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Preparation and Characterization of Mixed Ligands of Flucloxacillin and Proline and Theoretical Treatment to Form Coordination Complexes with Some Metal Ions

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Abstract

Flucloxacillin (Flx) derivative metal complexes(2S,5R,6R)-6-({[3-(2-chloro-5-fluorophenyl)-5-methylisoxazol-4-yl] carbonyl}amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo [3.2.0] heptane-2-methyl aster&proline with CoII, NiII, CuII, ZnII and CdII. They were synthesized and identified using physiochemical techniques, including (elemental analysis CHNS, metal analysis AA, Uv-Vis, FTIR, TGA, magnetic susceptibility, and conductivity measurement). This study aims to investigate the coordination behavior of flucloxacillin and proline, analyze their structural and electronic properties, and evaluate their potential biological activities against bacterial and fungal strains. Additionally, theoretical calculations using HyperChem software are conducted to assess the stability and electronic configurations of the synthesized complexes. Flucloxacillin reacted with metal ions as a bidentate ligand using the nitrogen of the β-lactone thiazolidine ring and the carboxylate ion, forming a five-membered ring, according to the results and from the oxygen and amine group of proline as mixed ligand complexes, with a six-coordination octahedral geometry proposed for all complexes. The Hyper Chem-8.0.7 software was employed to compute the Heat of Formation (Hf), Binding Energy (ΔEb), Total energy (ΔET), Vibration Spectra, and Electronic Spectra for the mixed ligand and its corresponding metal complexes. The antifungal activity of mixed ligands and their metal complexes was evaluated against Candida albicans, while their antibacterial activity against Staphylococcus aureus and Escherichia coli was also assessed. These tests were conducted at a concentration of 5 mM in a nutritional agar medium.

Keywords: Flucloxacillin derivatives, Metal ions complexes, Mixed ligand, Proline, Transition metal ions.

1. Introduction

In biological systems, it is common for metal ions to form ternary complexes, wherein the metal ion interacts with two or more different ligands (1). The recent increase in the popularity

of mixed chelation can be attributed to the presence of numerous possible ligands in biological fluids, which compete for metal ions *in vivo* (2). Ternary coordination complexes are widely recognized for their significant role in various biological processes, as evidenced by the fact that metal ions are known to activate enzymes in several instances. Ternary complexes that incorporate an amino acid as a secondary ligand hold considerable significance due to their potential as models for enzyme-metal ion-substrate complexes. The involvement of ternary complexes in the storage and transportation of active chemicals across membranes has been further impacted by the formation of these species and the electronic configuration of the metal ions involved in the process mentioned above (3). Flucloxacillin, alternatively referred to as floxacillin (Flx), is a widely utilized antibiotic in treating infections. Its mechanism of action involves inhibiting bacterial cell wall synthesis (4). Flucloxacillin, a β-lactam antibiotic, is commonly associated with a significant incidence of drug-induced cholestatic liver damage. While the precise pathogenic mechanisms responsible for developing the clinical phenotype remain unknown, it is plausible that both immune-mediated and nonimmune systems play a role. Forflucloxacillin-induced cholestasis, a nonimmune-mediated process characterized by the activation of HSP27 and subsequent sequential molecular events, has been documented (5). It is especially effective against benzylpenicillin-resistant *Staphylococci*. This category includes infections of the bones and joints, endocarditis, and soft tissues. In the event of a known penicillin hypersensitivity, it should be avoided. Patients having a history of hepatic impairment brought on by flucloxacillin should be avoided. In porphyria (associated with acute attacks), flucloxacillin is considered unsafe (6). Proline is a non-essential amino acid that the body synthesizes from glutamic acid. It is a key component of proteins, especially collagen, supporting skin, tendons, bones, and connective tissues. Vitamin C enhances its production and is found in citrus fruits, bell peppers, and parsley. Proline-rich foods include meat, fish, cabbage, soybeans, and peanuts. Also known as Pyrrolidine-2-carboxylic acid, it is a white solid with a molecular weight of 115.132 g/mole, a melting point of 205-228°C, and a molecular formula of $C_5H_9NO_2$. Proline was first isolated in 1900 by Richard Willstätter (7,9). This study aims to synthesize and characterize mixed ligand complexes of flucloxacillin and proline with transition metals (CoII, NiII, CuII, ZnII, CdII). It examines their coordination behavior, structural and electronic properties, and biological activities, especially antibacterial and antifungal effects. HyperChem software is used for theoretical calculations on stability and electronic configuration.

2. Materials and Methods

2.1. Instrumentation

Electrical conductivity measurements of the complexes were made with 10^{-3} M of DMF solvent and with the temperature of the laboratory in the laboratories of the Department of Chemistry, College of Science for Women, University of Baghdad. The melting point of the prepared ligands and complexes was measured using the Gallen Kamp M.F.B-60 thermoelectric melting device in the Department of Chemistry/ College of Science for Women/ University of Baghdad laboratory. The electronic spectra of the ligands and their ion metal complexes were recorded on an 1800-UV Shimadzu spectrophotometer in the range of 200–1100 nm using concentration 10^{-3} M by and DMF as a solvent at the laboratories of the Department of Chemistry, College of Science, University of Baghdad. Infrared spectra of

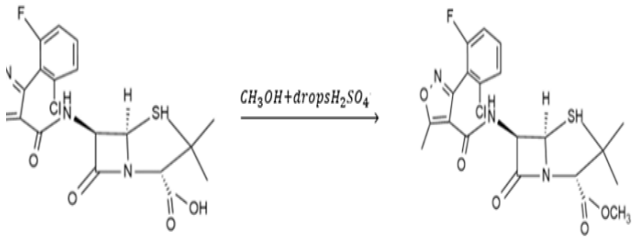
ligand in and their complexes were recorded using a device of the type (IR Prestige-21, single beam path) Fourier Transform Infrared spectrophotometer (Shimdizu) using a CsI disk within the range (200-4000) cm^{-1} at Central Environmental Laboratories, Department of Chemistry/ College of Science/ University of Baghdad. The prepared ligands' ^{1}H-NMR nuclear magnetic resonance spectra were recorded using a Bruker Avance 500 MHz NMR Spectrometer. The measurements were carried out at the University of Tehran/ Iran using DMSO as a solvent and TMS as an internal source. A study of the biological activity of each of the metal salts, ligands, and complexes prepared on special types of positive and negative bacteria and fungi. The tests were conducted in the laboratory of BPC Analysis Center/ Iraq-Baghdad.

2.2. Materials

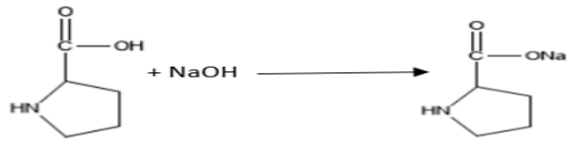
All materials used in the research are supplied by Fluka, with a purity of 99% (methanol, ethanol, $CoCl_2.6H_2O$, $NiCl_2.6H_2O$, $CuCl_2.6H_2O$, $ZnCl_2.6H_2O$, $CdCl_2.6H2O$, NaOH, H_2SO_4, proline, DMF, DMSO).

2.3. Preparation of mixed ligand

Flucloxacillin derivative was prepared by dissolving the drug (0.4 g) in (35 mL) methanol with drops of concentrated sulfuric acid to convert the COOH group to OCH_3.



About 0.11 g of Proline was reacted with (0.04 g) sodium hydroxide (NaOH) to prepare (Flx) salt.



2.4. Preparation of mixed ligand complexes

One mmol of Flucloxacillin (L) dissolved with (1 mmol) proline, formed (L-pro), which reacted with the salts of the elements (0.2 g) $CdCl_2.6H_2O$, (0.23 g) $CoCl_2.6H_2O$, (0.23 g) $NiCl_2.6H_2O$, (0.17 g) $CuCl_2.6H_2O$, (0.24 g) $ZnCl_2.6H_2O$) for the preparation of the ligand complexes (L-pro) (preparation ratio L:proline: M 1:1:1).

