201

Egypt. J. Chem. **Vol. 67**, No. 4 pp. 639 - 648 (2024)

Egyptian Journal of Chemistry

Synthesis, Characterization, Biological and Anticancer Activities Studies of Ternary Cephardine Pd (II) Complexes

Lamis O. Abo baker, Walaa H. Mahmoud, Abeer T. AbdEl-karim, Ahmed A. El-Sherif*

Department of Chemistry, Faculty of Science, Cairo University, Egypt

Abstract

This manuscript outlines the synthesis and spectral characterization of innovative Pd (II) coordination ternary metal complexes. The complexes are synthesized using aliphatic amines as the primary ligand L1 and Cephradine as the secondary ligand L2. The identification of these newly formed ternary metal complexes involves a comprehensive range of spectrophotometric and physico-chemical analyses, encompassing elemental analysis, Fourier-transform infrared spectroscopy (FT-IR), proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, determination of melting point, conductivity measurements, mass spectrometry, and thermal analysis (TG and DTG). Furthermore, the antimicrobial activity of these complexes is evaluated against various microorganisms, including Gram-positive bacteria such as *Staphylococcus aureus* and *Streptococcus mutans*, Gram-negative bacteria such as *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*, as well as fungi including *Candida albicans*, *Aspergillus Nigar*, and *Aspergillus Ochraceous*. To assess their efficacy, the complexes are compared to established standards in the field, namely Ampicillin and Gentamicin as antibacterial agents, and Nystatin as an antifungal agent. Moreover, the manuscript explores the anticancer properties of the ligands and their corresponding metal complexes using viability assays against human cancer cells (MCF-7 cells).

1. Introduction

Cephradine, classified as a first-generation cephalosporin antibiotic, is known for its effective chelating properties [2]. These antibiotics are vital for combating bacterial infections due to their favorable antibacterial efficacy, resistance to β-lactamases, and pharmacokinetic attributes [1-3]. The relationship between antibiotic effectiveness and their ability to form complexes with metal ions has prompted investigations into the complexation capabilities of antibiotics as ligands [7, 8]. Therefore, studying the binary and ternary complexes of cephradine contributes to a better understanding of the factors driving the formation of such complexes in biological systems. The interaction between metal complexes and antibiotics can either enhance or inhibit their antimicrobial activity, with many instances demonstrating an improvement in

pharmacological activity upon complexation with metal complexes compared to the free ligands [6, 7]. Alzheimer's disease (AD) is the leading cause of dementia among the elderly [1], and its treatment relies on cholinesterase inhibitors [2]. The pathogenesis of AD involves a reduction in acetylcholine, a neurotransmitter crucial for memory [3]. In the brain, acetylcholinesterase (AChE) regulates ACh activity by hydrolyzing it into acetal, and in AD, AChE activity either remains unchanged or increases [4]. While four cholinesterase inhibitors (tacrine, donepezil, rivastigmine, and galantamine) are approved by the US Food and Drug Administration, they come with adverse side effects [5]. Metal ions play diverse roles in brain function, participating in redox reactions, amyloid-β aggregation, and oxidative stress, which are central to AD pathogenesis [6]. Therefore, metal chelators are considered potential AChE and BuChE inhibitors [7, 8]. Palladium complexes containing amino acids

found in biological systems may mitigate toxic side effects or enhance the concentration of these complexes inside cells, thereby improving their antitumor properties [9]. In 1991, Mital et al. reported the synthesis of palladium (II) complexes incorporating 1, 10-phenanthroline and amino acids, finding that [Pd(phen)(valine)]$C_{12}H_2O$ had a lower IC_{50} value against sarcoma P388 lymphocytic leukemia cells compared to cisplatin [9]. More recently, our research has focused on the synthesis, characterization, biological activities, and anticancer and anti-Alzheimer's activities of select ternary Pd (II) complexes.

Materials and methods
2. Experimental
2.1. Chemicals and reagents
We utilized chemicals of the highest analytical grade, ensuring optimal purity for all experimental procedures. The substances employed included N,N'dimethylethylenediamine($C_4H_{12}N_2$), N-ethylethylenediamine ($C_4H_{12}N_2$), N,N'diethylethylenediamine ($C_6H_{16}N_2$) sourced from Sigma-Aldrich, and cephradine ($C_{16}H_{19}N_3O_4S$.$2H_2O$). Key reagent K_2PdCl_4 was obtained from Merck. In our organic solvent-based processes, ethyl alcohol (99%) and dimethylformamide (DMF) were utilized. Deionized water from glass equipment consistently served as the source for all preparations. For our research, we employed the MCF7 breast tumor cell line, which had been cryopreserved at -180°C in liquid nitrogen and obtained from the American Type Culture Collection. The maintenance and subculturing of these tumor celllines occurred at the National Cancer Institute in Cairo, Egypt.

2.2. Solutions
To generate newly prepared stock solutions of mixed ligand complexes with a concentration of 1×10^{-3} M, we accurately measured and dissolved the chelates in an appropriate volume of DMF. Following this, we assessed the conductivities of the resultant complex solutions. The metal salt solutions employed in ourexperiments underwent standardization using established and recommended procedures [9, 10].

