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Synthesis, characterization, biological, anticancer and antialzahimar activities studies of ternary pd(II) complexes

Lamis O. Abo baker[1], Walaa H.Mahmoud[2], Abeer T. AbdElkarim[2], Ahmed A. ElSherif [2]*

1 Basic Science department, Modern Academy for Engineering and Technology, Egypt

2Department of Chemistry, Faculty of Science, Cairo University, Egypt

Abstract

This paper details the synthesis and spectral characterization of novel Pd (II) coordination ternary metal complexes. These complexes are formed using Cefradine as the primary ligand and aromatic amines as secondary ligands. The identification of these new ternary metal complexes involves a comprehensive array of spectrophotometric and physico-chemical analyses, including elemental analysis, infrared spectroscopy (FT-IR), proton nuclear magnetic resonance (^{1}H NMR) spectroscopy, melting point determination, conductivity measurements, mass spectrometry, and thermal analysis (TG and DTG). Furthermore, the antimicrobial activity of these complexes was assessed against a range of microorganisms, including Gram-positive bacteria such as *Bacillus subtilis* and *Staphylococcus aureus*, Gram-negative bacteria including *Salmonella* species and *E. coli*, as well as fungi like *Candida albicans* and *Aspergillus fumigatus*. To gauge their effectiveness, the complexes were compared to well-known standards in the field: amikacin, an antibacterial agent, and ketoconazole, an antifungal agent. Remarkably, the Pd (II) ternary complexes exhibited noteworthy activity as potential anti-Alzheimer's drugs. Additionally, the paper explores the anticancer properties of the ligands and their corresponding metal complexes using viability assays against human cancer cells (MCF-7 cells). These prepared palladium complexes, which demonstrate high selectivity for acetylcholinesterase (AChE), warrant further investigation as potential candidates for the treatment of early-stage Alzheimer's disease symptoms.

Keywords: Cefradine, ternary complexes, thermogravimetric analyses, Antibacterial activity, anticancer activity, anti-alzahimar drugs.

1. Introduction

Cephradine, an antibiotic, falls under the category of first-generation cephalosporins. Cephalosporin antibiotics have long been recognized for their proficient chelating properties [2]. These antibiotics are crucial in combatting bacterial infections due to their favorable antibacterial efficacy, resistance to β-lactamases, and pharmacokinetic attributes [1-3]. The notion that antibiotic effectiveness is linked to their ability to form complexes with metal ions has prompted investigations into the complexation capabilities of antibiotics as ligands [7, 8]. Hence, exploring the binary and ternary complexes of cephradine contributes to understanding the driving forces behind the formation of such complexes in biological systems. Interaction between various metal complexes and antibiotics can either enhance or inhibit their antimicrobial activity, with many cases demonstrating an enhancement of pharmacological activity upon complexation with metal complexes as compared to the free ligand [6, 7]

Alzheimer's disease (AD) represents the leading cause of dementia among the elderly [1]. Its treatment relies on cholinesterase inhibitors [2]. AD pathogenesis arises from a reduction in acetylcholine, a neurotransmitter vital 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]. Four cholinesterase inhibitors (tacrine, donepezil, rivastigmine, and galantamine) are approved by the US Food and Drug Administration, but 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.

*Corresponding author e-mail: aelsherif@sci.cu.edu.eg/ aelsherif72@yahoo.com (A.A. El-Sherif)
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reported the synthesis of palladium (II) complexes incorporating 1, 10-phenanthroline and amino acids. Their findings indicated that [Pd(phen)(valine)]Cl2H2O had a lower IC50 value against sarcoma P388 lymphocytic leukemia cells compared to cisplatin [9]. More recently, we have presented research on the synthesis, characterization, biological activities, and anticancer and anti-Alzheimer's activities of select ternary Pd (II) complexes.

