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RESEARCH ARTICLE

Synthesis, Spectral Characterization, Cytotoxic Assay, and Antimicrobial Evaluation Studies of Some Transition Metal Complexes of Schiff Base Derived From Amoxicillin

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ABSTRACT

Four metal complexes of Schiff base ligand were prepared. To characterize both the ligand and the metal complexes, various techniques were employed, including elemental analysis, FT-IR spectroscopy, UV-Vis spectroscopy, molar conductivity measurements, magnetic moment determination, melting point, mass spectroscopy, ^{1}H-NMR and ^{13}C-NMR spectroscopy. The results showed that the metal complexes formed have the formula $[M(L)_2Cl_2]Cl_{(n)}.H_2O$, where L= Schiff base ligand and M=Cr(III), Rh(III), Pt(IV) and Au(III), n = 1, 2. Based on spectroscopic analysis, coordination with metal ions involves the 'N' donor atom of N atoms of the imine and the N-H amid group, and four complexes are suggested to have a six-coordinated octahedral structure. Molar conductivity of these complexes showed that they were electrolytic in nature. In this study, the anticancer of the ligand and its metal complexes were investigated against MDA-MB-231 cell lines. Moreover, the in vitro efficacy them against Gram-negative bacteria (*E. coli*) and Gram-positive bacteria (*S. aureus*), as well as the fungal strain *Candida albicans*, was evaluated using the disc diffusion method. The results indicated that the complexes demonstrated the highest levels of cytotoxicity against MDA-MB-231 cell lines and antimicrobial activity more than the free ligand. These findings suggest that these metal complexes may have promising applications in the development of novel anticancer, antioxidant and antimicrobial agents.

Keywords: Antimicrobial, Amoxicillin, Cell lines, Metal complexes, Schiff base

Introduction

Schiff-base compounds are chemical compounds containing an azomethine group. A reversible acid-catalyzed condensation process between a primary amine and a carbonyl molecule forms them. Hugo Schiff first reported this reaction in 1864.[1] Schiff base compounds are a significant and thoroughly studied group of substances because of their wide variety of biological uses, simplicity of synthesis, ability to form chelates, and stability.[2,3] Imine complexes are commonly utilized in treating medication resistance in cancer and are widely examined for their potential as antimalarial agents. Additionally, it can potentially be utilized for the immobilization of enzymes.[4] Schiff base complexes are highly regarded as a crucial stereochemical model in both main group and transition metal coordination chemistry due to their ease of formation and wide range of structural variations. Schiff bases are commonly employed as ligands due to the exceptional stability of the coordination compounds they form and their excellent solubility in commonly used solvents.[5,6] Studying metal-drug complexes and developing more effective

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pharmaceuticals is of great significance in contemporary times.[7] The interaction between metal ions and donor ligands yields organically effective molecules with different chemical structures.[8] The investigation of metal complexes formed with drugs acting as ligands is highly stimulating due to the amplification of the metal's efficacy in conjunction with the drug's activity.[9] Amoxicillin ($C_{16}H_{19}N_3O_5S$) is a semi-synthetic antibiotic belonging to the penicillin family, exhibiting minimal impact on gram-negative bacteria while significantly affecting gram-positive bacteria. It is utilized in both human medicine and veterinary treatments. The investigation of the presence and destiny of amoxicillin in the environment has been conducted.[10] This paper presents our findings from the research on the synthesis, structural analysis, and biological evaluation of many complexes formed by a Schiff base ligand. The present work describes the results of our investigations on the synthesis, structural studies, and biological studies of some complexes of Schiff base ligand.

Materials and methods

Amoxicillin (99.5%), P-bromobezaldehyde, metal chlorides (analytical grade), and solvents were purchased from Merck (Schnelldorf, Germany). The metal content was measured using an AA-6880 Shimadzu atomic absorption flame spectrophotometer (Shimadzu Corporation; Tokyo, Japan). To measure the ultraviolet-visible (UV-Vis) spectra in ethanol, a Shimadzu UV-1601 spectrophotometer (Shimadzu Company; Tokyo, Japan) was used. The FT-IR 8300 Shimadzu spectrophotometer (Shimadzu Corporation; Tokyo, Japan) was used to record the Fourier transform infrared (FTIR) spectra. Direct Probe captured mass spectra. The melting points in open glass capillaries were examined. Using EA-034.mth, the elemental analyses (C.H.N.S.) were obtained. Conductivity measurements were performed using a Corning conductivity meter 220 and were done in an ethanol solvent with a concentration of (10^{-3} M).

Synthesis of schiff base ligand

A reported methods[11] was used to prepare Amoxicillin derivative (5R)-6-((R)-2-((4-bromo benzylidene)amino) -2-(4-hydroxyphenyl) acetamido) -3,3-dimethyl-7-oxo-4-thia-1 azabicyclo[3.2.0]heptane-2-carboxylic acid as ligand.

Synthesis of metal complexes

The synthesis of metal complexes was done using a Schiff base ligand (L) and four different metal ions, Cr(III), Rh(III), Pt(IV), and Au(III). The procedure involves dissolving (2 mmol, 1.066 g) of the Schiff base ligand in 10 mL of absolute ethanol, followed by the addition of 1 mmol of the metal ion (0.158 g $CrCl_3.6H_2O$, 0.209 g $RhCl_3.H_2O$, 0.409 g $H_2PtCl_6.6H_2O$, and 0.411 g $HAuCl_4.H_2O$). The resulting mixture is refluxed for 2 hours, during which the color of the solution changes. This color change is likely due to the formation of the metal complex. After refluxing, the solvent is evaporated to yield a precipitate with a different color.

Cytotoxic studies-MTT assay

The cytotoxicity of free ligands and their metal complexes was studied against MDA-MB-231 cell lines by in vitro MTT cytotoxicity assay.[12] Cell lines were evaluated 24 hours after exposure to the compounds at various concentrations. Results from the MTT testing utilizing a desiccator were shown for ligands and their metal complexes. All the compounds produced were characterized using spectroscopic, analytical, and physical methods. Various concentrations (400, 200, 100, 50 μg/ml) were compared to the untreated negative control culture medium.

Antimicrobial activity studied

The agar well diffusion technique was employed to evaluate the antibacterial activity of the ligand and their complexes.[13] Muller Hinton agar medium was uniformly filled with a bacterial suspension at a standard concentration of 0.5 McFarland, and wells were prepared using a sterile cork borer. Each antibiotic sample weighed 10 mg, and plates were incubated at 37 C for 24 hours. A caliper measured the area around the well where bacterial growth was inhibited. Overall, this study offers significant new understandings of the antibacterial properties of different compounds and their potential as agents to combat bacterial infection.

Results and discussion

Synthesis and characterization of the ligand and its metal complexes

The data in Table 1, suggests that ligand (L) and its metal ion complexes agree with calculated values. The suggested molecular structure is formulated and characterized by subsequent spectral and magnetic moment and conductivity measurements.

Spectral analysis

The FT-IR spectrum of the free ligand and its complexes are shown in Figs. 1 to 3, and Table 2. The (COOH) and (C=O) stretching vibration (C=O) bands

Table 1. Color, melting point, yield, and elemental composition of ligand and metal complexes.