3. Results and Discussion

The complexes are characterized by their stability at room temperature and distinct melting points and colors. The chloride content of the prepared complexes was measured using the Moore-Titro processor method in Ibn Sina laboratories in Baghdad. The physical properties are illustrated in **Table 1**.

3.1. Infrared spectra of the complexes prepared from the first mixed ligand

The band seen at a wave number of 1735 cm^{-1}, corresponding to the β-lactam carbonyl group (C=O) in the ligand, remains unaltered in all complexes. It is important to note that the oxygen atom of the carbonyl group does not participate in any coordination interactions. In contrast, the participation of the amide nitrogen atom in the β-lactam compound occurs due to its favorable

positioning for forming a 5-membered ring. This nitrogen atom is also bent out of a plane about the other three C atoms in the β-lactam ring (10-12), as shown in **Table 2**.

Table 1. Analytical and some physical data for the ligand and its metal complexes.

Compounds	Elemental analysis cal. (found) %							M.W	M.P ◦C	Color	% yield	μ_{scm}-1
	C	H	N	S	M	Cl	O					
$C_{22}H_{27}Cl\ FN_3O_5S$	52.85	5.44	8.40	6.41	---	7.09	16.00	499.98	210	Light yellow	92	---
L	(52.66)	(5.21)	(8.30)	(6.22)	---	(7.00)	(15.98)					
$C_5H_8NO_2$	52.62	7.07	12.27	-----	---	---	28.04	114.12	208	White	85	---
Pro	(52.40)	(7.00)	(12.10)	-----	---	---	(27.96)					
$[C_{27}H_{37}Cl_2CoFN_4O_8S]\ Cl_2.2H2O$	44.64	5.13	7.71	4.41	8.11	9.76	17.62	726.51	225	Pink	78	18.4
L (pro) Co	(44.32)	(5.01)	(7.31)	(4.22)	(7.97)	(9.14)	(17.02)					
$[C_{27}H_{37}Cl_2NiFN_4O_8]\ Cl_2.2H_2O$ **L (pro) Ni**	44.65	5.13	7.71	4.42	8.08	9.76	17.62	726.27	230	Light green	88	12.0
	(44.05)	(4.93)	(7.06)	(4.09)	(8.01)	(9.03)	(17.33)					
$[C_{27}H_{37}Cl_2CuFN_4O_8S]\ Cl_2.2H_2O$	44.36	5.10	7.66	4.39	8.69	9.70	17.51	731.12	235	Dark green	67	14.7
L (pro) Cu	(44.18)	(4.82)	(7.28)	(4.02)	(8.05)	(9.56)	(17.20)					
$[C_{27}H_{39}ClZnFN_4O_9]\ Cl_2.2H_2O$	45.32	5.49	7.83	4.48	9.14	4.95	20.12	715.55	243	Light yellow	85	76.2
L (pro) Zn	(44.97)	(5.03)	(7.29)	(4.09)	(9.00)	(4.10)	(19.95)					
$[C_{27}H_{39}ClCdFN_4O_9]\ Cl_2.2H_2O$	42.53	5.16	7.35	4.20	14.74	4.65	18.88	762.55	252	Light yellow	87	89.5
L (pro) Cd	(42.06)	(4.78)	(7.03)	(4.10)	(14.56)	(4.20)	(18.33)					

The coordination between the oxygen atom of the carbonyl group (C=O) and the nitrogen atom of the β-lactam (C-N) exhibited a decrease in frequency, falling within the range of 5-35 cm^{-1} for both the ligand (L) and all complexes. Additionally, the C-N band in proline was observed within the range of 2-21 cm^{-1}. This coordination was further substantiated by identifying M-O and M-N bands in the complex spectra, which were observed within the range of 400-550 cm^{-1} as reported in the literature (13). **Table 3** and **Figures 1-3** show the FT-IR spectrum's additional significant characteristic peaks.

Table 2. Fourier transform infrared spectroscopy bands of L and proline.

Compound	νNH	ν(COO)asy ν(COO)sym	ν C-N	C=O Ester β-Lactam L1	νM-N νM-O	νM-Cl
L	3380	----	1335	1743 1735	----	---
Pro	3083	1573 1492	1342	-----	---	---

Table 3. Fourier transform infrared spectroscopy bands of complexes.

Compound	N-H (L) (pro)	COO (asy) COO (sym) (pro)	C-N (L) (pro)	C=O Ester β-Lactam L	M-N (pro) (L)	M-O (pro) (L)	M-Cl
L (pro) Co	3033 3411	1575 1458	1300 1340	1793 1735	521 547	474 418	---
L (pro) Ni	3101 3390	1573 1460	1330 1355	1745 1735	512 543	476 416	---
L (pro) Cu	3078 3421	1577 1456	1320 1342	1780 173**5**	505 512	462 406	---
L (pro) Zn	3058 3390	1510 1458	1315 1321	1781 1733	501 510	472 451	235
L (pro) Cd	3122 3417	1508 1438	1320 1353	1770 1735	509 530	480 420	248

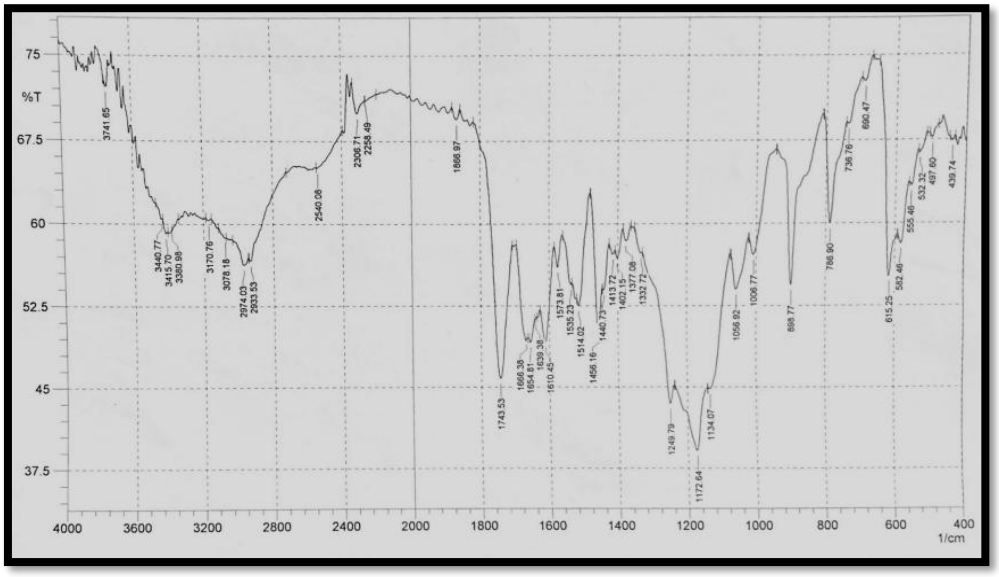


Figure 1. Spectrum of L FTIR.