Materials and methods

2. Experimental

2.1.1. Chemicals and reagents

We employed chemicals of the highest analytical grade with the utmost purity available for all our experiments. These chemicals encompassed N-benzylethylenediamine (C9H14N2), picolyl amine (C6H8N2), Bipyridine-C (C10H8N2) from Sigma-Aldrich, and cephradine (C16H19N3O4S .2H2O). Additionally, we utilized K2PdCl4 from Merck as a key reagent. In our organic solvent-based processes, we relied on ethyl alcohol (99%) and dimethylformamide (DMF). For all preparations, we consistently used deionized water sourced from glass equipment.

To conduct our research, we employed the breast tumor cell line MCF7, which had been preserved in a frozen state in liquid nitrogen (-180°C) and was acquired from the American Type Culture Collection. The maintenance and subculturing of these tumor cell lines took place at the National Cancer Institute in Cairo, Egypt.

2.1.2. Solutions

To create fresh stock solutions of mixed ligand complexes at a concentration of 1×10^{-3} M, we precisely weighed the chelates and dissolved them in the suitable volume of DMF. Subsequently, we measured the conductivities of these complex solutions. The metal salt solutions used in our experiments were standardized in accordance with established and recommended procedures [9, 10].

2.1.3. Instrumentation

We conducted microanalyses of carbon, hydrogen, and nitrogen using a CHNS932 (LECO) Vario Elemental analyzer at the Microanalytical Center, located at Cairo University, Egypt. For measuring melting points, we employed a triforce XMTD-3000 apparatus. Fourier transform infrared (FT-IR) spectra were recorded using a Perkin-Elmer 1650 spectrometer spanning the range of 4000–400 cm−1, with KBr disks as the medium. In solutions of dimethyl sulfoxide-d6 (DMSO-d6), we recorded 1H NMR spectra at room temperature using a 300-MHz Varian-Oxford Mercury spectrometer, employing tetramethylsilane as an internal standard. For assessing the molar conductivity of solid complex solutions at a concentration of 10−3 M in ethanol, we utilized a Jenway 4010 conductivity meter. Mass spectra were recorded via the electron ionization technique at 70 eV, employing an MS-5988 GS-MS Hewlett-Packard instrument, available at the Microanalytical Center in Egypt. Our antimicrobial assays were carried out at the Microanalytical Center, Cairo University, Egypt.

Anticancer activity experiments were conducted at the National Cancer Institute, specifically in the Department of Cancer Biology and the Department of Pharmacology, Cairo University, Egypt. Optical density (OD) measurements for each well were determined spectrophotometrically using an enzyme-linked immunosorbent assay (ELISA) microplate reader, set at 564 nm (Meter Tech. R960, USA).

The assessment of anti-Alzheimer's activity, particularly butyrylcholinesterase (BuChE) inhibition, was performed at the Biotechnology Research Center, Al-Azhar University.

1.1. Procedures

Synthesis of the metal complexes

To prepare mixed ligand complexes of palladium with aliphatic diamines (utilizing a 1:1 metal-to-diamine molar ratio), we followed this procedure: Initially, $PdCl_2$ was mixed with KCl in a 1:2 ratio, followed by the addition of distilled water with continuous stirring and heating up to 50°C until complete dissolution occurred, resulting in a clear solution. Subsequently, the solution was filtered to remove any impurities or undissolved substances. Next, the amine was added drop by drop with continuous stirring at a 1:1 ratio to the filtered solution. The resulting precipitates were then filtered. The solids obtained, ranging in color from yellow to brown, were filtered and subsequently dried under vacuum conditions.

In the case of mixed ligand complexes involving cephradine, the preparation proceeded as follows: A mixture of binary complexes (Pd:amine) was dissolved (1 mmol in 10 ml of DMF) and then heated with stirring, followed by the immediate addition of cephradine solution (1 mmol in 15 ml of distilled water). The stirring process was continued for 3 hours, yielding the corresponding 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 are detailed in Table 1.