2.3. Instrumentation
We performed elemental analyses of carbon, hydrogen, and nitrogen using a CHNS932 (LECO) Vario Elemental analyzer at Cairo University's Microanalytical Center in Egypt. Melting points were measured using a triforce XMTD-3000 apparatus. Fourier transform infrared (FT-IR) spectra spanning

the 4000–400 cm^{-1} range were recorded with a Perkin-Elmer 1650 spectrometer, using KBr disks as the medium.

^{1}H NMR spectra in dimethyl sulfoxide-d$_6$ (DMSO-d$_6$) solutions at room temperature were recorded using a 300-MHz Varian-Oxford Mercury spectrometer, with tetramethylsilane as an internal standard. Molar conductivity of solid complex solutions at a concentration of 10^{-3} M in ethanol was assessed using a Jenway 4010 conductivity meter. Mass spectra were obtained through electron ionization at 70 eV, employing an MS-5988 GS-MS Hewlett-Packard instrument at Cairo University's Micro analytical Center.

Antimicrobial assays were conducted at Cairo University's Microanalytical Center, while anticancer activity experiments were performed at the National Cancer Institute, specifically in the Department of Cancer Biology and the Department of Pharmacology. Optical density (OD) measurements for each well were determined spectrophotometrically at 564 nm using an enzyme-linked immunosorbent assay (ELISA) microplate reader (Meter Tech. R960, USA).

2.4. Procedures
Synthesis of the metal complexes
To synthesize mixed ligand complexes of palladium with aliphatic diamines using a 1:1 metal-to-diamine molar ratio, the following procedure was employed: Initially, $PdCl_2$ was combined with KCl in a 1:2 ratio. The mixture was then subjected to continuous stirring and heating up to 50°C until complete dissolution occurred, resulting in a clear solution. Following this, the solution underwent filtration to eliminate any impurities or undissolved substances. Subsequently, the aliphatic diamine was added dropwise with continuous stirring at a 1:1 ratio to the filtered solution. The resulting precipitates were filtered, and the solids obtained, ranging in colorfrom yellow to brown, were subsequently dried under vacuum conditions.

For the preparation of mixed ligand complexes involving cephradine, the procedure was as follows: A mixture of binary complexes (Pd:amine) was dissolved (1 mmol in 10 ml of DMF) and then heated with stirring. Cephradine solution (1 mmol in 15 ml of distilled water) was immediately added, and the stirring process was continued for 3 hours. This resulted in the formation of ternary complexes with molar ratios of 1:1:1 for metal, amine, and cephradine. The resulting dark brown complexes were filtered, washed with ethanol, and subsequently dried under

vacuum conditions. The yields of each complex can be found in Table 1.

2.5. Antimicrobial activity

The in vitro assessment of the antibacterial and antifungal properties of Ampicillin, Gentamicin, and Nystatin was conducted using the disc diffusion technique, serving as positive controls for Gram-positive bacteria, Gram-negative bacteria, and fungi, respectively [21, 22]. Gram-positive bacterial strains included *Staphylococcus aureus* and *Streptococcus mutans*, Gram-negative bacterial strains included *Escherichia coli*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa*, and fungal strains included *Candida albicans*, *Aspergillus Niger*, and *Aspergillus Ochraceous*.

To prepare stock solutions (1 mmol), the ligands and their complexes were dissolved in DMSO. For antibacterial activity assessment, a nutrient agar medium was prepared and cooled to 47°C before seeding with microorganisms. After solidification, 5-mm-diameter holes were created using a sterile cork borer. The investigated compounds (ligands and metal complexes), dissolved in DMSO at 1×10^{-3} M, were added to Petri dishes (only 0.1 m). The growth plates were incubated for 20 hours at 37°C, and then the inhibition zones were measured in millimeters. The antimicrobial activity experiments were conducted in triplicate, and the average of the final readings was determined for each assessment [23].
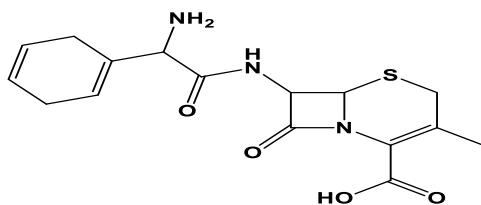
Scheme 1: Structure of cephardine drug

2.6. Antitumor activity

spectrophotometrically at 564 nm using an ELISA microplate reader. Following this, the mean background absorbance was automatically subtracted, and the mean values for each drug concentration were computed.

To establish the survival we assessed the potential cytotoxicity of the compounds using the methodology outlined by Skehan and Storeng [14]. Initially, cells were seeded in a 96-multiwell plate at a density of 10,000 cells per well and allowed to adhere to the well surface for 24 hours. Following this, varying concentrations of the tested compounds (ranging from 0, 5, 12.5, 25, 50, to 100 μg/mL) were introduced to the cell monolayer, with triplicate wells prepared for each concentration. The cells were then subjected to a 48-hour incubation period under conditions of 37°C and 5% CO_2.

After the incubation period, the cells underwent fixation, washing, and staining with sulforhodamine B stain. Any excess stain was eliminated using acetic acid, and the stain adhering to the cells was recovered using a Tris-EDTA buffer. The optical density (OD) of each well was measured curve of the breast tumor cell line for each compound; we plotted the relationship between the surviving fraction and the drug concentration. The cell survival percentage was calculated using the provided formula:

Survival fraction = (OD of treated cells) / (OD of control cells)

The analysis involved determining the IC_{50} values, which indicate the concentrations of the ligand or its complexes needed to achieve a 50% inhibition of cell growth. To ensure the reliability and consistency of the results, the experiment was repeated three times.

