds could reduce the effective interaction of the complex with biological targets, or the solubility and cellular uptake of this particular complex may be less favorable. The Cu(II) complex exhibited an IC₅₀ of 308.6 µg/mL toward HFF normal cells, giving a selectivity index (SI = IC₅₀ HFF / IC₅₀ MCF-7) of about 1.33, which is rather low. For the Ni(II) complex, the IC₅₀ toward HFF could not be determined within the tested concentration range (denoted as N/A), suggesting

significantly lower toxicity to normal cells, though higher-concentration testing would be needed to establish a precise selectivity index.

Overall, these results demonstrate that the nature of the central metal ion plays a decisive role in determining both the magnitude and selectivity of the cytotoxic effect. The Ni(II) nano-complex stands out as a particularly promising candidate for further investigation, given its potent activity against MCF-7 cells combined with an apparently favorable toxicity profile toward normal fibroblasts.

CONCLUSION

A novel tetrakis-azo nano-ligand (PHBTA) was successfully synthesized in good yield through the diazotization–coupling of biphenyltetraamine with propyl 4-hydroxybenzoate, and its Ni(II) and Cu(II) nano-complexes were prepared under reflux conditions. The combined spectroscopic and analytical evidence including CHN, FTIR, UV-Vis, and ¹H-NMR consistently indicated that the ligand coordinates in a dibasic tetradentate fashion via the deprotonated phenolic oxygens and the azo nitrogen atoms, with the ester groups remaining outside the coordination sphere. The magnetic and electronic data supported a regular high-spin octahedral geometry for the Ni(II) complex and a Jahn–Teller distorted octahedral structure for the Cu(II) analogue. Nano-structural investigations by FESEM and XRD revealed clear morphological and crystallographic changes upon complexation, with nanocrystalline aggregates forming in both compounds. The *in vitro* MTT assay demonstrated that the Ni(II) nano-complex possesses remarkable anticancer activity against MCF-7 breast cancer cells (IC₅₀ = 7.153 µg/mL), far exceeding that of the free nano-ligand and the Cu(II) nano-complex. These findings underscore the importance of the metal ion identity in modulating biological activity and highlight the Ni(II)–PHBTA nano-complex as a lead compound worthy of more detailed pharmacological and mechanistic studies.

CONFLICT OF INTEREST

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

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