1.2. Antimicrobial activity

The disc diffusion technique was used to assess the in vitro antibacterial and antifungal

properties of gentamycin, ampicillin, and amphotericin B, which served as positive controls for Gram-positive, Gram-negative bacteria, and fungi, respectively [21, 22]. The bacterial organisms used were Gram-positive bacteria (*Bacillus subtilis*, *Bacillus cereus*, and *Staphylococcus aureus*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, and *Neisseria gonorrhoeae*), and fungal strains (*Candida albicans* and *Aspergillus flavus*). The Schiff base ligand and its complexes were dissolved in DMSO to prepare the stock solutions (1 mmol). For the antibacterial activity assessment, a nutrient agar medium was prepared, cooled to 47°C, and seeded with microorganisms. After the material had solidified, a sterile cork borer was used to drill 5-mm-diameter holes. The investigated compounds (the Schiff base ligand and its metal complexes) after being dissolved in DMSO at 1×10^{-3} M were added to Petri dishes (only 0.1 m). The growth plates of bacteria and fungi were then placed in an incubator for 20 hours at 37°C. Then the inhibition zones were subjected to diameter measurements in millimeters. The average of the final reading of antimicrobial activity assessments was determined by carrying out the antimicrobial activity experiments in triplicate [23].

1.3. Antitumor activity

We evaluated the potential cytotoxicity of the compounds using the Skehan and Storeng method [14]. Initially, cells were seeded in a 96-multiwell plate (104 cells per well) and allowed to attach to the well surface for 24 hours. Subsequently, various concentrations of the tested compounds (ranging from 0, 5, 12.5, 25, 50, to 100 µg/mL) were added to the cell monolayer, with triplicate wells prepared for each concentration. The cells were then incubated with the compounds for 48 hours under conditions of 37°C and 5% CO2.

After the 48-hour incubation period, the cells were fixed, washed, and stained with sulforhodamine B stain. Any excess stain was removed 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 spectrophotometrically at 564 nm employing an ELISA microplate reader. Subsequently, the mean background absorbance was automatically subtracted, and the mean values for each drug concentration were computed.

To determine the survival 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 following formula:

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

The IC50 values, representing the concentrations of the ligand or its complexes required to achieve 50% inhibition of cell growth, were determined through this analysis, and the experiment was repeated three times to ensure consistency and reliability of the results.

1.4. G. Butyrylcholinesterase Activity Assay and Inhibition Studies (Anti-Alzhaimar activity).

The hydrolysis of butyrylthiocholine iodide (BTC, 0.05-0.4 mM) by butyrylcholinesterase (BChE) was assessed using spectrophotometry in a MOPS buffer (50 mM, pH 8) at a temperature of 25°C. This process was conducted in the presence of 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB, 0.125 mM), following the Ellman method [15]. The reactions were initiated by introducing 0.2 U/mL of BChE into the mixture. The rate of absorbance increase was continuously monitored at 405 nm using a Biosystem-310 spectrophotometer.

To determine enzyme activity, we considered the linear segments of the progress curves within the initial 60-second period and applied an extinction coefficient of 14.2 mM−1/cm−1.

For the study of BChE inhibition, we introduced varying concentrations of 1, 9-dimethyl-methylene blue (DMMB) into the reaction mixture (final volume 1.2 mL) [16]. Importantly, the presence of methanol (≤1.25% (v/v)) in the reaction mixture had no impact on enzyme activity.

Results and Discussion

Elemental analyses and molar conductivity measurements.

The Pd (II) ternary complexes formed in this study exhibit remarkable stability in ambient air conditions. In practical terms, they display relatively high solubility in polar organic solvents such as ethanol (EtOH), methanol (MeOH), dimethylformamide (DMF), and dimethyl sulfoxide (DMSO). However, these complexes are insoluble in water. Furthermore, the stoichiometry and composition of the cephradine (L) ligand and its metal complexes were validated, confirming a consistent metal-to-amine-to-cephradine drug ratio of 1:1:1 in these complexes. This confirmation was achieved through elemental analysis, which involved determining the metal content as well as the levels of carbon, hydrogen, nitrogen, and chlorine (as shown in Table 1). The results from the elemental analyses closely match the proposed structural compositions.

TABLE 1. Yields of Mixed Ligand Complexes of Palladium with Aliphatic Diamines.
(L =cephardine , (1) cephardine(N-benzylethylenediamine)Pdchloride ,(2)) cephardine(picolyl amine)Pd chloride, (3) cephardine(Bipyridine)Pdchloride).