					Elemental analysis found(Calc.)				*Metal %*	
Comp.	*Color*	*m.p. °C*	*Yield %*	*M.Wt g.mol* $^{-1}$	*C*	*H*	*N*	*S*	*found (Calc.)*	*Suggested Chemical Formula*
L	Orange	195–197	82	532.41	51.89 (51.96)	4.16 (4,23)	7.89 (7.97)	6.02 (6.14)	—-	$C_{23}H_{22}BrN_3O_5S$
Cr L	Green	280–282	52.92	1239.17	44.57 (44.59)	3.55 (3.58)	6.76 (6.78)	5.14 (5.18)	4.17 (4.20)	$C_{46}H_{44}Br_2Cl_3N_6O_{11}S_2Cr$
Rh L	Brown	236–238	55.7%	1285.90	42.80 (42.83)	3.41 (3.44)	6.48 (6.51)	4.94 (4.97)	7.95 (7.98)	$C_{46}H_{44}Br_2Cl_3N_6O_{11}S_2$ Rh
Pt L	Yellow	310d	82	1324.79	41.55 (41.58)	3.15 (3.19)	6.29 (6.32)	4.80 (4.83)	14.65 (14.68)	$C_{46}H_{42}Br_2Cl_2N_6O_{10}S_2$ Pt
Au L	Dark Orange	291–293	80	1384	39.92 (39.88)	3.17 (3.20)	6.04 (6.07)	4.60 (4.63)	14.20 (14.23)	$C_{46}H_{44}Br_2Cl_3N_6O_{11}S_2$ Au

Table 2. Selected FT-IR absorption bands of ligand and its metal complexes.

Comp.	νNH vC=N	ν C=O	ν C=O amide	ν NH amide	ν OH phenol	ν OH aliphatic + aromatic	ν C-N	ν M-N	Others
L	1641	1770	1680	3095	3520	2972 2995	1265	—	—
CrL	1650	1772	1695	3224	3500	2970 2993	1270	586	ν OH(H_2O) = 3421 ν Cr-Cl = 352
RhL	1660	1774	1703	3235	3500	2933 2978	1276	551	ν OH(H_2O) = 3396 ν Rh-Cl = 318
PtL	1662	1776	1696	3226	3500	2935 2983	1275	549	ν OH(H_2O) = 3441 ν Pt-Cl = 352
AuL	1668	1776	1697	3203	3500	2883 2983	1250	549	νOH(H_2O) = 3203 ν Au-Cl = 376

appeared at 1770 cm^{-1} and 1680 cm^{-1}, respectively. The stretching absorption at 1619 cm^{-1}, 2995 cm^{-1}, 1174 cm^{-1}, and 2972 cm^{-1} was assigned to the (C=C) aromatic, (C–H) aromatic, (C–C) aliphatic, and (C–H) aliphatic bands, in that order. (C–N) stretching vibration is responsible for the absorption at 1265 cm^{-1}. The band at 1641 and 3095 cm^{-1} due to the C=N stretching band confirming the formation of Schiff's base and (amide N–H) respectively.[14] The band at 1770 cm^{-1} of the β-Lactam carbonyl group (C=O) does not shift in all complexes, indicating that carbonyl oxygen doesn't participate in coordination. The bands ascribed to the ν NH amid the ligand shifted to higher wave numbers in all complexes by approximately (9–18) cm^{-1}, as did the bands attributed to the C=N of the ligand. This demonstrates that the ligand functions as a neutral bidentate via the N atoms of the imine and the N-H amid. The coordination of amide and imine through the nitrogen atom is further confirmed by the presence of M-N bands in the complexes spectra in the range (472–586) cm$^{-1.}$[15] All complexes exhibited bands between 3203 and 3441 cm^{-1} that corresponded to the stretching band of H_2O uncoordination.[16] In the spectra of complexes, new weak bands appeared in the frequency range (318–376) cm^{-1}, which corresponds to the stretching frequency of (M-Cl).[17]

Electronic spectral

The electronic spectra of the ligand, Fig. 4, presents two absorption bands at 39062 and 32258cm^{-1}, respectively, attributed to $\pi \rightarrow \pi^*$ and n→ π^*, respectively, determined by the C=O and C=N groups. These absorption bands also appear in the electronic spectra of the complexes but they are shifted to lower values, which proves the coordination of the ligand to the central metallic ions.[18]

[CrL]: The electronic spectrum of the Cr(III) complex derived from the ligand, Fig. 5, showed a band at 17421cm^{-1}, which is due to 4A2g→4T2g. The complexes displayed a band at 28169 and 36638 cm^{-1} due to 4A2g →4T1g (F) and 4A2g → 4T1g(p) transitions, respectively, in an octahedral geometry.[19] Measurements of conductivity indicated that the compound will exhibit ionic behavior, Table 3.

[RhL]: Three main absorption bands were observed in the electronic spectra of the Rh(III) complex in an ethanol solution, and these bands observed at 1100, 36701, and 42052 cm-1 were assigned to the transitions 1A1g → 3T1g, 3T2g, 1A1g→1T2g, and $^{1}A_{1}g\rightarrow$ $^{1}T_{1}g$ respectively, in an octahedral geometry.[20] The magnetic moment for the solid complex exhibits diamagnetic behavior.

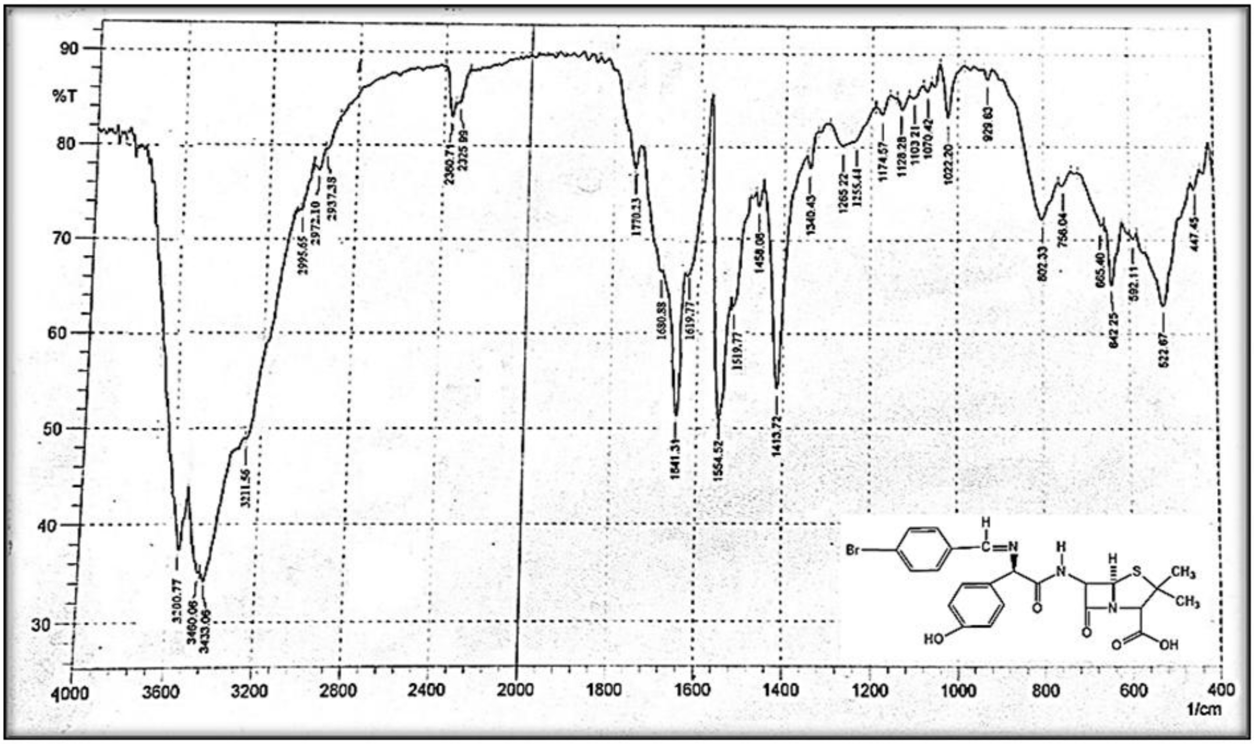


Fig. 1. FTIR spectrum of ligand.