3. Results and Discussion
3.1. Elemental analyses and molar conductivity measurements

The Pd (II) ternary complexes investigated in this study demonstrate notable stability under standard atmospheric conditions. In practical terms, these complexes exhibitnotable solubility in polarorganic solvents like ethanol (EtOH),methanol (MeOH),dimethylformamide (DMF), and dimethyl sulfoxide (DMSO). However, their insolubility in water is a distinctive feature. Additionally, the stoichiometry and composition of the cephradine (L) ligand and its metal complexes have been substantiated, confirming a consistent metal-to-amine-to-cephradine drug ratio of 1:1:1 in these compounds. This validation was accomplished through elemental analysis, encompassing the determination of metal content, as well as levels of carbon; hydrogen, nitrogen, and chlorine (refer to Table 1). The findings from the elemental analyses closely align with the proposed structural compositions. Three ternary complexes demonstrated notable molar conductance values, with readings ranging from 64 to 72 Ω^{-1} mol^{-1} cm^2, respectively.

The indicated values imply the electrolytic characteristics of the substances, pointing to the existence of anions in the external coordination sphere [17].

3.2. IR spectra

The infrared (IR) spectra offer valuable insights into the coordination of cephradine ligand and amines with Pd (II) [18]. Table (2) summarizes the key IR spectral data [19] and Fig. 1 shows the chart of cephardine and its ternery complexes. In the free cephradine ligand's IR spectrum, distinct bands at 1764 cm^{-1} (C=O of lactam group) and 1650 cm^{-1} (amide carbonyl group) are observed. Interestingly, these bands remain mostly unchanged in the ternary complexes, suggesting limited involvement in the coordination process [20].

Significantly, the bands at 1395 cm⁻¹ (carboxylate symmetrical stretching) and 1188 cm⁻¹(ternary-N) in the ternary complexes shift to lower frequencies (1382-1383 cm⁻¹ and 1103-1107 cm⁻¹, respectively) compared to the free ligand. This shift implies electron density transfer from the donor nitrogen (-N) and oxygen (COO-) atoms to the metal ion, weakening the absorption bands of (-N) and (C=O). Conversely, the free ligand's carboxylate a symmetrical stretching band at 1582 cm⁻¹shifts to higher frequencies (1613-1618 cm⁻¹), suggesting possible bond stabilization. The N-H stretching frequency band at 3220 cm⁻¹ remains largely unchanged in the ternary complexes

[21]. Notably, medium-intensity bands in the region of 588-573 cm⁻¹ (υ (M-O)) and 422-466 cm⁻¹ (υ (M-N)) emerge, indicating cephradine coordination with Pd-comeplxes through the nitrogen (-N) and (C=O) carboxylate group [22].

Additionally, the cephradine IR spectrum exhibits abroad band at 3400 cm⁻¹attributed to O–H groups of aqua ligands. In the three ternary complexes, a broad band in the range of (3426-3430 cm⁻¹) appears due to stretching vibrations of uncoordinated water molecules [23].

Table 1: Show the physical and analytical data of the palladium Ternary Complexes

Compound (chemical formula)	Color Yield (%)	M.p. (°C)	Found (Calcd)					A_m (Ω^{-1} mol⁻¹ cm²)
			C (%)	H (%)	N (%)	S (%)	M (%)	
[Pd(N,N\ D Men)ceph] Cl.3H₂O	Brown (89)	223	37.67 (37.95)	5.84 (5.85)	10.89 (11.07)	5.01 (5.06)	16.63 (16.83)	67
[Pd(NEen)ceph] Cl.2H₂O	Brown (76)	217	39.01 (39.08)	5.51 (5.54)	10.97 (11.40)	5.16 (5.21)	17.19 (17.33)	72
[Pd(N,N\ D Een)ceph] Cl.2H₂O	Brown (83)	225	41.00 (41.10)	6.01 (6.07)	10.38 (10.89)	4.75 (4.98)	16.46 (16.50)	64

Table 2: Show the key IR spectral data of the palladium Ternary Complexes

Compound	υ N–H	υ(co) lactum	υ(co) amide	υ(co) Asymmetric	υ(co) symmetric	Tertiary N	υ(M-O)	υ(M-N)
Cephardine	3220	1764	1650	1582	1395	1188	-	-
[Pd(N,N\ D Men)ceph] Cl.3H₂O	3221	1765	1652	1613	1383	1103	574	422
[Pd(NEen)ceph] Cl.2H₂O	3222	1765	1555	1618	1383	1107	573	466
[Pd(N,N\ D Een)ceph] Cl.2H₂O	3220	1765	1551	1617	1382	1105	588	455

(1)

(1)

(3)
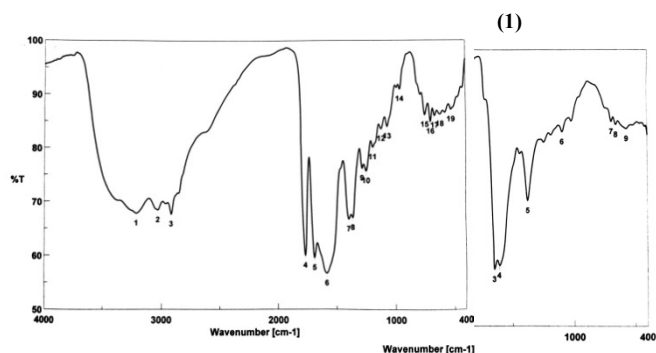
(2)