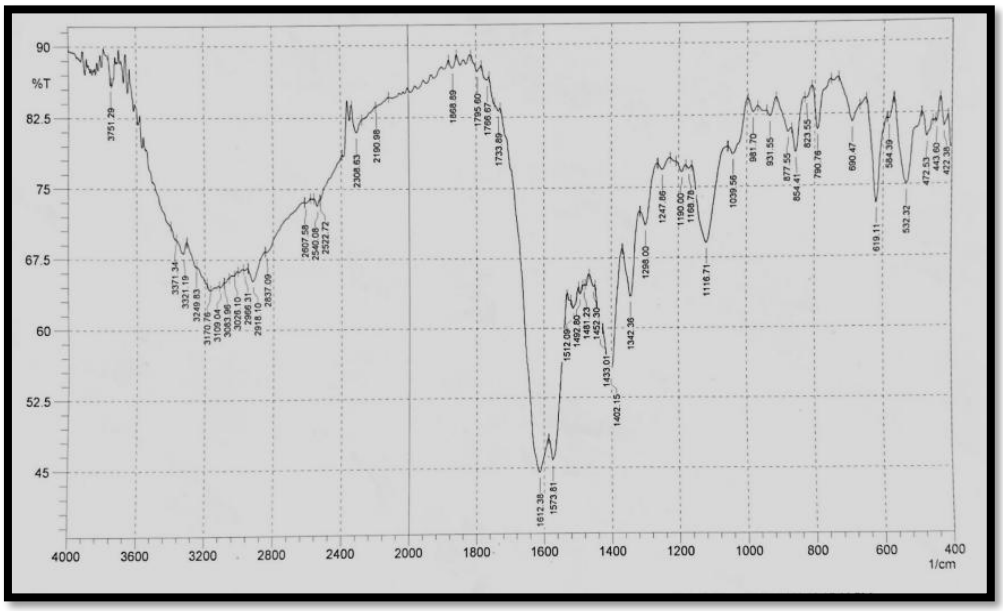


Figure 2. The FTIR spectrum of Pro.

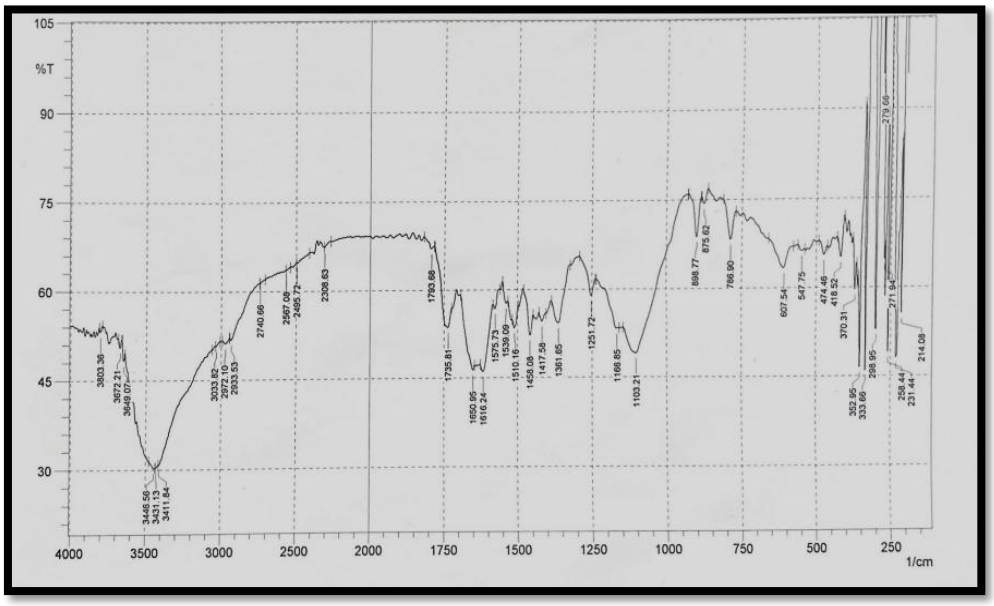


Figure 3. The FTIR spectrum of L (pro) Co.

3.2. The UV-Vis spectra of mix ligand and its metal complexes

The n→π* and π→ π* transitions to the C-O, C-N, and C=C transitions, respectively, were assigned to the peaks between 220 and 350 nm in the electronic spectrum of the mix of ligands in **Figure 4**. The utilization of spectra facilitated the prediction of the planned geometry by analyzing the characteristics of the observed peaks in shape and quantity (14). In **Table 4**, L_1(pro)Co complex shows 4 bands in the wave number (14755,16447,23255,34722) cm^{-1}. L_1(pro) Ni complex has 4 bands (11173,15873,20576,34843) cm^{-1}. L (pro)Cu complex shows 3 bands in the wave number (12987,2270,41322) cm^{-1}. L (pro)Zn complex shown 2 bands in the wave number (39216) cm^{-1}. L (pro) Cd complex shows 2 bands in the wave number (34722,29411) cm^{-1} (15,16). The Uv-visb spectra of L (pro) Cu is shown in **Figure 5**.

Table 4. The Uv-visible bands of prepared compounds.

Compound	Λ nm	Absorption Bands cm^{-1}	Assignment
L	328	30487	π-π* (C=C)
	227	44528	n-π*(CO, CN)
	221	45248	
pro	331	30211	π-π* (C=C)
	281	35871	n-π*(CO, CN)
L (pro)Co	686	14755	$^4T_1g \rightarrow {}^4T_2g$
	608	16447	$^4T_1g \rightarrow {}^4T_1g$
	430	23255	$^4T_1g \rightarrow {}^4A_2g$
	288	34722	I.L
L (pro) Ni	895	11173	$^3A_2g \rightarrow 3T_2g$
	630	15873	$^3A_2g \rightarrow {}^3T_1g_{(F)}$
	486	20576	$^3A_2g \rightarrow {}^3T_1g_{(P)}$
	287	34843	I.L
L (pro) Cu	770	12987	$^2Eg \rightarrow {}^2T_2g$
	361	2770	Charge Transfer
	242	41322	ILCT
L (pro)Zn	255	39216	ILCT
	327		ILCT
L (pro)Cd	288	34722	ILCT
	340	29411	ILCT

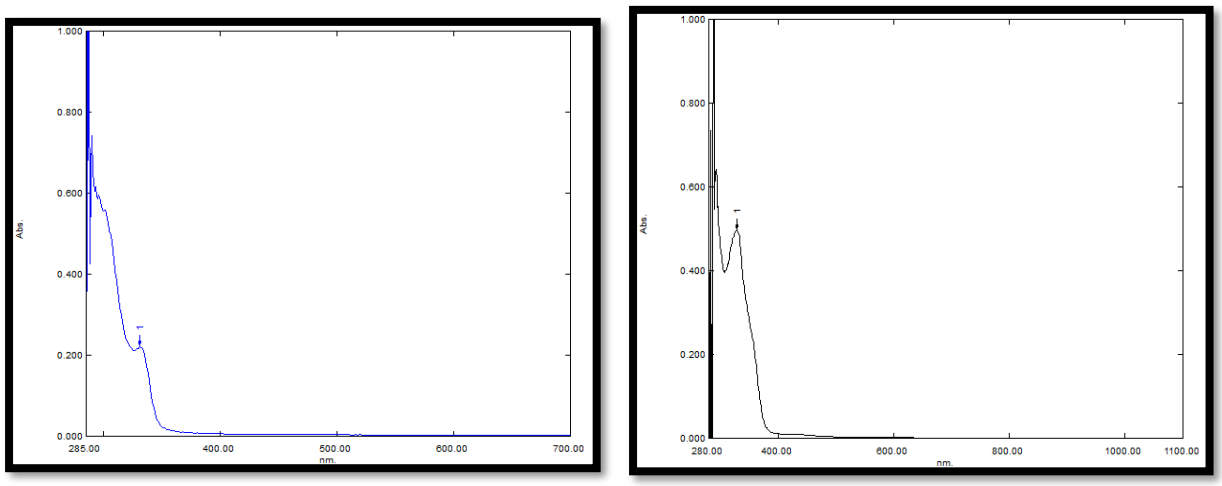


Figure 4. The Uv-visb spectra of FLU and Pro.