Compound (chemical formula)	Color Yield (%)	M.p. (°C)	Found (Calcd)					A_m (Ω^{-1} mol^{-1} cm^2)
			C (%)	H (%)	N (%)	S (%)	M (%)	
L ($C_{16}H_{19}N_3O_4S$)	Pale yellow (93)	140 to 142	54.87 (55.01)	5.13 (5.44)	12.03 (11.36)	8.73 (9.17)		--------
Pd(N ben)cephCl	Brown (93)	210	44.11 (44.32)	5.12 (5.32)	10.01 (10.34)	4.38 (4.73)	15.36 (15.71)	76
Pd(pic)cephCl	Brown (93)	210	41.34 (41.64)	4.61 (4.73	10.83 (11.04)	4.93 (5.05)	16.31 (16.78)	82
Pd(Bipy)cephCl	Brown (93)	210	44.13 (44.57)	4.26 (4.57)	9.76 (10.00)	4.36 (4.57)	14.82 (15.20)	86

Notably, ternary complexes 1, 2, and 3 exhibited relatively high molar conductance values, ranging from 76 to 86 Ω^{-1} mol^{-1} cm^2, respectively.

These values suggest their electrolytic nature, indicating the presence of anions in the outer coordination sphere [17]. The summarized results can be found in Table 1.

IR spectra

The position of bands in the infrared (IR) spectra provides valuable insights into the bonding sites of the cephradine ligand and amines when coordinated to Pd (II) [18]. The key IR spectral data are summarized in Table (2) [19]. In the IR spectrum of the free drug ligand, two distinct bands are observed: one attributed to the carbonyl of the lactam group (C=O) at 1764 cm^{-1} and another associated with the amide carbonyl group (C=O) at 1650 cm^{-1}. Remarkably, in the ternary complexes, these bands remain largely unchanged, indicating that these groups do not actively participate in the coordination process [20].

Furthermore, two important bands are noteworthy: one at 1395 cm^{-1}, corresponding to carboxylate symmetrical stretching, and another at 1188 cm^{-1}, corresponding to ternary-N. These bands experience a shift to lower frequencies (1383-1382 cm^{-1}) and (1155-1100 cm^{-1}), respectively, when compared to the free ligand. This shift suggests a transfer of electron density from the donor nitrogen (-N) and oxygen (COO-) atoms to the metal ion, thereby weakening the absorption bands of (-N) and (C=O). In contrast, for the free ligand, the band at 1582 cm^{-1} corresponding to carboxylate a symmetrical stretching shifts to higher frequencies (1617-1600 cm^{-1}), possibly indicating bond stabilization. Additionally, the N-H stretching frequency band is observed at 3220 cm^{-1} without significant change in the ternary complexes [21].

Notably, new medium-intensity bands emerged in the region of 595 - 577 cm^{-1}, which can be attributed to υ (M-O), and at 466-450 cm^{-1} for υ (M-N). These findings indicate that the L1 ligand coordinates with Pd-complexes through the nitrogen (-N) and (C=O) carboxylate group [22].

Furthermore, the IR spectrum of cephradine reveals a broad band at 3400 cm^{-1}, attributed to the O–H groups of aqua ligands. In the ternary complexes (1, 2, 3), a broad band in the range of (3428-3424 cm^{-1}) appears due to stretching vibrations of uncoordinated water molecules [23].

Table 2: IR assignments of cephardine Schiff base ligand, and ternary pd(II)) complexes

Compound	N N–H	υ(co) lactum	υ(co) amide	υ(co) Asymmetric	υ(co) symmetric	Tertiary N	υ(M-O)	υ(M-N)
L	**3220sh**	**1764sh**	**1650sh**	**1582 sh**	**1395sh**	**1188m**		
1	3228m	1768sh	1651sh	1611m	1382m	1100m	578ss	450w
2	3225m	1765sh	1653sh	1617m	1383m	1100m	577s	466w
3	3222m	1765sh	1650sh	1600 m	1382w	1155m	595s	465w