(4)
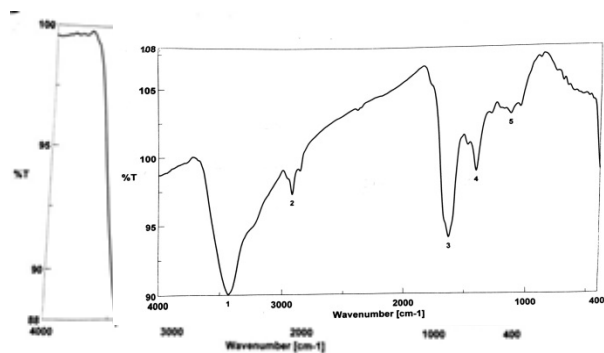
Figure 1: IR spectra of cephardinel and its complexes ,(1) cephardine ,(2) [[Pd(N,N$^\backslash$ D Men)ceph] Cl.3H$_2$O,(3)[Pd(NEen)ceph] Cl.2H$_2$O,(4)[Pd(N,N$^\backslash$ D Een)ceph] Cl.2H$_2$O
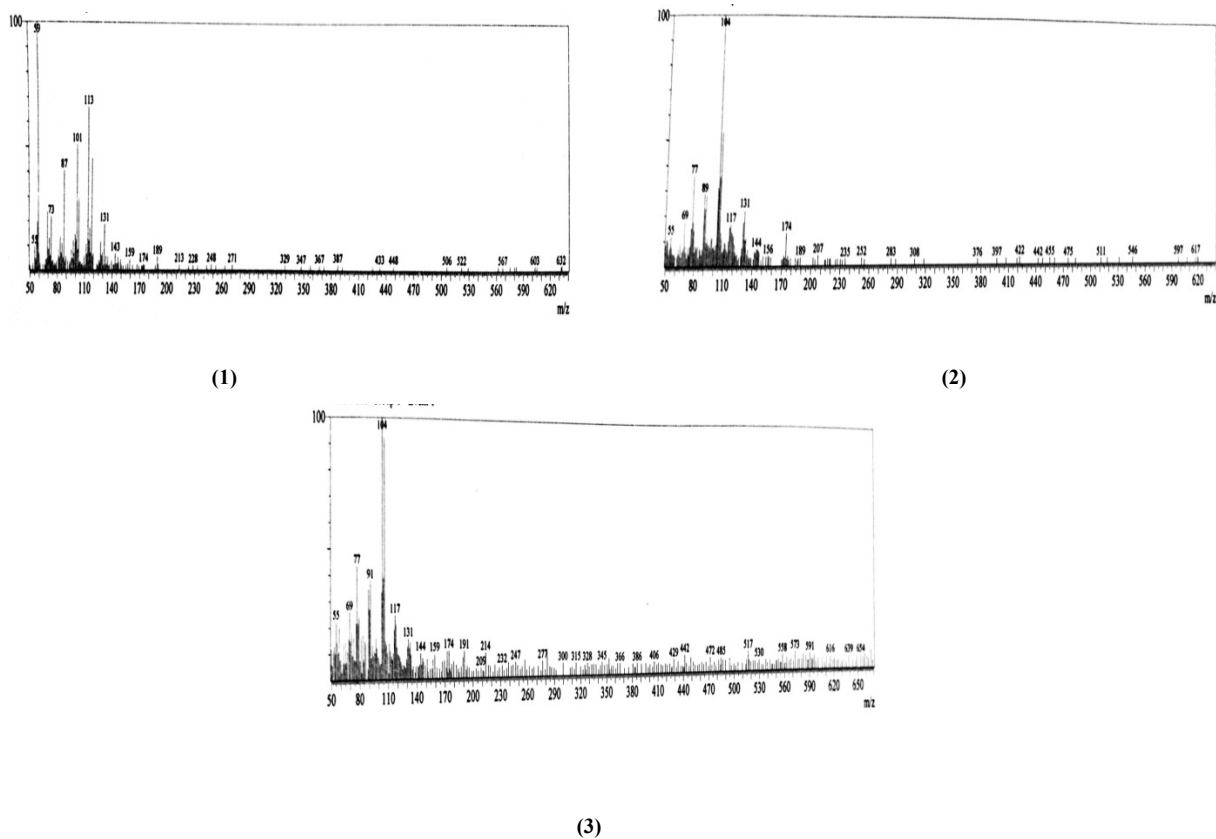
3.3. Mass spectra

The mass spectra analysis of the ternary complexes was conducted using electron ionization mode with a 70 eV energy level. The key observations for each complex are summarized below and show in Fig.2

(1) Ternary complex involving cephradine (N,N'dimethylethylenediamine) Pd CL. 3H$_2$O displayed three distinctive peaks in the mass spectrum. The first peak at m/z 632.1 represented the ternary complex (calculated mass: 632.1 g/mol). The second peak at m/z 347 confirmed the presence of the second ligand, cephradine (calculated mass: 349.4 g/mol). The third peak at m/z 88.2 indicated the presence of the first ligand (N,N'dimethylethylenediamine) (calculated mass: 88 g/mol). These results are in accordance with the expected molecular weight, providing validation for the proposed (1:1:1) Pd: L1: L2 stoichiometric ratio [24].