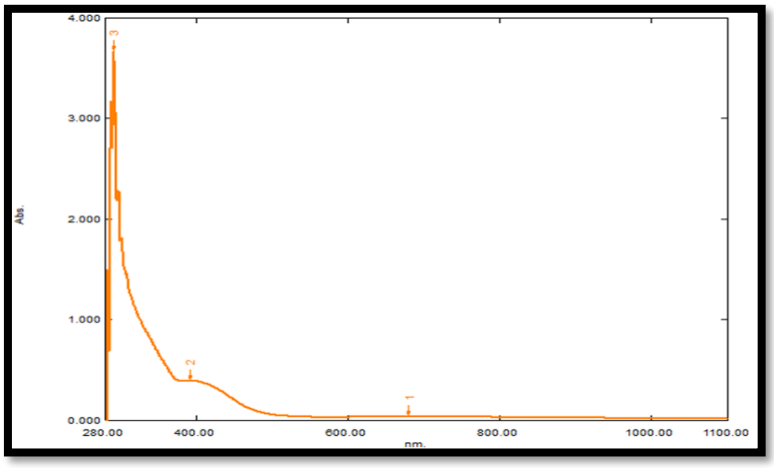


Figure 5. The Uv-visb spectra of L (pro) Cu.

3.3. The ^{1}H NMR spectrum of L(pro)

The ^{1}HNMR spectrum of the mixed ligand L (pro) was measured using DMSO as a solvent, as illustrated in **Figure 6**, where the mono signal at σ (2.53) ppm refers to the DMSO solvent and the mono signal at (3.35) that belongs to 3H, CH_3-O and the treble signal at σ (3.67) ppm which belongs to 9H, CH3, (7.30) ppm due to CH_2-N for β-lactam cycle, and the mixed ligand spectrum showed a signal at σ (8.52) ppm, which is attributed to 3H, NH, amine. It also showed a mono signal at σ (9.20) ppm due to 1H-NH amide. **Table 5** shows the apparent signals and the chemical shifted values measured in ppm (17,18).

Table 5. The ^{1}H NMR spectrum of L(pro) $C_{25}H_{27}ClFN_4O_7S$.

Compound	No of proton	σ(ppm)
L_2(pro)	S(3H, CH_3-O)	3.35
	t(9H ,CH3)	3.67
	S(2H, SCH)	4.82
	2d(7H, cyclo)	5.83 -6.73
	t (2H, CH_2-N) β-lactam	7.30
	d (3H, NH, amine)	8.52
	s (1H, NH, proline)	9.20

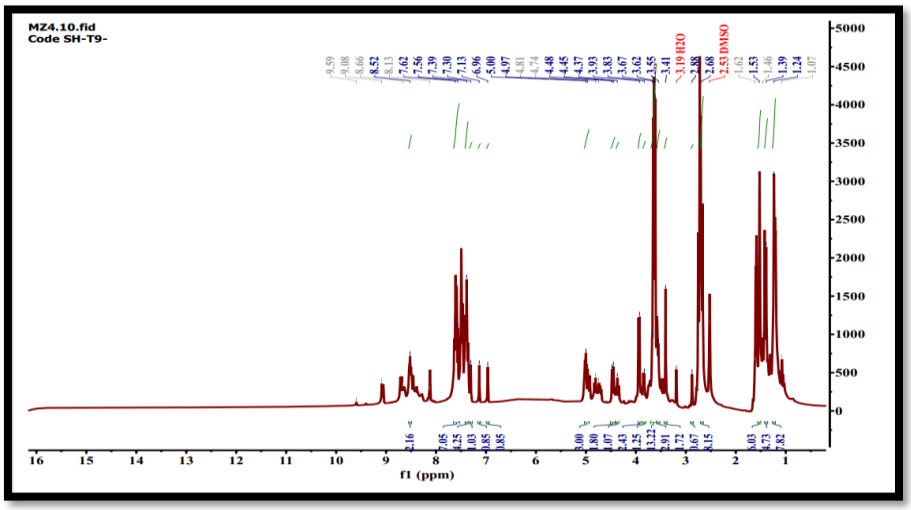


Figure 6. The [1]HMNR spectrum of L.

3.4. Theoretical study

HyperChemprogramme is an advanced molecular modeler, editor, and potent computational suite renowned for its quality, adaptability, and user-friendliness (19). To optimize geometry, it can calculate electrostatic potential, total charge density, and thermodynamic functions. The semi-empirical method is one of the most essential methods employed. This information is valuable for assessing reactivity and establishing a correlation between computational outcomes and experimental data.

3.5. Electrostatic potential

The PM3 method was used to measure the electrostatic potential (E.P) of a molecular system with a positively charged point, with the objective of identifying the most active interaction sites within the positively charged particle species. These entities tend to target a molecule with a highly negative and electrophilic electron pair (20,21). The E.P of the ligands (Flx, Proline) were determined and visualized through 2D and 3D contour plots. These plots were utilized to analyse the reactive sites within the molecules, as illustrated in **Figure** 7.

3.6. Energies and optimized geometries of metal complexes for the ligand

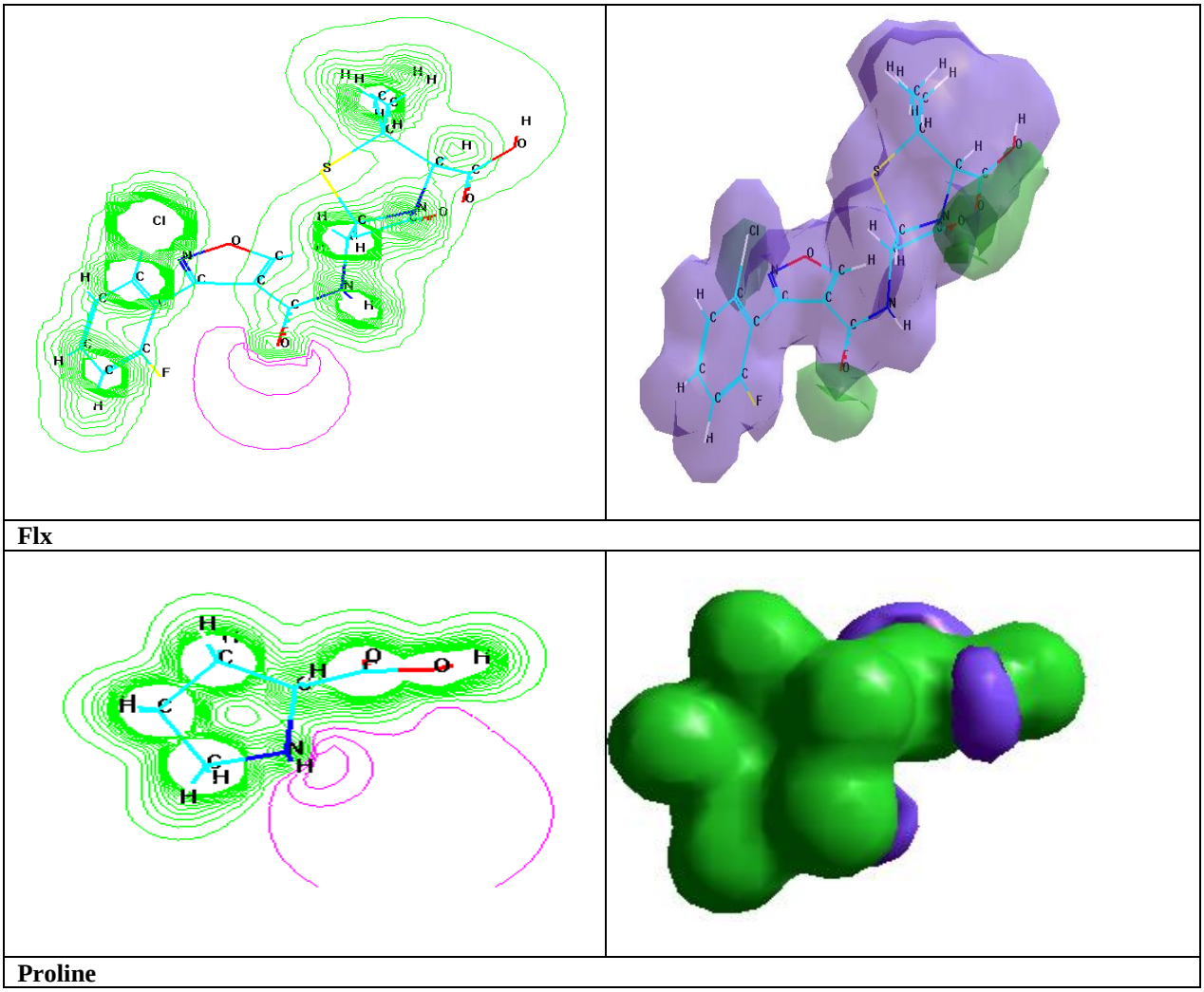
Theoretical treatment was used to find the most stable structures for the metal complexes with ligands (22,23). Depending on the method (PM3), the geometric configurations depicted in Figure 8 correspond to the most favorable computation geometries for both the ligand and its complexes. Semi-empirical techniques are used to calculate the thermodynamic functions (ΔHf, ΔEb, and ΔET) of compounds in the gas phase at 0 °K, as tabulated in **Table 6**.