Mass spectra

The mass spectra of the ternary complexes were analyzed using electron ionization mode at 70 eV. Here are the key findings for each of the complexes:
(1) In the ternary complex involving **cephradine (N-benzylethylenediamine) Pd chloride**, three distinct peaks were observed in the mass spectrum. The first peak appeared at m/z 678.5, corresponding to the ternary complex (calculated mass: 676.9 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, detected at m/z 151, indicated the presence of the first ligand (N-benzylethylenediamine) (calculated mass: 150.22 g/mol). These findings align with the proposed molecular weight of the synthesized complex, validating the (1:1:1) Pd: L1: L2stoichiometric ratio [24].
(2) In the ternary complex involving **cephradine (picolyl amine) Pd chloride**, three prominent peaks were observed in the mass spectrum. The first peak, at m/z 637 (M^{+3}), corresponded to the ternary complex (calculated mass: 634 g/mol). The second peak at m/z 350 confirmed the presence of the second ligand, cephradine (calculated mass: 349.4 g/mol). The third peak, observed at m/z 108, indicated the presence of the first ligand, cephradine (picolyl amine) Pd (calculated mass: 108 g/mol). These findings substantiate the proposed molecular weight and the (1:1:1) Pd: L1: L2 stoichiometric ratio [24].
(3) In the ternary complex involving **cephradine (Bipyridine) Pd chloride**, the mass spectrum revealed three important peaks. The first peak appeared at m/z 700, corresponding to the ternary complex (calculated mass: 700 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, observed at m/z 156, indicated the presence of the first ligand, cephradine (Bipyridine)Pd (calculated mass: 156 g/mol). These findings are consistent with the proposed molecular weight and support the (1:1:1) Pd: L1: L2 stoichiometric ratio [25].

^{1}HNMR spectra.

The structural analysis of the cephradine ligand (L) and its mixed palladium (II) complexes was conducted through NMR data. The data are summarized in Table (3). The ^{1}H NMR spectra of cephradine (L) and its palladium complexes were recorded in DMSO-*d6* using tetramethylsilane (TMS) as the internal standard. Here are the key observations:
In the ^{1}H NMR spectrum of cephardine ligand, a single peak at 9.60 ppm corresponding to C-OH (s) was identified. To confirm that this peak belongs to the acidic group COOH, D_2O solvent was employed, causing the disappearance of this band. In the ternary complexes 1, 2, and 3, this peak vanishes due to the ionization of COO-, providing evidence for the coordination of cephradine to the binary complex through C=O and the formation of uninegative complexes. This observation aligns with the conclusions drawn from the IR spectral data [26]. Additionally, in the ^{1}H NMR spectrum of Ligands, signals in the multiplet range of 7.29-7.44 ppm (m) correspond 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-METHINE (d), 3.70 ppm for CH_2-CH (s), and 2.49 ppm for CH_3 (s).
In the case of the ternary complexes, a slight downfield shift was noticed in the signals, attributed to the coordination of cephradine to Pd (II) complexes [27, 28].

Table 3. ^{1}H NMR assignmen ts of cephardine Schiff base ligand, and ternary pd(II)) complexes

Compound	Chemical shift, (δ) ppm	Assignment
L	9.60	(s, H ,OH)
	8.53	(s,2H , CH-NH_2)
	8.32	(s,H ,CH-NH-CH)
	7.44-7.29	(m,5H,CH benzen)
	5.67	(d,H ,CH-CH-NH)
	5.10	(d,H ,S-CH-CH-NH)
	4.05	(S, H, CH METHINE)
	3.70	(d,2H ,CH_2-CH)
	2.49	(s,3H ,CH_3)
1	Disappear	(s, H ,OH)
	8.30	(s,2H , CH-NH_2)
	7.95	(s,H ,CH-NH-CH)
	7.36-7.28	(m,5H,CH benzen)
	5.67	(d,H ,CH-CH-NH)
	5.18	(d,H ,S-CH-CH-NH)
	4.05	(S, H, CH METHINE)
	3.30	(d,2H ,CH_2-CH)