(2) Ternary complex involving cephradine (N-ethylethylenediamine) Pd CL. 2H$_2$O exhibited three prominent peaks in the mass spectrum. The first peak at m/z 617 (M+3) corresponded to the ternary complex (calculated mass: 614 g/mol). The second peak at m/z 349 confirmed the presence of the second ligand, cephradine (calculated mass: 349.4 g/mol). The third peak at m/z 88.2 indicated the presence of the first ligand, (N-ethylethylenediamine) (calculated mass: 88 g/mol). These findings support the proposed molecular weight and the (1:1:1) Pd: L1: L2 stoichiometric ratio [24].

(3) Ternary complex involving cephradine (N,N'diethylethylenediamine) Pd CL. 2H$_2$O showed three significant peaks in the mass spectrum. The first peak at m/z 643 (M+1) corresponded to the ternary complex (calculated mass: 642.31 g/mol). The second peak at m/z 350.6 (M+1) confirmed the presence of the second ligand, cephradine (calculated mass: 349.4 g/mol). The third peak at m/z 116.2 indicated the presence of the first ligand, N,N'diethylethylenediamine (calculated mass: 116 g/mol). These results align with the proposed molecular weight, providing support for the (1:1:1) Pd: L1: L2 stoichiometric ratio [25].

Figure 2: Mass spectra of ternary complexes(1) [Pd(N,N' D Men)ceph] Cl.3H$_2$O, (2)[Pd(NEen)ceph] Cl.2H$_2$O, (3)[Pd(N,N' D Een)ceph] Cl.2H$_2$O

3.4. ^{1}HNMR spectra

The structural analysis of the cephradine ligand (L2) and its mixed palladium (II) complexes was carried out using NMR data. The ^{1}H NMR spectra for cephradine (L2) and its palladium complexes were recorded in DMSO-d$_6$ with tetramethylsilane (TMS) serving as the internal standard. Notably, in the 1H NMR spectrum of the cephradine ligand, a singular peak at 9.60 ppm corresponding to C-OH (s) was identified. The verification that this peak is associated with the acidic COOH group was established by employing D$_2$O as a solvent, resulting in the disappearance of this band. In the ternary complexes the disappearance of this peak indicates the ionization of COO-, providing supporting evidence for cephradine coordination to the binary complex through C=O and the formation of uninegative complexes. This finding is consistent with conclusions drawn from IR spectral data [26].

Additionally, the ^{1}H NMR spectrum of cephradine revealed signals in the multiplet range of 7.44-7.29 ppm (m), corresponding to the aromatic protons. Singlet peaks were observed at 8.53 ppm for CH-NH2 (s), 8.32 ppm for CH-NH-CH (s), 5.67 ppm for CH-

CH-NH (d), 5.10 ppm for S-CH-CH-NH (d), 4.05 ppm for CH-methionine (d), 3.70 ppm for CH$_2$-CH (s), and 2.49 ppm for CH$_3$ (s).

In the ternary complexes, a subtle downfield shift in the signals was noted, which is attributed to the coordination of cephradine to Pd (II) complexes [27, 28].

3.5. Thermal analyses

The thermal analysis of ternary complexes was carried out using thermogravimetric analysis (TGA) within a temperature range of 10°C to 800°C, employing a heating rate of 5°C/min. The TGA results summarized in Table (3) delineate temperature intervals and the corresponding percentages of mass loss for the investigated complexes.

The observed weight reduction primarily arises from the elimination of uncoordinated water molecules and coordinated species. This mass loss aligns with the elemental analysis findings. Ultimately, the breakdown of residual carbon atoms and metal oxides contributes to the final mass loss [29].

In the case of the ternary complex cephradine (N,N'dimethylethylenediamine) Pd chloride 3H$_2$O,

the TGA profile indicates decomposition occurring between 35°C and 123°C, resulting in a mass loss of 8.54% (equivalent to 54 g/mol). This corresponds to the removal of three water molecules, indicating the elimination of outer coordination water. Further decomposition is observed in the range of 130°C to 622°C, constituting 67.83% (455.5 g/mol) of mass loss. Beyond 800°C, the remaining mass residue decomposes into PdO +2C [30].

For the ternary complex cephradine (N-ethylethylenediamine) Pd chloride $2H_2O$, decomposition takes place between 33°C and 125°C, leading to a mass loss of 5.85% (36 g/mol), attributed to the loss of two water molecules. Additional decomposition occurs in the range of 126°C to 375°C,

accounting for 68.27% (419.5 g/mol) of mass loss. Beyond 800°C, the remaining mass residue decomposes into PdO and 3°C.

In the instance of the ternary complex cephradine (N,N'diethylethylenediamine) Pd chloride $2H_2O$, decomposition is observed between 39°C and 116°C, resulting in a mass loss of 5.60% (54 g/mol), corresponding to the loss of three water molecules. Further decomposition occurs in the range of 116°C to 249°C, constituting 15.00% (96.5 g/mol) of mass loss. In the subsequent range of 249°C to 308°C, there is a mass loss of 42.19% (267 g/mol), and finally, in the range of 309°C to 779°C, a mass loss of 15.10% (97 g/mol) is observed. Beyond 800°C, the remaining mass residue decomposes into (PdO +2C).