Table 6. Energies of shapes (KJ.mol^{-1}) for the (L1,L2 and metal complexes).

Compounds	**PM3**		
	ΔHf	**ΔEb**	**ΔET**
Flx	-475.05	-19770.44	-507738.67
Proline	-418.29	- 6930.79	- 143078.96
L (Pro) Co	-2656.6	- 29610.09	- 785939.97
L (Pro) Cu	- 1645.13	- 28507.77	- 823673.68
L (Pro) Ni	- 2379.65	- 29334.82	- 810222.17
L (Pro) Zn	- 794.31	- 27449.58	- 711345.43
L (Pro) Cd	- 893.47	- 27530.1	- 710949.28

3.7. Optimized vibrational spectra

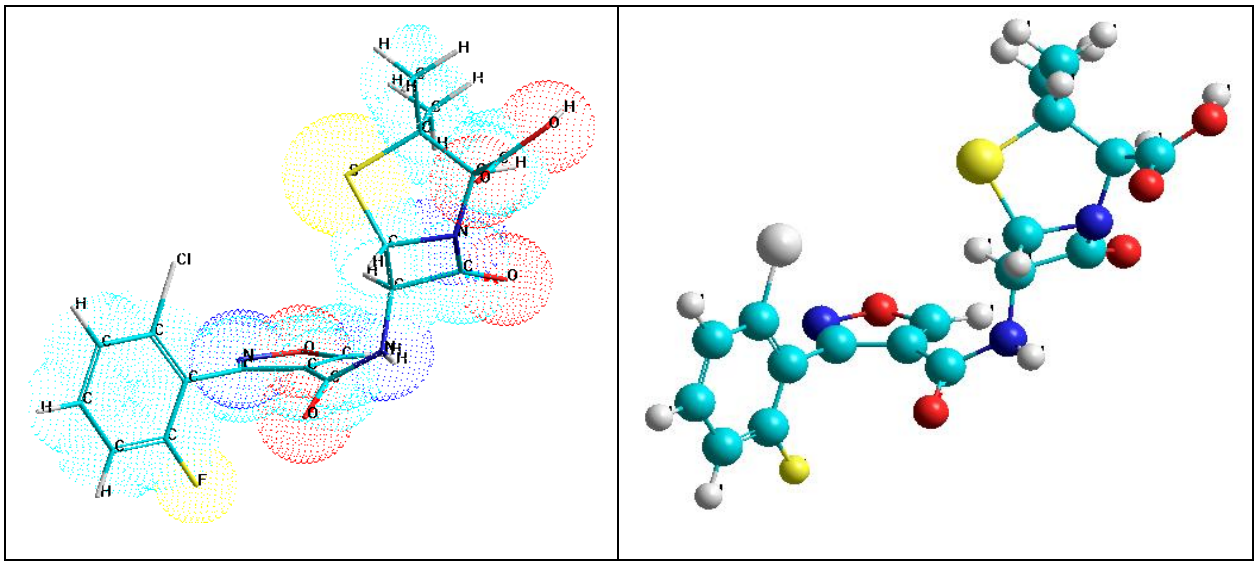
Theoretically, calculated molecular frequencies of vibration deviate from experimentally determined values (24). These deviations are generally accepted in theoretical calculations and are outlined in **Table 7**. **Figures 7-1**0 show some theoretical vibrational modes of L and proline (19,20). The Suggested structure of the prepared complexes illustrated in **Figure 11**.

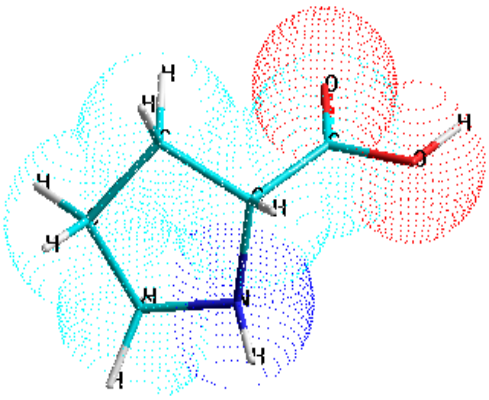
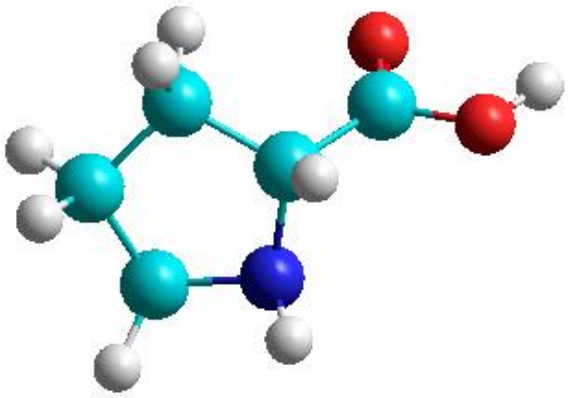
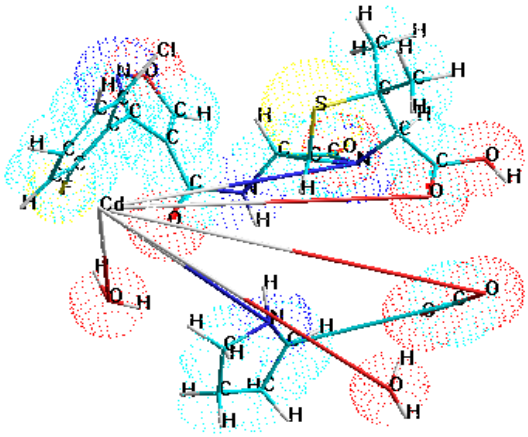
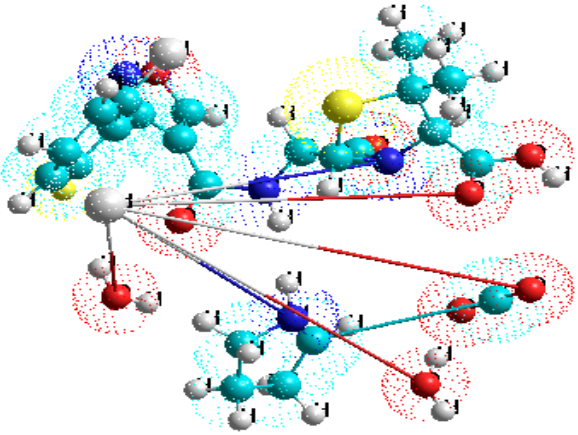
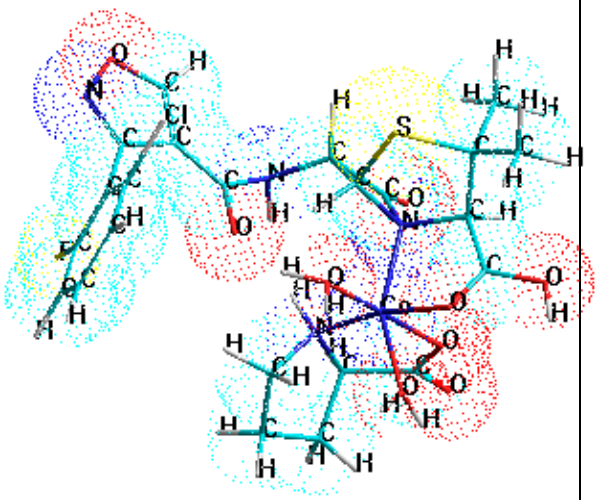
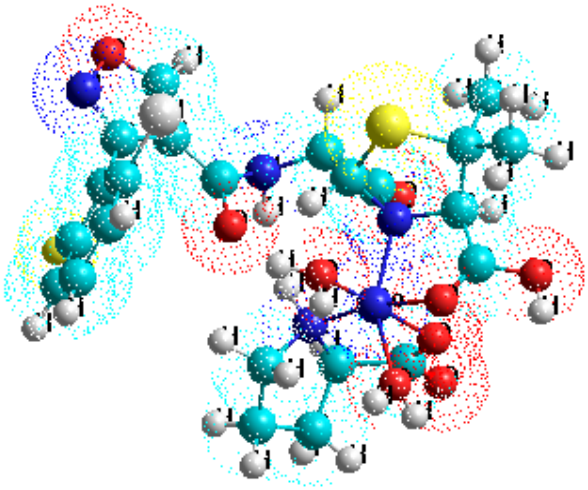