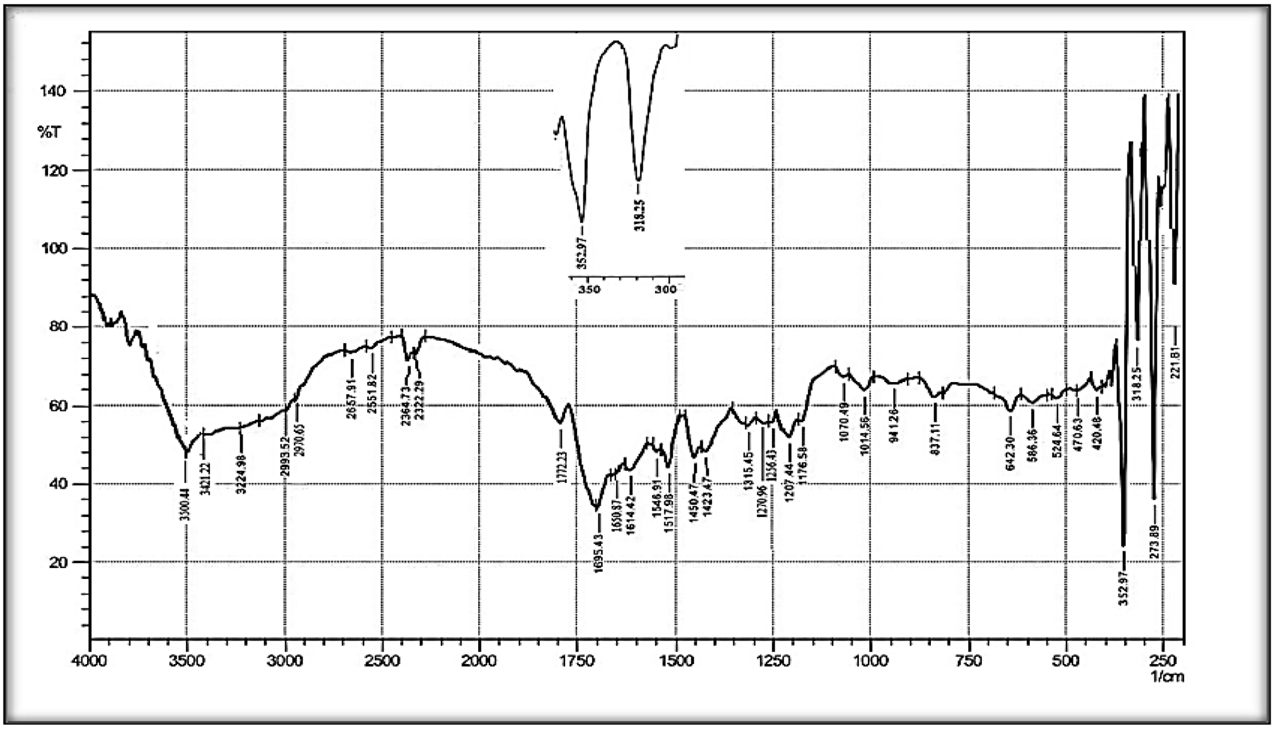


Fig. 2. FTIR spectrum of Cr (III) complex.

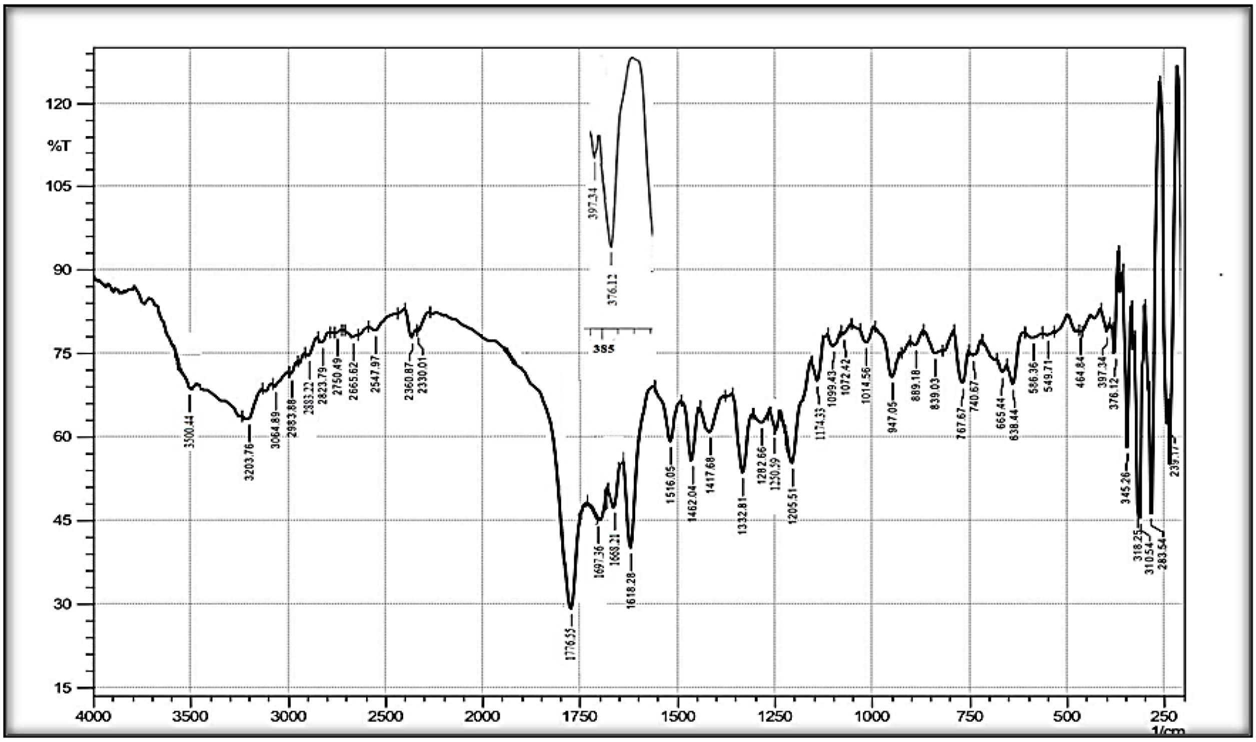


Fig. 3. FTIR spectrum of Au (III) complex.

Measurements of conductivity indicated that the compound will exhibit ionic behavior.

[PtL]: The electronic spectra of the resulting yellow platinum (IV) complex reveals three absorption bands at 11011, 26455, 36101, and 45662 cm^{-1} that correspond to the $^1A_1g \rightarrow {}^3T_1g$, ^3T_2g, $^1A_1g \rightarrow {}^1T_2g$, $^1A_1g \rightarrow {}^1T_1g$, and L→Pt CT transitions respectively, in an octahedral geometry.[21] The conductance behavior indicates an electrolyte.