Table 3: Thermal Characterization of Ternary Complexes (1–3) Temperature Intervals and Mass Loss Percentages

Complex	TG-range (°C)	DTG max	n*	Mass loss Estim(calcd)% (Totalmass Loss)	Assignment	Residues
1	(35-123) (130-622)	83 267-520	1 2	8.54(8.41) 68.39(67.83) 76.93(76.24)	Loss of $3H_2O$ Loss of $C_{18}H_{30}ClN_5O_3S$	Pdo +2C
2	(33-125) (126-375)	50 261,333	1 2	5.85 (5.98) 68.37(67.83) 74.22 (73.81)	Loss of $2H_2O$ Loss of $c_{17}H_{31}ClN_5O_3S$	PdO +3C
3	(39-116) (116-249) (249-308) (309-779)	57 237 255,283 335,767	1 1 2 2	5.39(5.60) 15.51(15.00) 41.7(42.19) 15.00(15.10) 77.60 (77.89)	Loss of $2H_2O$ Loss of HCL, CO_2, CH_4 Loss of $C_{14}H_{27}N_4O$ Loss of C_4H_3NS	PdO +2C

3.6. Antimicrobial activity

The synthesized Pd(II) complexes were subjected to screening to evaluate their antimicrobial activities against a spectrum of bacterial and fungal strains, encompassing Gram-positive bacteria (Staphylococcus aureus and Streptococcus mutans), Gram-negative bacteria (Escherichia coli, Klebsiella pneumonia, and Pseudomonas aeruginosa), as well as fungal strains (Candida albicans, Aspergillus Niger, and AspergillusOchraceous). The antibacterial and antifungal properties were assessed using the disc diffusion method, and the summarized results are presented in Table 4.The study revealed that the Pd-amine binary complexes investigated exhibited significant activity against both Gram-positive and Gram-negative bacterial strains [31, 32]. Notably, the complex involving (N,N'dimethylethylenediamine) Pd demonstrated robust antibacterial activities, surpassing the other complexes. Furthermore, the synthesized Pd(N,N'dimethylethylenediamine)ceph chloride complex emerged as the sole complex displaying antifungal activities (17.3±0.5) in

comparison to the parent ligand and other complexes [33]

3.7. Anticancer activity

The potential anti-cancer properties of the synthesized complexes were investigated through cytotoxicity assessments on the human breast cancer cell line, MCF7. Utilizing the MTT colorimetric assay, a noteworthy anti-cancer effect against MCF7 cells was observed after 24-hour incubation, as summarized in Table (5). It is essential to highlight that the anti-cancer effects exhibited a concentration-dependent pattern, with a decrease in cell viability as the concentrations of the complexes increased.

Significantly, the complexation of the ligand with metal ions resulted in an augmentation of anti-cancer activity [34, 36].

Among the various complexes, N,N'dimethylethylenediamine based complexes demonstrated the highest cytotoxicity against MCF7 cells. Particularly noteworthy is the remarkably low IC_{50} value less than 20 μg/ml indicating its potent anti-cancer activity.

Table 4: Biological activity of palladium binary and ternary complexes

Sample	Inhibition zone diameter (mm / mg sample)							
	Gram positive bacterial species		Gram negative bacterial species			Fungi		
	Staphyloc occus aureus (ATCC:13 565)	*Streptoco ccus mutans (ATCC:2 5175)*	*Escherich ia coli (ATCC:1 0536)*	*Klebsiella pneumoni a (ATCC:1 0031)*	*Pseudom onas aeruginos a (ATCC:2 7853)*	*Candida albicans (ATCC:1 0231)*	*Asperagillus Nigar (ATCC:164 04)*	*AsperagillusOch raceous (ATCC:22947)*
Cephardine	31.3±*0.6*	29.6±*0.6*	20.3±0.5	24.3±0.6	NA	NA	NA	NA
Pd(N,N D Men)	19.3±0.5	24.6±0.6	25.6±0.6	22.6±0.6	22.3±0.6	NA	NA	NA
[Pd(N,N' D Men)ceph] Cl.3H$_2$O	19.6	NA	NA	NA	NA	17.3±0.5	NA	NA
Pd(NEen)	18.6±0.5	23.0±1.0	25.3±0.6	21.3±0.6	20.6±0.6	NA	NA	NA
[Pd(NEen)ceph] Cl.2H$_2$O	9.3±*0.5*	NA	NA	NA	NA	NA	NA	NA
Pd(N,N D Een)	19.6±0.5	20.6±0.6	22.3±0.6	22.6±0.6	19.6±0.5	NA	NA	NA
[Pd(N,N' D Een)ceph] Cl.2H$_2$O	10.3±*0.5*	8.6±*0.5*	NA	NA	NA	NA	NA	NA
Stand ard — *Ampicil lin*	22±*0.1*	30±*0.5*						
Stand ard — *Gentam icin*			27±*0.5*	25±*0.5*	28±*0.3*			
Stand ard — *Nystati n*						21±*0.5*	19±*0.5*	20.3-±*0.5*