	2.39	(s,3H ,CH_3)
2	Disappear	(s, H ,OH)
	8.7	(s,2H , CH-NH_2)
	8.3	(s,H ,CH-NH-CH)
	7.416-7.36	(m,5H,CH benzen)
	5.6	(d,H ,CH-CH-NH)
	5.00	(d,H ,S-CH-CH-NH)
	4.70	(S, H, CH METHINE)
	3.1-2.9	(d,2H ,CH_2-CH)
	2.41	(s,3H ,CH_3)
3	Disappear	(s, H ,OH)
	8.69	(s,2H , CH-NH_2)
	8.37	(s,H ,CH-NH-CH)
	7.87-7.43	(m,5H,CH benzen)
	5.70	(d,H ,CH-CH-NH)
	5.12	(d,H ,S-CH-CH-NH)
	3.31	(S, H, CH METHINE)
	2.89	(d,2H ,CH_2-CH)
	2.50	(s,3H ,CH_3)

Thermal analyses.

Thermal characterization of the ternary complexes (1–3) was conducted through thermogravimetric analysis (TGA) over a temperature range from 10°C to 800°C, with a heating rate of 5°C/min. The TGA data, illustrated in Fig. (), and summarized in Table (4), reveal temperature intervals and the corresponding mass loss percentages for these complexes.

The observed mass loss primarily stems from the removal of uncoordinated water molecules and coordinated molecules. This weight loss is consistent with the data obtained from elemental analysis. Ultimately, the breakdown of residual carbon atoms and residual metal oxides leads to the final mass loss [29].

For the ternary complex cephradine (N-benzylethylenediamine) Pd, the TGA curve indicates decomposition between 35°C and 130°C, resulting in a mass loss of 5.14% (equivalent to 36 g/mol). This corresponds to the loss of two water molecules, suggesting the removal of outer coordination water. Further decomposition occurs in the range of 130°C to 350°C, accounting for 11.42% (80.5 g/mol) of mass loss. Between 350°C and 574°C, a significant decomposition of 63.97% (559.4 g/mol) is observed. Beyond 800°C, the decomposition of the remaining mass residue is attributed to PdO [30].

For the ternary complex cephradine(picolyl amine)Pd, decomposition occurs between 33°C and 104°C, resulting in a mass loss of 5.12% (36 g/mol), attributed to the loss of two water molecules. Further decomposition is observed in the range of 104°C to 391°C, accounting for 64.88% (415.5 g/mol) of mass loss. Beyond 800°C, the decomposition of the remaining mass residue is attributed to PdO and carbon (PdO+5C).

In the case of the ternary complex cephradine(Bipyridine)Pd, decomposition takes place between 30°C and 150°C, leading to a mass loss of 7.43% (54 g/mol), corresponding to the loss of three water molecules. Further decomposition occurs in the range of 150°C to 750°C, accounting for 70.67% (499.5 g/mol) of mass loss. Beyond 800°C, the decomposition of the remaining mass residue is attributed to PdO and carbon (PdO+2C).

Table 4: Thermal Characterization of Ternary Complexes (1–3) Temperature Intervals and Mass Loss Percentages.

Complex	TG-range (oC)	DTG max	n*	Mass loss Estim (calcd)% (Total mass Loss)	Assignment	Residues
Pd(Nben)ceph	(35-130) (130-350) (350-620)	80 270 450,574	1 1 2	5.32(5.14) 11.82(11.42) 64.49(63.96) 81.63(80.52)	Loss of 2H2O Loss of HCl ,CO2 Loss of C24H31N5OS	Pdo
Pd(pic)ceph	(33-103) (104-391)	54 261,333	1 2	5.68 (5.12) 65.54(64.89) 71.22 (70.01)	Loss of 2H2O Loss of c17H26 ClN5O	PdO +5C
Pd(Bipy)ceph	(30-150) (150-750)	90 430,573	1 2	7.43(7.71) 70.67(71.36) 78.10(79.07)	Loss of 3H2O Loss of C24H26ClN5O3S	PdO+2C

Antimicrobial activity.