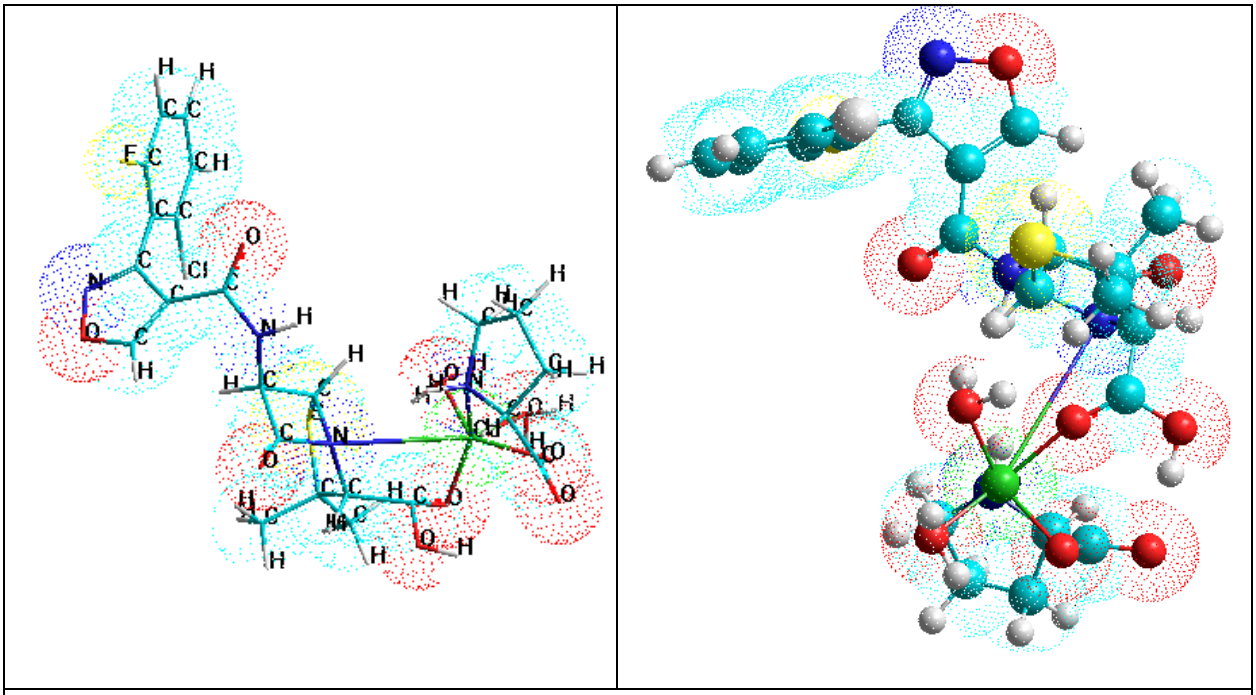
Flx

Proline

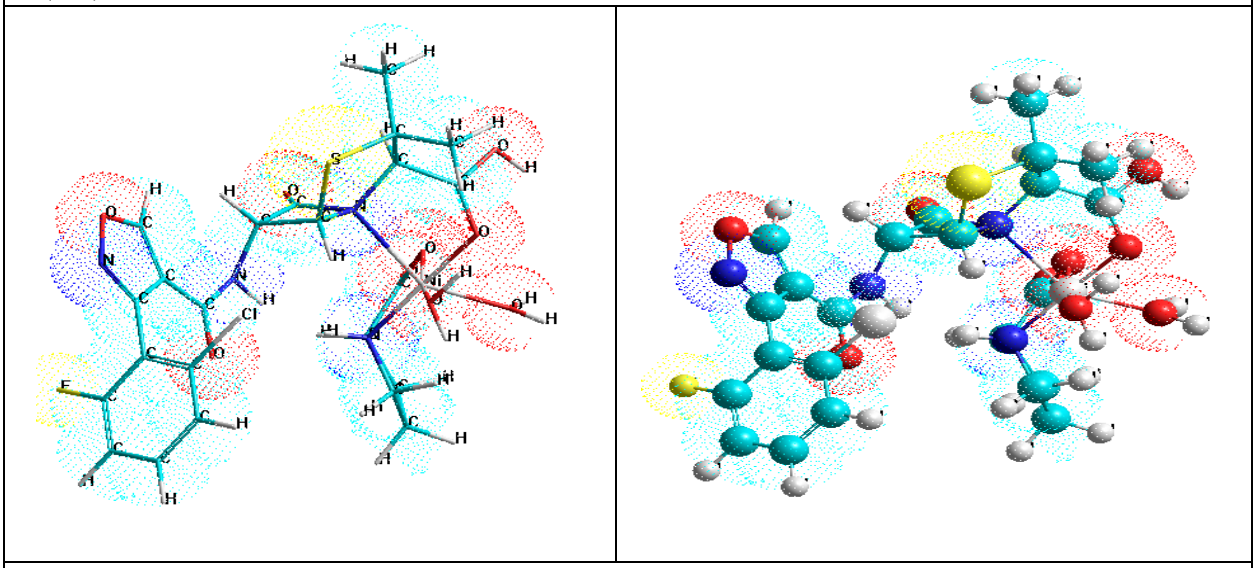
Figure 7. The E.P of the (Flx) and (proline) using PM3 methods.