Table 5: Anticancer activity of palladium binary and ternary complexes against breast cancer

samples	Concentration (µg/mL)				
	Surviving fraction (%)				
	12.5	25	50	100	IC$_{50}$
Cefhardine	86	64	59	51	103
Pd(N,N D Men)	54	45	33	27	18.2
[Pd(N,N D Men)ceph] Cl.3H$_2$O	61	52	37	31	18.6
Pd(N Een)	80	73	33.6	21.5	39.5
[Pd(N Een)ceph] Cl.2H$_2$O	85	78	38	27	42.9
Pd(N,N D Een)	70	62	25.6	23	34
[Pd(N,N\ D Een)ceph] Cl.2H$_2$O	73.5	96.5	60	47	86.7

References
1. Small, G., & Bullock, R. (2011). Defining optimal treatment with cholinesterase inhibitors in Alzheimer's disease. *Alzheimer's & Dementia*, *7*(2), 177–184.
2. Han, H. J., Lee, J. J., Park, S. A., Park, H. Y., Kim, J. E., Shim, Y. S., Shim, D.-S., Kim, E.-J., Yoon, S. J., & Choi, S. H. (2011). Efficacy and safety of switching from oral cholinesterase inhibitors to the rivastigmine transdermal patch in patients with probable Alzheimer's disease. *Journal of Clinical Neurology*, *7*(3), 137–142.
3. Adsersen, A., Kjølbye, A., Dall, O., &Jäger, A. K. (2007). Acetylcholinesterase and butyrylcholinesterase inhibitory compounds from Corydalis cava Schweigg. &Kort. *Journal of Ethnopharmacology*, *113*(1), 179–182.
4. Greig, N. H., Lahiri, D. K., &Sambamurti, K. (2002). Butyrylcholinesterase: an important new target in Alzheimer's disease therapy. *International Psychogeriatrics*, *14*(S1), 77–91.
5. Mukherjee, P. K., Kumar, V., Mal, M., & Houghton, P. J. (2007). Acetylcholinesterase inhibitors from plants. *Phytomedicine*, *14*(4), 289–300.
6. Budimir, A., Humbert, N., Elhabiri, M., Osinska, I., Biruš, M., & Albrecht-Gary, A.-M. (2011). Hydroxyquinoline based binders: Promising

ligands for chelatotherapy? *Journal of Inorganic Biochemistry*, *105*(3), 490–496.

7. J Bonda, D., Liu, G., Men, P., Perry, G., A Smith, M., & Zhu, X. (2012). Nanoparticle delivery of transition-metal chelators to the brain: oxidative stress will never see it coming! *CNS & Neurological Disorders-Drug Targets (Formerly Current Drug Targets-CNS & Neurological Disorders)*, *11*(1), 81–85.

8. Masoud, M. S., Ali, A. E., Ghareeb, D. A., & Nasr, N. M. (2013). Synthesis and characterization of cephradine metal complexes as Alzheimer disease therapeutic agent: an in vitro kinetic study on acetylcholinesterase and monoamine oxidase. *Chemical & Pharm. Res*, *5*(12), 1325–1334.

9. Vogel, A. I. (1962). *A Text Book of Quantitative Inorganic Analysis Inlcuding Elementary Instrumental Analysis*. English Language Book Society.

10. Abdelkarim, A. T., Mahmoud, W. H., & El-Sherif, A. A. (2021). Potentiometric, thermodynamics and coordination properties for binary and mixed ligand complexes of copper (II) with cephradine antibiotic and some N-and O-bound amino acids (α-alanine and β-alanine). *Journal of Molecular Liquids*, *328*, 115334.

11. Albert, A. (1979). Selective toxicity. The physico-chemical basis of therapy. *Selective Toxicity. The Physico-Chemical Basis of Therapy., 6th edition.*

12. Chandra, S., Jain, D., Sharma, A. K., & Sharma, P. (2009). Coordination modes of a Schiff base pentadentate derivative of 4-aminoantipyrine with cobalt (II), nickel (II) and copper (II) metal ions: synthesis, spectroscopic and antimicrobial studies. *Molecules*, *14*(1), 174–190.

13. Asla, K. A., Abdelkarimm, A. T., El-Reash, G. M. A., & El-Sherif, A. A. (2020). Potentiometric, Thermodynamics and DFT Calculations of Some Metal (II)-Schiff Base Complexes Formed in Solution. *International Journal of Electrochemical Science*, *15*(5), 3891–3913.

14. Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S., & Boyd, M. R. (1990). New colorimetric cytotoxicity assay for anticancer-drug screening. *JNCI: Journal of the National Cancer Institute*, *82*(13), 1107–1112.

15. Ellman, G. L., Courtney, K. D., Andres Jr, V., & Featherstone, R. M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical Pharmacology*, *7*(2), 88–95.

16. Sezgin, Z., Biberoglu, K., Chupakhin, V., Makhaeva, G. F., &Tacal, O. (2013). Determination of binding points of methylene blue and cationic phenoxazine dyes on human butyrylcholinesterase. *Archives of Biochemistry and Biophysics*, *532*(1), 32–38.

17. Zhang, J., Xu, L., & Wong, W.-Y. (2018). Energy materials based on metal Schiff base complexes. *Coordination Chemistry Reviews*, *355*, 180–198.