[AuL]: UV-vi's spectrum of orange Au(III) complex. Fig. 6 shows two bands at 26455 and 34843 cm^{-1} assigned to $^3A_2g \rightarrow {}^3T_2g$, $^3A_2g \rightarrow {}^3T_1g$ transition, respectively, and other bands that appeared at 40650 cm^{-1} which could be due to the L→Au CT. The Racah parameter B value has been calculated by fitting the ratio ν_2/ν_1 to the Tanabe-Sugano diagram for the octahedrald system.[8] The B' will be 874, and the value of the constant field splitting Dq=2709.4 cm-1 and 10 Dq will be 27094 cm-1, which is in agreement with the octahedral environment reported.[22] So, the third transition was calculated theoretically from the equation 15B = v3 + v2-3v1 and found to be 52919 cm^{-1}, attributed to the $^3A_2g \rightarrow {}^3T_1g$ (p) transition. The conductivity measurement for this complex shows to be ionic.

Fig. 7 suggests the distorted octahedral prepared for this complex based on the analysis of data and spectroscopy techniques and all results.

Mass spectroscopy

The mass spectrum of the ligand shown in Fig. 8 can be described by four major events, the most important pathway, and some pathways that fragmented further. The mass spectrum of the ligand appeared to have a molecular ion peak at m\z = 532.9, which confirms the molecular weight m\z value of the ligand and that is compatible with the experimental ligand formula $C_{23}H_{22}BrN_3O_5S$. The 100% intensity for the base peak m\z = 345.3 was estimated for cleavage of ($C_{16}H_{12}BrN2O_2{}^+$), leading to give notable peak at m/z = 301.3 estimated for ($C_{15}H_{11}BrNO^+$). The appearance of variable absorption peaks was recorded in the mass spectrum at m/z = 201.3 for ($C_8H_{10}NO_3S^+$) and m/z = 95.2 for ($C_6H_5O^{\bullet+}$).

^{1}H-NMR spectra

The ^{1}H-NMR spectral comparison of the Schiff base ligand and its Pt(IV) complex was made to confirm the binding mode of the ligand with metal ions. The ^{1}H-NMR spectra of the ligand are shown in Fig. 9. The ^{1}H-NMR spectral peak at 2.7 ppm in the spectra of ligand is assignable to DMSO-d$_6$ solvent protons. The azomethine proton of the ligand resonated as a sharp singlet at 9.35 ppm,[23] and on complexation Fig. 9,

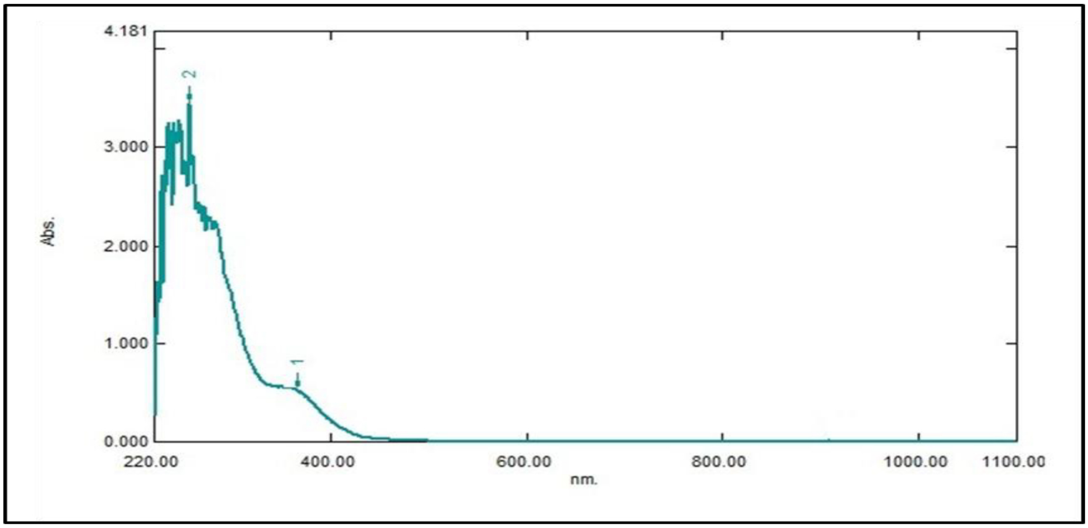


Fig. 4. UV-VIS spectrum of L.

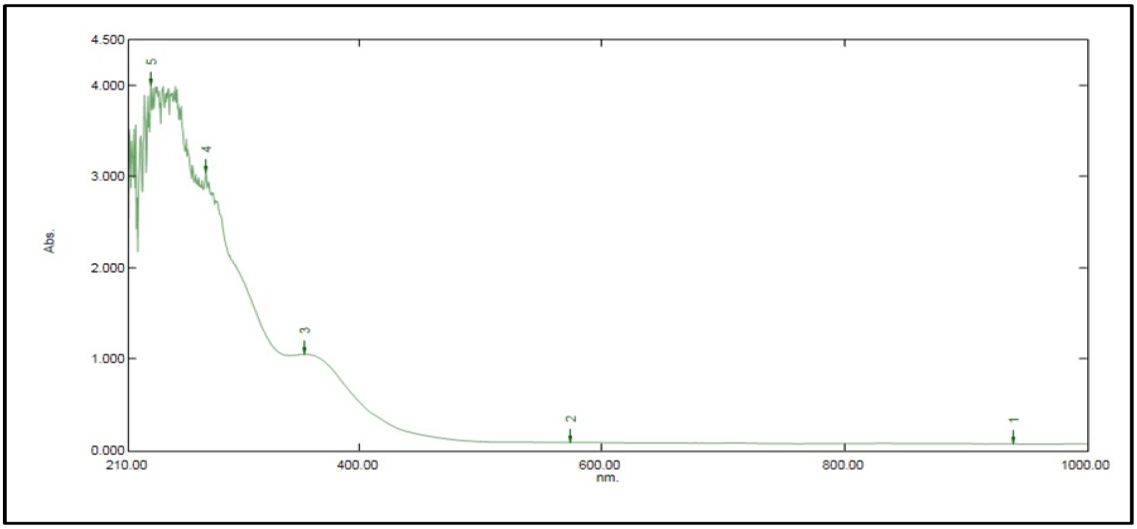


Fig. 5. UV-VIS spectrum of Cr(III) complex.

this signal was absent. This indicates the involvement of the azomethine group in the chelation process. The signal for amide NH proton has appeared at 8.57 ppm in the ^{1}H-NMR of ligand, which is absent in the spectrum of Pt(IV) complex, Fig. 10. This confirms the coordination of amide NH with metal atom.[24] The peaks in the region of 0.6–1.2 ppm suggest the peak for methyl protons present in the amoxicillin moiety near the lactam ring.[25] In addition to this, methyl protons of the ester group of amoxicillin show singlet in the region of 3.8–4.5 ppm.

^{13}C-NMR spectra of the ligand and its Pt(IV) complex

In the ^{13}C-NMR spectrum in Fig. 11, the signals due to (CH=N) azomethine and CH_3 are observed at 128.57–135.31 and 19.03 ppm, respectively. Comparison of ^{13}C-NMR spectrum Pt(IV) complex with the free Schiff base ligand demonstrates that those carbon atoms connected with bonding sites were slightly shifted to the downfield region in the Pt(IV) complexes, Fig. 12. The (CH=N) azomethine showed within 131.68–132.8.

Biological activity

Invitro anticancer activities

Malignant diseases like cancer are characterized by aberrant cell proliferation. Chemotherapy and surgical intervention are the two most popular treatments for this disease, both of which have negative side effects. Despite their usage in cancer

Table 3. Electronic transitions, conductivity in ethanol, and suggested geometry of metal complexes.