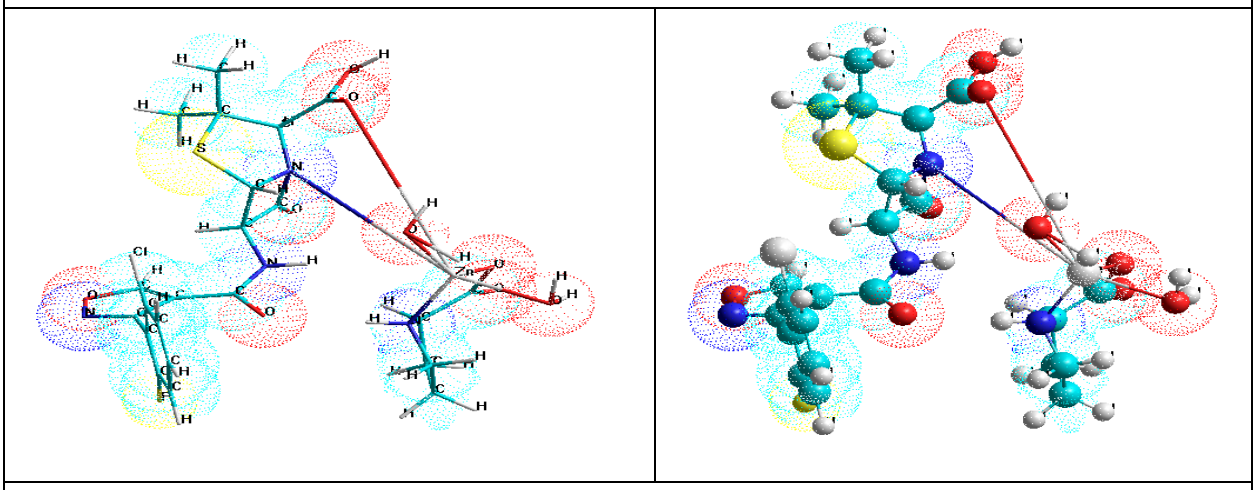
Flx	
	
Proline	
	
L (pro)Cd	
	
L (Pro) Co	



L (Pro) Cu



L (Pro) Ni



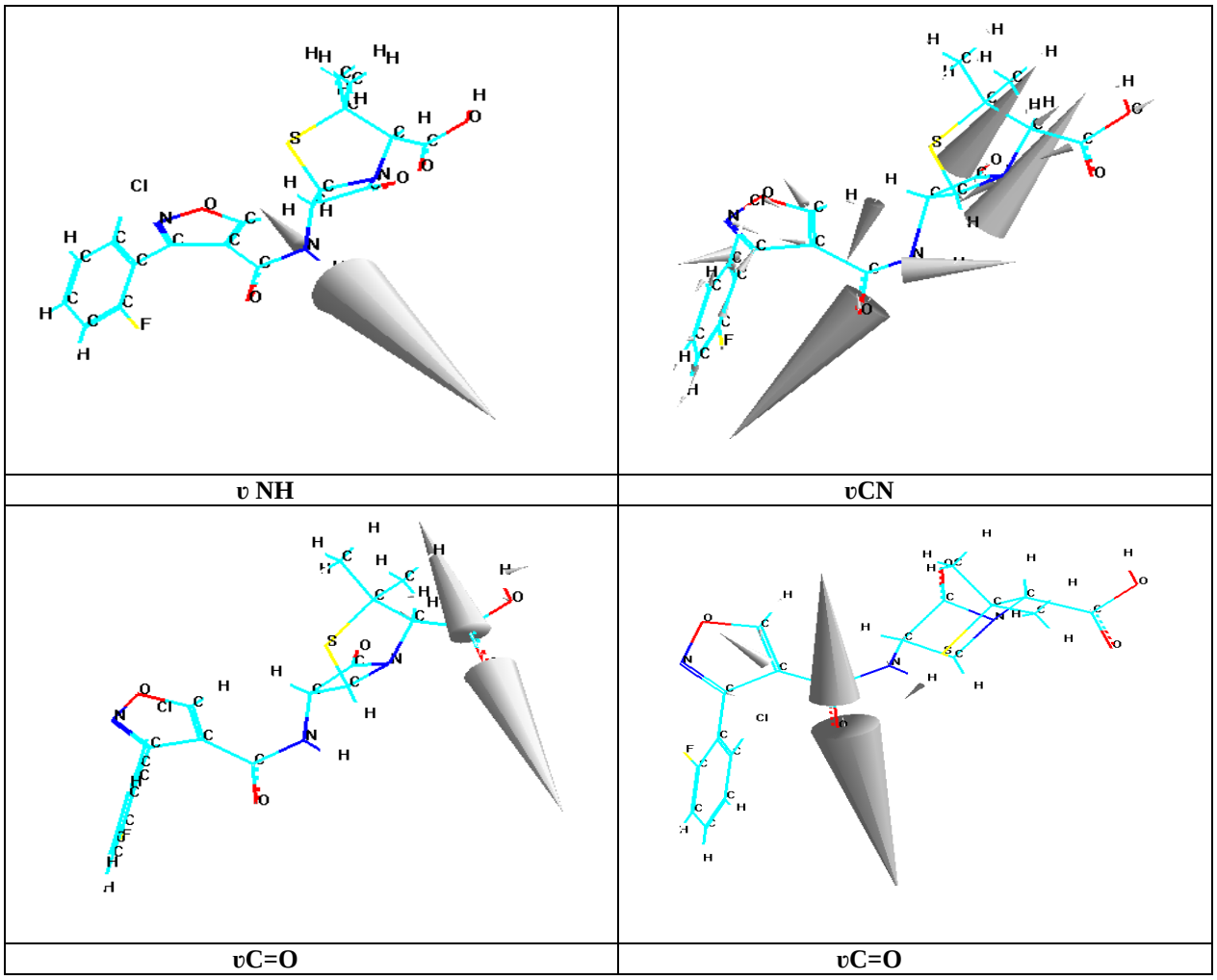
L (Pro) Zn

Figure 8. Geometric shapes of (L(Pro)) and its complexes using PM3 method.

Table 7. Data of theoretical wave number of (L, proline) compounds (cm^{-1}) and their comparison with experimental vibrations.

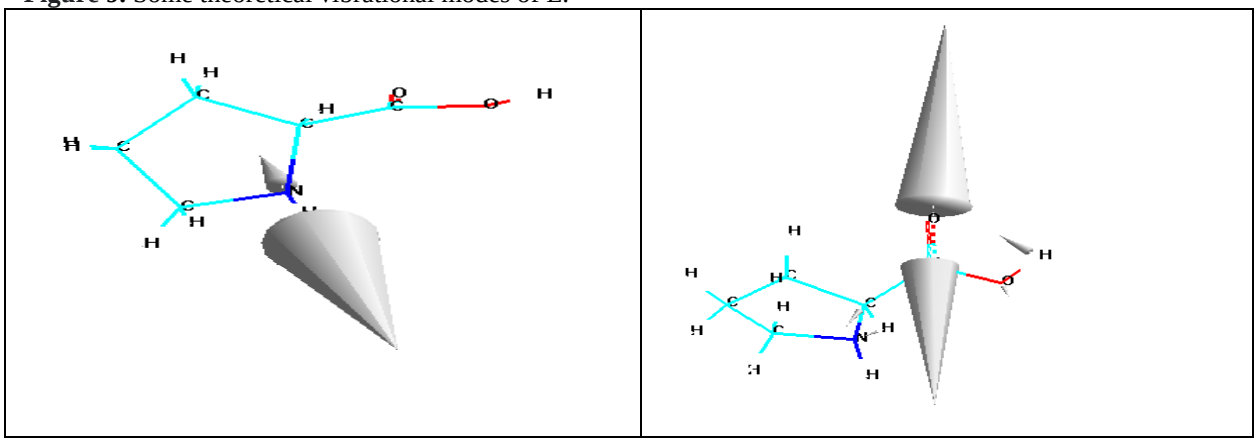
Compound	υ NH	υC=O	υCOO	υC-N
	3380*	1743*		1335*
L	3339**	1952**		1442**
	0.012***	-0.107***		0.074***
	3083*		1573*	1342*
Proline	3393		1441**	1326**
			-0.09***	-0.012***

*experimental, **theoretical, ***Error.



υ NH	υCN
υC=O	υC=O

Figure 9. Some theoretical vibrational modes of L.