18. Refat, M. S., Saad, H. A., Gobouri, A. A., Alsawat, M., Adam, A. M. A., & El-Megharbel, S. M. (2022). Charge transfer complexation between some transition metal ions with azo Schiff base donor as a smart precursor for synthesis of nano oxides: An adsorption efficiency for treatment of Congo red dye in wastewater. *Journal of Molecular Liquids*, *345*, 117140.

19. Anacona, J. R., & Acosta, F. (2006). Synthesis and antibacterial activity of cephradine metal complexes. Journal of Coordination Chemistry, 59(6), 621–627.

20. El-Said, A. I., Aly, A. A. M., El-Meligy, M. S., & Ibrahim, M. A. (2009). Mixed ligand zinc (II) and cadmium (II) complexes containing ceftriaxone or cephradine antibiotics and different donors. *Journal of the Argentine Chemical Society*, *97*(2), 149–165.

21. De, A., Ray, H. P., Jain, P., Kaur, H., & Singh, N. (2020). Synthesis, characterization, molecular docking and DNA cleavage study of transition metal complexes of o-vanillin and glycine derived Schiff base ligand. Journal of Molecular Structure, 1199, 126901.

22. Miyasaka, H., Saitoh, A., & Abe, S. (2007). Magnetic assemblies based on Mn (III) salen analogues. Coordination Chemistry Reviews, 251(21–24), 2622–2664.

23. Ahmed, S.M.; Aly, A.A.; El-Apasery, M.A.; Ragai, S. M Decolorization of Reactive Dyes, Part VIII: Eco-Friendly Approach of Reactive Red 195 Dye Effluents Decolorization Using Geopolymer Cement Based on Metakaolin backed by slag. Egyptian Journal of Chemistry, 2023, 66, 21–27. 10.21608/EJCHEM. 2023.181057.7336

24. Tofiq, D. I., Hassan, H. Q., &Abdalkarim, K. A. (2021). Preparation of a novel Mixed-Ligand divalent metal complexes from solvent free Synthesized Schiff base derived from 2, 6-Diaminopyridine with cinnamaldehyde and 2, 2′-Bipyridine: Characterization and antibacterial activities. Arabian Journal of Chemistry, 14(12), 103429.

25. Aljahdali, M. S., & El-Sherif, A. A. (2020). Synthesis and biological evaluation of novel Zn (II) and Cd (II) Schiff base complexes as antimicrobial, antifungal, and antioxidant agents. *Bioinorganic Chemistry and Applications*, *2020*, 1–17.

26. Kavitha, A., Easwaramoorthy, D., Thangeeswari, T., Parthipan, G., Shanmugan, S., & Ansari, T. (2021). Synthesis and characterization of tritendate Schiff base rare earth nano metal complexes. *Materials Today: Proceedings*, *34*, 453–459.

27. Rajakkani, P., Alagarraj, A., &Thangavelu, S. A. G. (2021). Tetraazamacrocyclic Schiff base metal complexes bearing pendant groups: Synthesis, characterization and bioactivity studies. *Inorganic Chemistry Communications*, *134*, 108989.

28. Kargar, H., Torabi, V., Akbari, A., Behjatmanesh-Ardakani, R., Sahraei, A., &Tahir, M. N. (2020). Pd (II) and Ni (II) complexes containing an asymmetric Schiff base ligand: Synthesis, X-ray crystal structure, spectroscopic investigations and computational studies. *Journal of Molecular Structure*, *1205*, 127642.

29. Amal A. Aly, Safia A. Mahmoud and Morsy A. El-Apasery," Decolorization of reactive dyes, Part I: Eco-friendly approach of reactive dye effluents decolorization using cationized sugarcane bagasse," Pigment & Resin Technology, 47 (2), (2018) 108-115.

30. Mir, M. A. (2022). Synthesis, Catalysis and Antimicrobial activity of 5d-metal chelate complex of Schiff base ligands. *Inorganic Chemistry Communications*, *142*, 109594.

31. Emad S. Mousa, Walaa H. Mahmoud, ApplOrganometal Chem. 33:e4844 (2019) 1-18.

32. De, A., Ray, H. P., Jain, P., Kaur, H., & Singh, N. (2020). Synthesis, characterization, molecular docking and DNA cleavage study of transition metal complexes of o-vanillin and glycine derived Schiff base ligand. Journal of Molecular Structure, 1199, 126901.

33. Sathyanarayana, D. N. (2001). Electronic absorption spectroscopy and related techniques. Universities Press.

34. Ghosh, A. K., Mitra, M., Fathima, A., Yadav, H., Choudhury, A. R., Nair, B. U., &Ghosh, R. (2016). Antibacterial and catecholase activities of Co (III) and Ni (II) Schiff base complexes. Polyhedron, 107, 1–8.

35. Tofiq, D. I., Hassan, H. Q., &Abdalkarim, K. A. (2021). Preparation of a novel Mixed-Ligand divalent metal complexes from solvent free Synthesized Schiff base derived from 2, 6-Diaminopyridine with cinnamaldehyde and 2, 2′-Bipyridine: Characterization and antibacterial activities. Arabian Journal of Chemistry, 14(12), 103429.

36. Grosdidier, A.; Zoete, V.; Michielin, O. SwissDock, a protein-small molecule docking web service based on EADock DSS. Nucleic Acids Res. 2011, 39, 270–277. [CrossRef] [PubMed]