The synthesized Pd(II) complexes underwent screening to assess their antibacterial and antifungal activities against a range of bacterial and fungal strains, including Gram-positive bacteria (B. cereus, B. subtilis, and S. aureus), Gram-negative bacteria (*E. coli, N. gonorrhoeae,* and *P. aeruginosa*), and fungal strains (*A. flavus* and *C. albicans*). The disc diffusion method (Fig. 3) was employed to evaluate their antibacterial and antifungal properties, and the results are summarized in Table (5).

The findings demonstrate that the complexes examined in this study exhibit notable activity against both Gram-positive and Gram-negative bacterial strains [31, 32]. Specifically, Pd (pic) ceph metal complexes displayed strong antifungal activity against Candida albicans. Notably, the synthesized Pd (pic) ceph chloride complex exhibited significantly higher antibacterial and antifungal activities compared to the parent ligand and the other complexes. These enhanced antibacterial and antifungal activities of the synthesized metal Schiff base complexes can be attributed to the chelation theory [33].

Anticancer activity

The anticancer potential of the synthesized complexes was evaluated by assessing their cytotoxicity against the human breast cancer cell line (MCF7). The MTT colorimetric assay revealed significant anticancer activity against MCF7 cells following a 24-hour incubation, as summarized in Table (6). Importantly, the anticancer effects were concentration-dependent, with decreasing cell viability observed as complex concentrations increased. Notably, complexation of the ligand with metal ions led to an enhancement in anticancer activity [34, 36].

Among the complexes, Pd (pic) ceph chloride exhibited the highest cytotoxicity against MCF7 cells. The IC50 value for Pd (pic) ceph was notably low, at 24.3 µg/ml indicating its strong anticancer activity.

Anti-alzahimar activity

Acetylcholinesterase inhibitors have been widely used in the treatment of neurodegenerative disease. The synthesized palladium mixed complexes were tested to evaluate their ability to inhibit AChE. Among the tested compounds, Pd (pic) ceph chloride complex showed the best ability to inhibit AChE with IC50 6.01 µM, while the weakest inhibitory activity was found in the case of cephradine (Bipyridine) Pd chloride (IC50 20.08 µM). The reported data clearly indicate that the main features influence the enzyme inhibition is the nature of the R substituent of amine which plays a key role in the inhibitory potency [37].

Table 5: Antimicrobial Activity of Synthesized Pd(II) Complexes

Sample	Inhibition zone diameter (mm / mg sample)							
	Gram positive bacterial species		Gram negative bacterial species			Fungi		
	Staphylococcus aureus (ATCC:13565)	*Streptococcus mutans (ATCC:25175)*	*Escherichia coli (ATCC:10536*	*Klebsiella pneumonia (ATCC:10031*	*Pseudomonas aeruginosa (ATCC:27853)*	Candida albicans (ATCC:10231)	Asperagillus Nigar (ATCC:16404)	Asperagillus Ochraceous (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 ben)	22.6±*0.6*	23.3±*0.6*	*23.3±0.6*	*22.0±1.0*	*17.3±0.5*	NA	NA	NA
Pd(N ben)ceph	31.6±*0.6*	13.6±*0.5*	*12.6±0.5*	*13.6±0.5*	*20.6±0.6*	NA	NA	NA
Pd(pic)	26.3±*0.6*	23.3±*0.6*	*26.3±0.6*	*23.6±0.6*	*23.6±0.6*	27.3±0.6	25.6±0.6	22.3±0.6
Pd(pic)ceph	28.0±*1.0*	13.3±*0.5*	*11.6±0.5*	*12.3±0.5*	*23.3±0.6*	25.6±0.6	28.6±0.6	30.6±0.6
Pd(Bipy)	22.3±*0.6*	20.3±*0.6*	*21.6±0.6*	*21.3±0.6*	*18.3±0.5*	NA	NA	NA
Pd(Bipy)ceph	13.3±*0.5*	14.3±*0.5*	*8.6±0.5*	*10.3±0.5*	*9.6±0.5*	12.6±0.5	NA	NA

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