Comp.	Absorption cm^{-1}	Absorption nm	Assignments	B°	B'	B	15 B'	10Dq	$\mu s.cm^{-1}$	$\mu\ eff$ B.M	Suggested Geometry
L	39062	256	$\pi \rightarrow \pi *$	—	—	—	—	—		—	—
	32258	310	$n \rightarrow \pi *$								
Cr L	17421	574	$^4A_2g \rightarrow ^4T_2g$	918	952	0.96	14280	17153	33	3.80	Distorted Octahedral
	28169	355	$^4A_2g \rightarrow ^4T_1g$								
	36638	272	$^4A_2g \rightarrow ^4T_1g(p)$								
	43859	228	L → Cr C.T								
Rh L	11001	909	$^1A_1g \rightarrow ^3T_1g$, ^3T_2g	—-	—	—	—	—-	45	0.00	Distorted Octahedral
	36701	272	$^1A_1g \rightarrow ^1T_1g$								
	42052	237	$^1A_1g \rightarrow ^1T_2g$								
Pt L	11011	908	$^1A_1g \rightarrow ^3T_1g, ^3T_2$	—	—	—	—	—	78	0.00	Distorted Octahedral
	26455	378	$g^1A_1g \rightarrow ^1T_1g$								
	36101	277	$^1A_1g \rightarrow ^1T_2g$								
	45662	219	(L) → Pt C.T								
Au L	26455	378	$^3A_2g \rightarrow ^3T_2g$	—	874	—	—	27094	48	2.80	Distorted Octahedral
	34843	287	$^3A_2g \rightarrow ^3T_1g$								
	52919(cal.)	188 (cal.)	$^3A_2g \rightarrow ^3T_1g(p)$								
	40650	246	(L) → Au C.T								

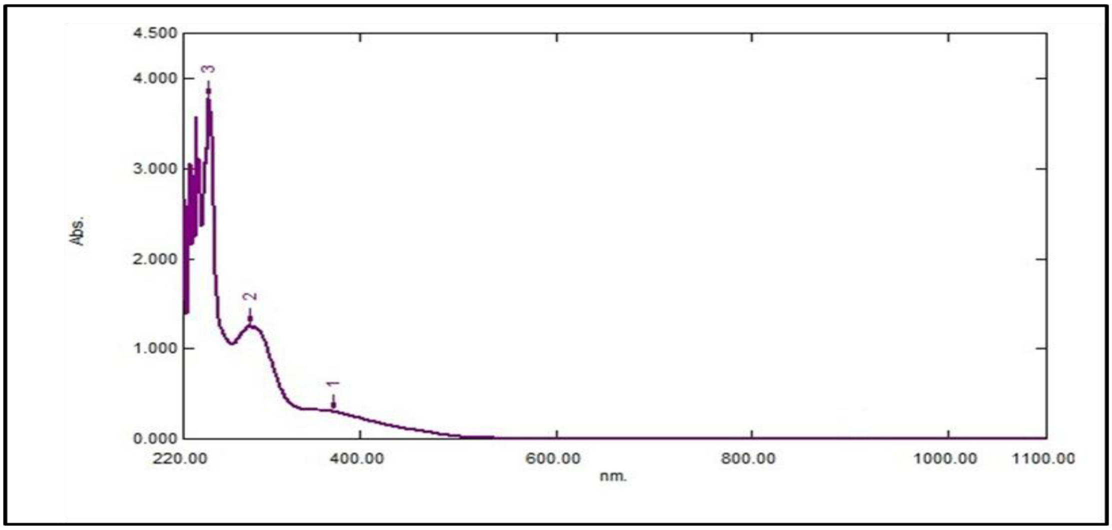


Fig. 6. UV-VIS spectrum of Au(III) complex.

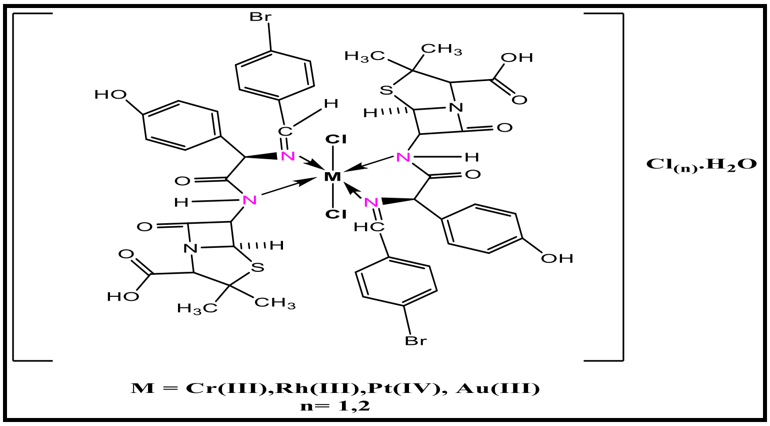


Fig. 7. Suggested structure for the prepared complexes.

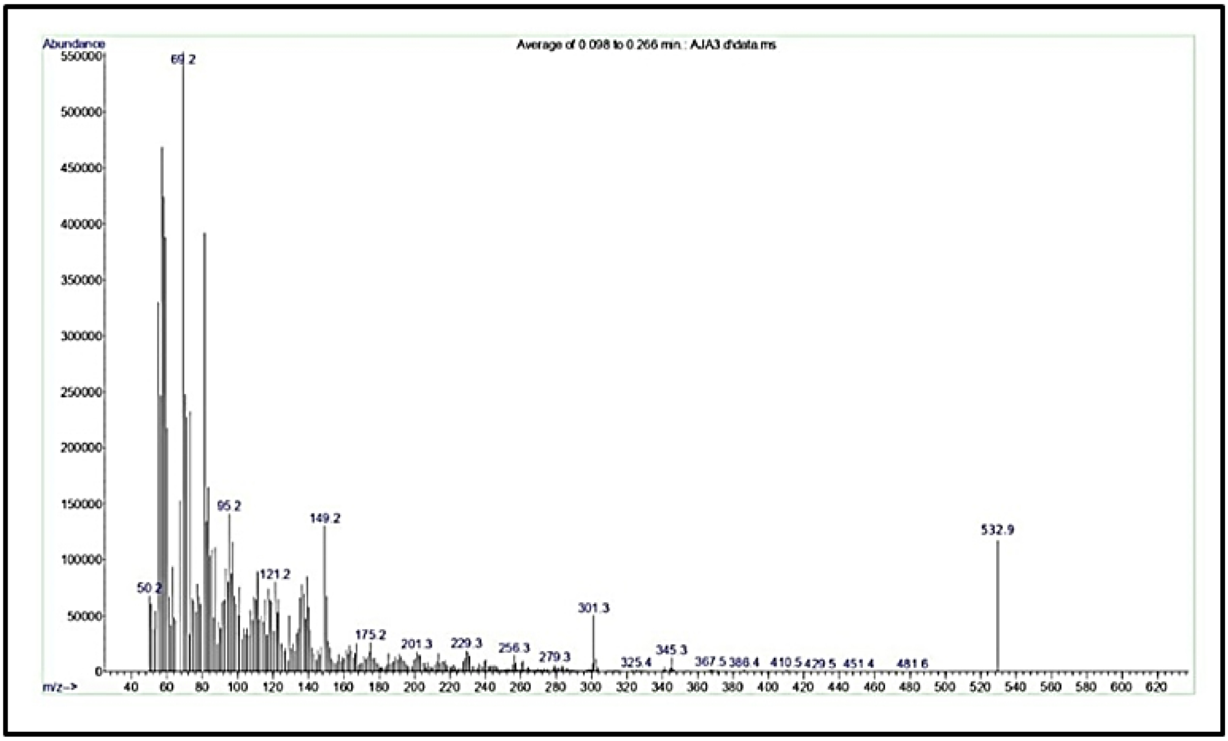


Fig. 8. Mass spectrum of ligand.