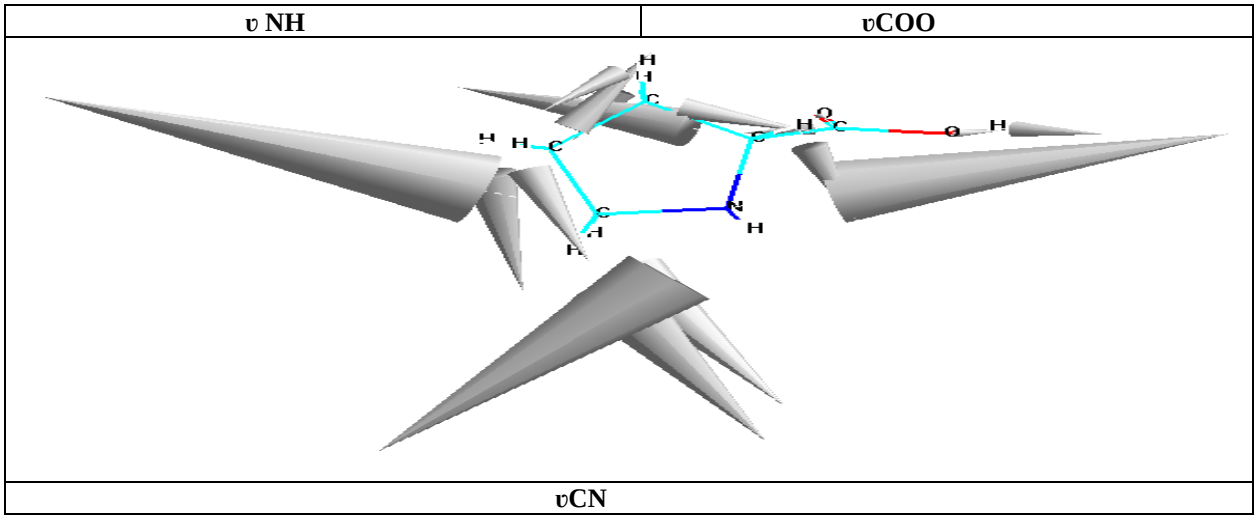


Figure 10. Some theoretical vibrational modes of proline.

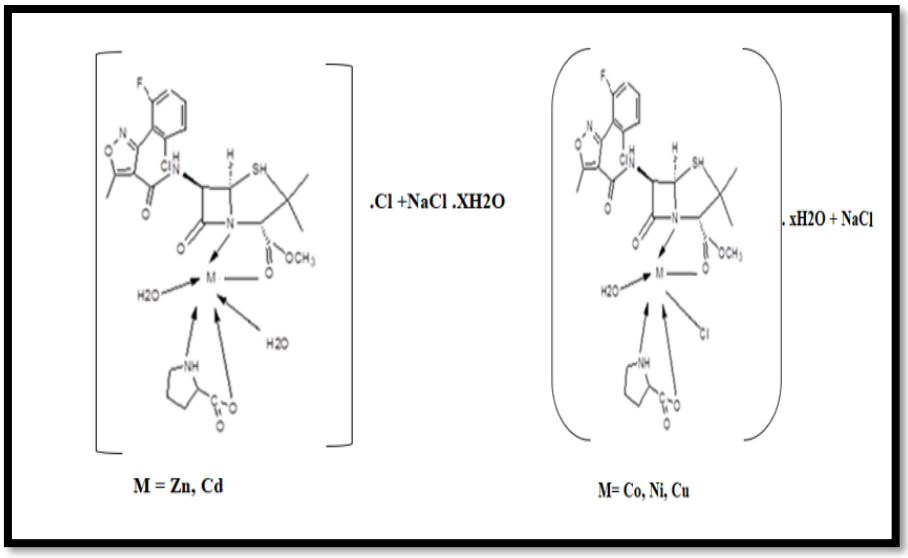


Figure 11. The suggested structure of the prepared complexes.

3.8. Evaluation of antibacterial activity

Mix ligand and metal complexes were examined for their *in vitro* biological screening effects against microorganisms. This was accomplished using the disc diffusion method with nutrient agar as the medium (25,26). The test solution was introduced into the well using a micropipette, and subsequently, 24 hours were spent incubating the dishes at 37°C. Using a ruler in millimeters (mm), the inhibition zone of each pore was measured. To record the results, the growth inhibition surrounding the disc was estimated. Chelation theory and the concept of overtones can explain why the inhibition zone of metal complexes is more significant than that of ligands. The chelation process reduces the polarity of the metal ion due to the overlapping of the ligand orbital and the partial sharing of the positive charge of the metal by the donor groups. Furthermore, it facilitates the dispersion of π-electrons throughout the entirety of the chelating ring and promotes the infiltration of complexes into lipid membranes. Additionally, it obstructs the metal-binding sites of microorganism enzymes. The results obtained for biological activity for the preparation ligand and its metal complexes are shown in **Table 8**. The free

ligand showed a slightly antimicrobial and antifungal activity against both bacteria. The nickel (II) complex shows higher inhibition against both organisms and antifungal effect than free ligands in both concentrations. The copper (II) and cobalt (II) complexes showed moderate and non-activity, respectively, against all organisms used in this study, which is clarified in **Figure 12**. The higher inhibition zone of the metal complexes than the ligand can be explained based on chelation theory and overtone concept (27-30).

Table 8. The diameter of the inhibition circle in (mm) after being injected (24 hrs.) at 37 ^{0}C for some mixed ligand complexes prepared from ligand (L2) and proline.

Compound	*Escherichia coli*	*Staphylococcus aureus*	*Candida albicans*
Pro	10	28	19
L	10	27	14
L (pro)Ni	31	34	8
L (pro)Cu	17	25	16
L (pro)Zn	15	9	9
L (pro)Co	14	8	10
L (pro)Cd	13	14	27

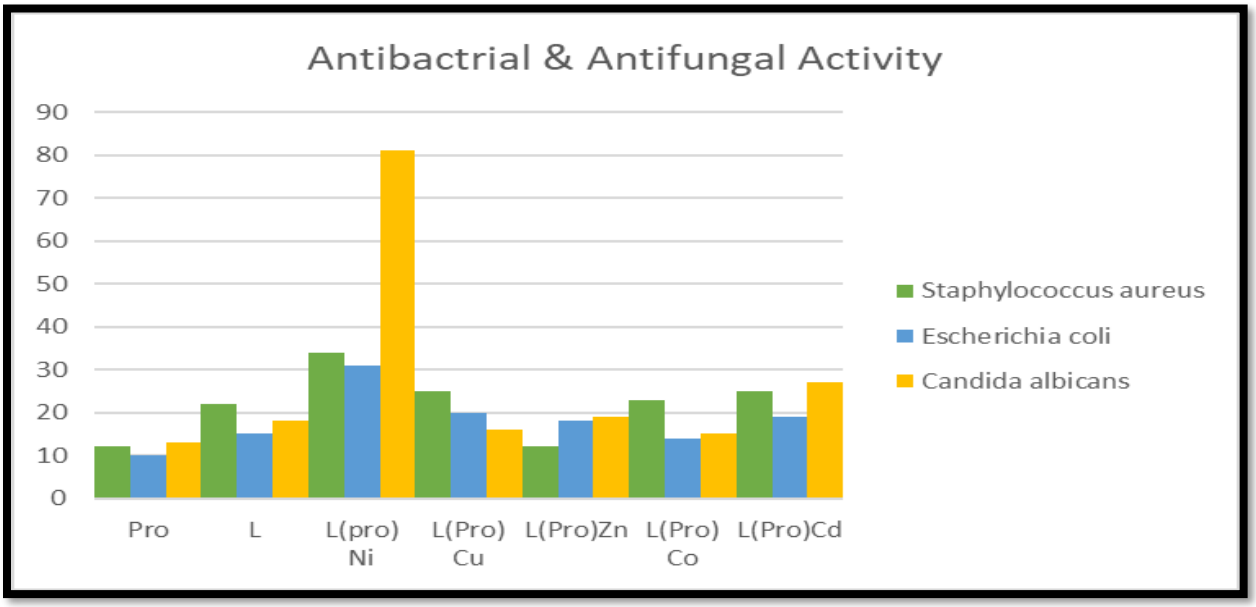


Figure 12. The antibacterial activity of prepared compounds against *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*. in 5 mM.

4. Conclusion

A new mixed ligand was prepared from a derivative of Floclxaciline and the amino acid (proline). The form and type of bonding between the ligand and the prepared complexes were revealed using spectroscopic methods and nuclear magnetic resonance. The shapes of the

formed complexes were suggested to be octahedral. The Hyperchem-8.07 software was used to forecast the structural engineering of all compounds in the gas phase, with physical properties calculated using the PM3 program.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

Ethical Clearance

The study is included in the 2022–2023 academic year research plan form at the University of Baghdad.

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