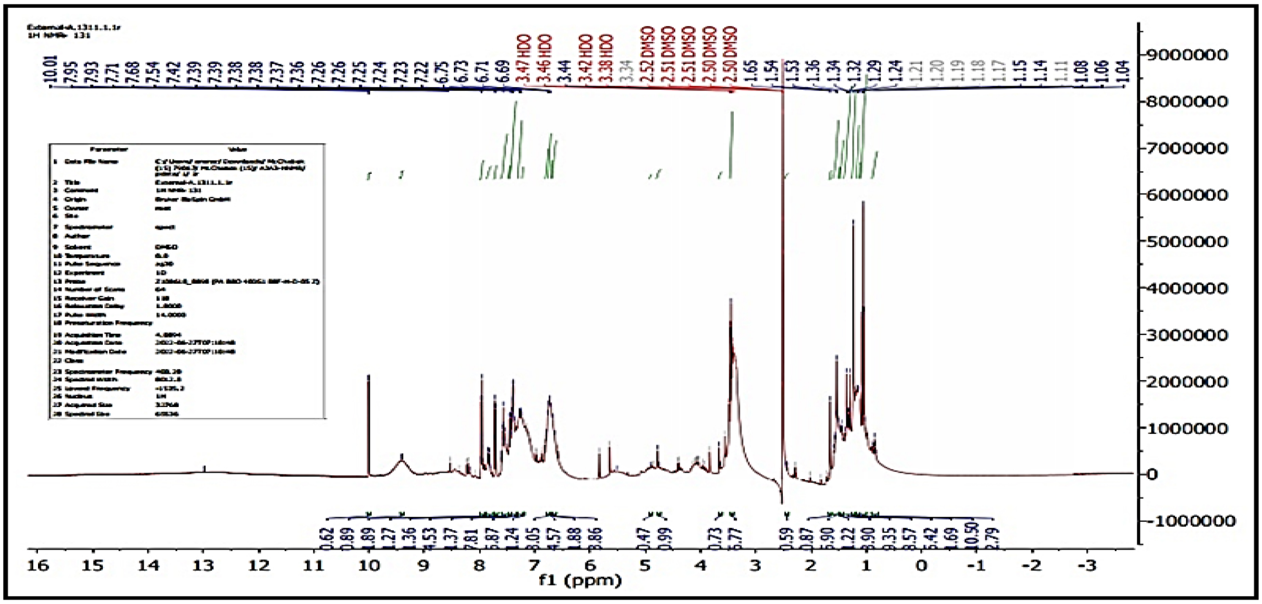


Fig. 9. ^{1}H-NMR spectrum of the ligand.

treatment, platinum-based compounds like cis-platin have limited effectiveness. To explore potential alternatives, in vitro experiments were conducted to test chromium and rhodium complexes against cancer MDA-MB-231 cell lines.

Schiff base ligand and their complexes were tested for cytotoxicity in the 50–400 (g/ml) concentration range. Fig. 13, which lists the LSD values for each complex, reveals the results. In our study, we prepared the complexes which recorded a high inhibition rate reduced progressively from high to low concentration for AuL (62, 55, 42, and 30%) with LSD values of 7.44, CrL (60, 50, 45, and 28.0%) with LSD value 8.69 and for ptL (60, 46, 39, and 20%) with LSD value

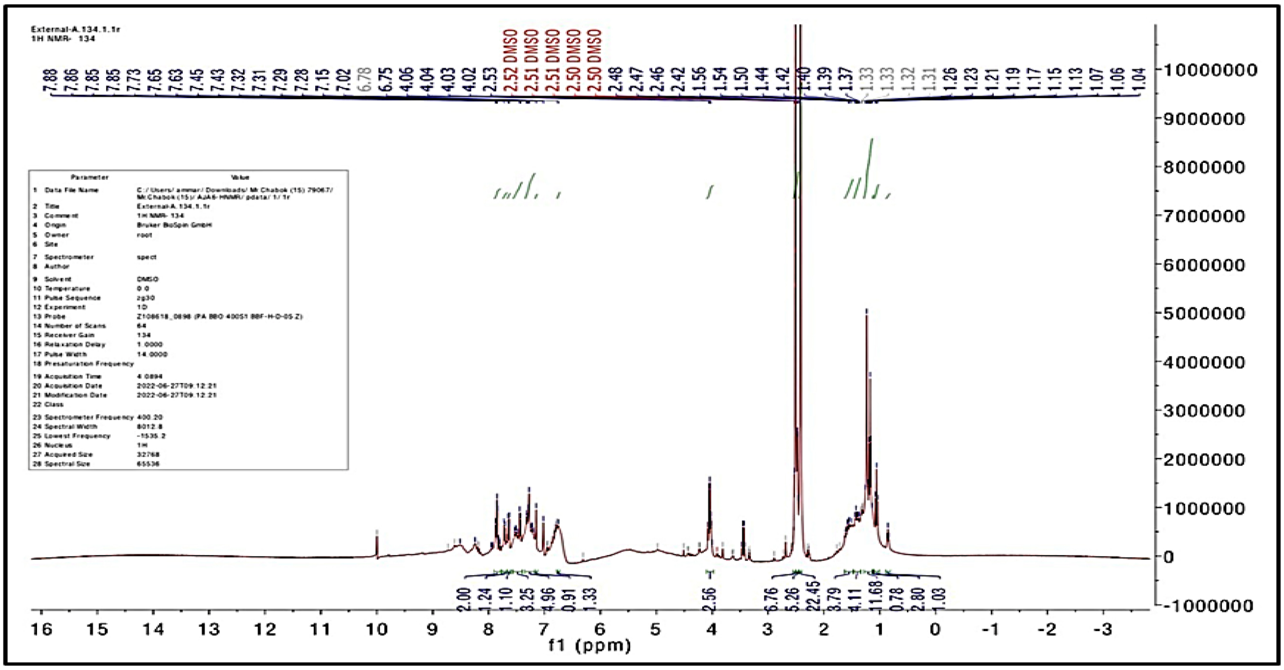


Fig. 10. The ^{1}H-NMR spectrum of the Pt (IV) complex.

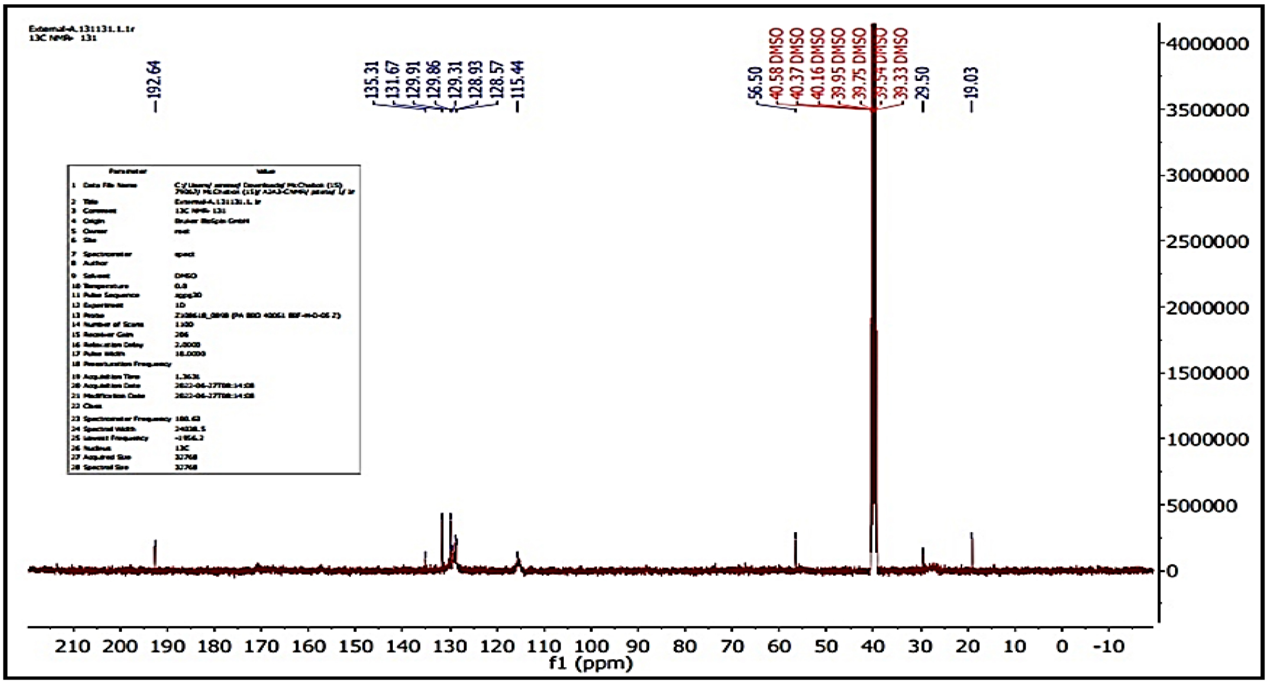
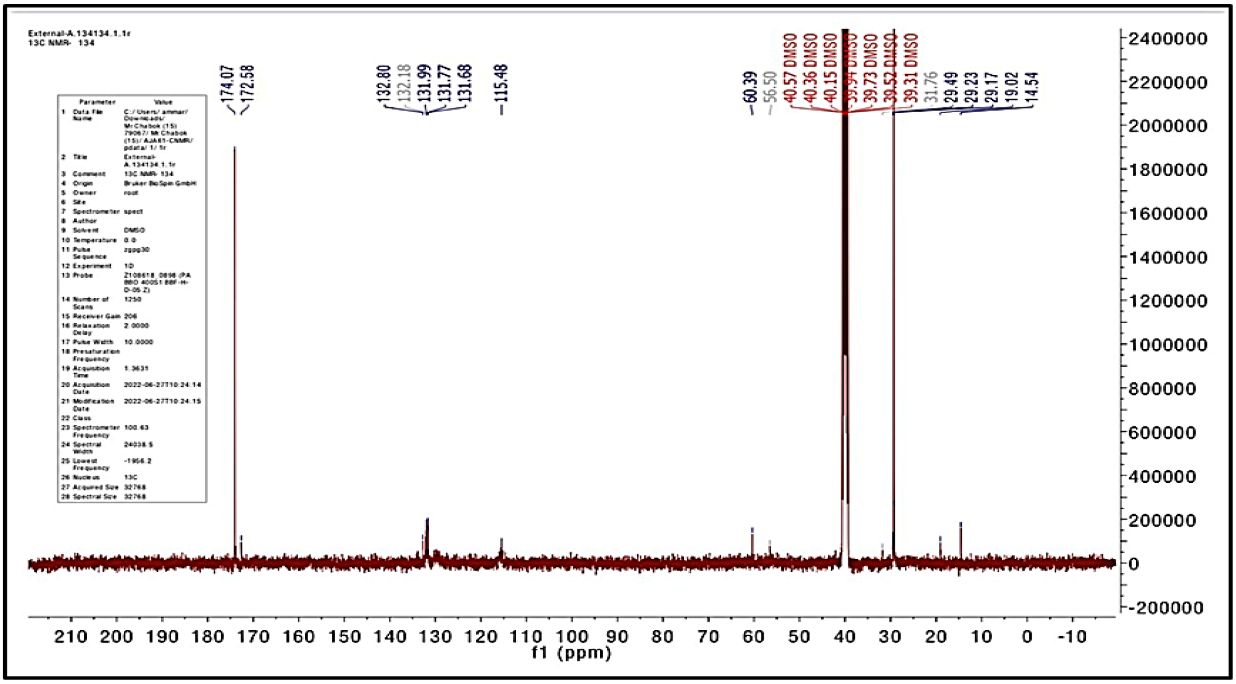


Fig. 11. The ^{13}C-NMR spectrum of the ligand.

8.51. The free ligand showed a lower cytotoxicity effect (56, 40, 34, and 13 %) with a value of 8.07. The cytotoxicity tests demonstrated that all the examined complexes exhibited potential against MDA-MB-231 cancer cells. These findings suggest that these complexes hold promise as effective anticancer agents and warrant further investigation and study.

Antimicrobial activity

The *in-vitro* susceptibility of certain strains of the bacterium, such as S. aureus and E. coli, and antifungal against one species, candida albicans, toward Schiff base ligand and their metal complexes were studied. The investigated compounds, ligand, and



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7	Spectrometer	spect
8	Author	
9	Solvent	DMSO
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12	Experiment	1D
13	Probe	Z108618_0898 (PA BBO 400S1 BBF-H-D-05 Z)
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15	Receiver Gain	206
16	Relaxation Delay	2.0000
17	Pulse Width	10.0000
18	Presaturation Frequency	
19	Acquisition Time	1.3631
20	Acquisition Date	2022-06-27T10:24:14
21	Modification Date	2022-06-27T10:24:15
22	Class	
23	Spectrometer Frequency	100.63
24	Spectral Width	24038.5
25	Lowest Frequency	-1956.2
26	Nucleus	13C
27	Acquired Size	32768
28	Spectral Size	32768

Fig. 12. The ^{13}C-NMR spectrum of the Pt (IV) complex.

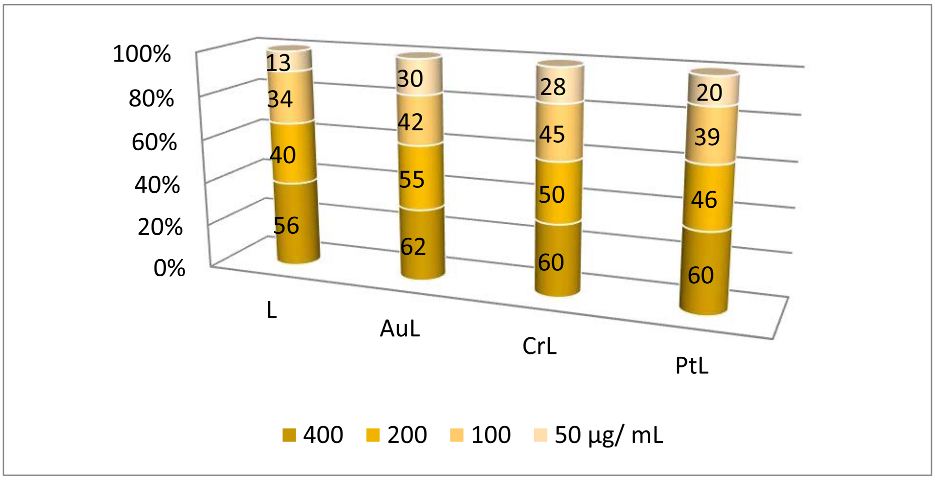

Fig. 13. The percentage inhibition rate in (400, 200, 100 & 50 μg/ mL) after exposure to L and its metal complexes for 24 hrs.

their metal complexes were dissolved (30 μg) in DMF (0.01 ml). The results obtained are shown in Fig. 14.

Schiff's bases constitute a significant class of pharmacologically active molecules that have attracted the attention of medicinal chemists due to their diverse pharmacological properties. A number of Schiff's base derivatives have been reported to exert notably antibacterial, antifungal, and DNA-binding activities.[26] It is believed that different biological activities of Schiff bases, like antibacterial, antifungal, and herbicidal activities, are attributed to the azomethine linkage. Various Schiff base derivatives of - lactam antibiotics have been synthesized and evaluated for their antimicrobial activity in an effort to increase their "potency and broaden their antibacterial activity spectrum. Pharmacological research is interested in the design of metal–drug complexes because it was discovered that metal–drug combinations increase the activity of the drug and reduce the incidence of toxic side effects.[27]

At a minimum inhibitory concentration (MIC) of 100 mg/ml, the Pt(IV) complex had the greatest significant antibacterial activity against all chosen microbial. This demonstrates that the Pt (IV) complex

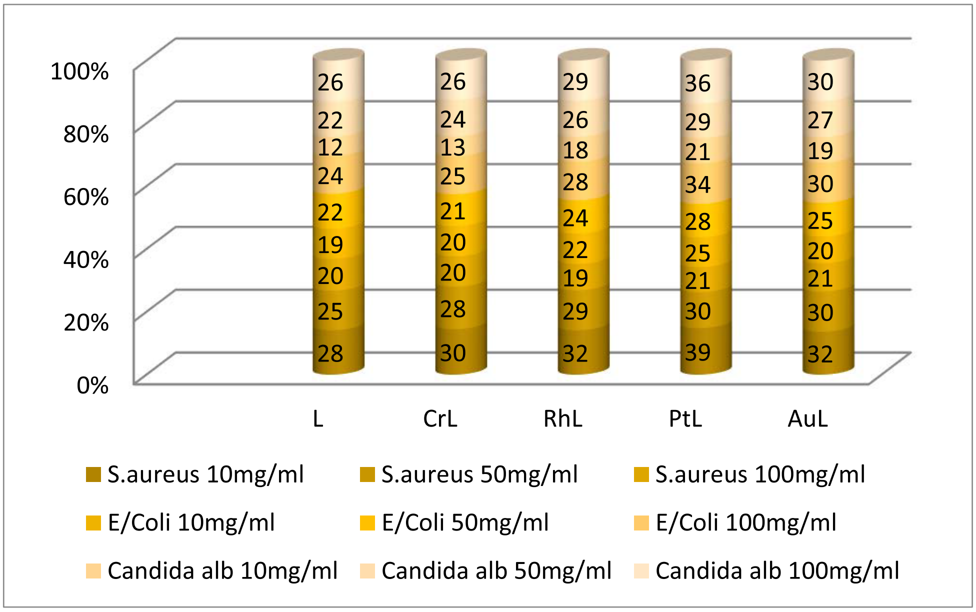

Fig. 14. Antimicrobial activity of L and their metal complexes against three types of selected microorganisms.

is a powerful antibacterial compound with strong microbial growth inhibition properties. Pt (IV) complex was more active than ligand and other complex, on the other hand. The metal ion's small size could result in less chelation with the ligand and reduced antibacterial action. The activity of metal chelates is significantly influenced by chelate theory. Metal ions are bound to several donor groups during chelation to produce stable complexes. This complex can have unique chemical properties that differ from those of the metal ion or ligand alone. These properties may affect the ability of the complex to interact with its bacterial targets and inhibit their growth. Overall, the activity of metal chelates against microorganisms is a complex process that depends on several factors including the type of metal and oxidation state of metal ions, lipophilicity, and chelation theory. Further research on these factors may aid in the development of new and more effective antimicrobial agents.[28]

Conclusion

In conclusion, studies have been conducted on the anticancer and antibacterial effects of Schiff ligand and their Cr(III), Rh(III), Au(III), and Pt(IV) complexes. All synthesized metal complexes displayed moderate cytotoxicity against MDA-MB-231 cancer cell lines. According to *in-vitro* biological investigations, the Au(III) complex exhibited the most promising anticancer efficacy against the MDA-MB-231 cell line, with an LSD value of 7.44. The Pt(IV) complex, when compared with ligand and other prepared complexes, demonstrated the best biological activity against G^+ and G^- bacteria and fungi, demonstrating its promising therapeutic potential.

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Authors' declaration

- Conflicts of Interest: None.
- We hereby confirm that all figures and tables in the manuscript are ours. Furthermore, figures and images that are not ours have been included with the necessary permission for re-publication, which is attached to the manuscript.
- No human studies are presented in the manuscript.
- No animal studies are presented in the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee at the Ministry of Higher Education and Scientific Research.

Authors' contribution statement

A.J.A., did the conception, design, acquisition of data. A. S. S. and E FM made in the methodology of ligand synthesis M F. A., did the analysis, interpretation, drafting the MS, revision, and proofreading.

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تحضير، تشخيص طيفي، قياس السمية الخلوية ودراسات تقييم مضادات الميكروبات لبعض معقدات العناصر الانتقالية لقاعدة شف المشتقة من الأموكسيسيلين

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الخلاصة

تم تحضير اربعة معقدات فلزية مشتقة من ليكاند قاعدة شف. لتوصيف كل من الليكاند والمعقدات الفلزية، تم استخدام تقنيات مختلفة ، بما في ذلك التحليل الدقيق للعناصر، والتحليل الطيفي للأشعة تحت الحمراء، والتحليل الطيفي للأشعة المرئية وفوق البنفسجية، وقياسات الموصلية المولية، وتحديد العزم المغناطيسي، وتحليل درجة الانصهار. أظهرت النتائج أن المعقدات المتكونة لها الصيغة $[M(L)_2Cl_2]\ Cl_{(n)}.H_2O$، حيث L = ليكاند قاعدة شف، M = Cr(III) , Rh(III) ,Pt(IV) ,Au(III)، n= 1,2. استنادًا إلى نتائج التحليل الطيفي، يكون التناسق مع ايونات المعادن من خلال ذرتي نتروجين مانحة لكل من الايمين و N-H و الشكل ثماني السطوح سداسي التناسق، اظهرت الموصلية المولارية ان المعقدات ذات طبيعة الكتروليتية. في هذه الدراسة ، تم فحص الإمكانات المضادة للسرطان علاوة على ذلك، تم تقييم فعالية الليكاند ومعقداته في المختبر ضد البكتيريا سالبة الجرام (*E. coli*) والبكتيريا موجبة الجرام (*S. aureus*)، وكذلك السلالة الفطرية *Candida albicans* ، باستخدام طريقة الانتشار القرصي. أشارت النتائج إلى أن المعقدات أظهرت أعلى مستويات السمية الخلوية ضد خطوط الخلايا MDA-MB-231 ونشاط مضاد للميكروبات أكثر من الليكاند الحر. تشير هذه النتائج إلى أن هذه المعقدات قد يكون لها تطبيقات واعدة في تطوير عوامل جديدة مضادة للسرطان ومضادات الميكروبات.

الكلمات المفتاحية: مضادات الميكروبات، اموكسيسيلين، خطوط خلوية، معقدات فلزية، قاعدة شيف.