

Synthesis, Structural characterization, and Alkaline phosphatase inhibition studies of transition metal complexes of (e)-4-((3,5-dichlorophenyl) amino)-4-oxobut-2-enoic acid

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Abstract

Ligand, its sodium salt and four new metals (Zn (II), Cu (II), VO(IV) and Cd (II)) carboxylate complexes have been prepared and characterized by FT-IR, conductometry, and UV-visible spectroscopy. The prepared compounds are pure and obtained in the solid state. They have sharp melting points and have good yield. FT-IR spectroscopy proved that carbonyl and amine groups are not involved in metal-ligand coordination. The ligand was coordinated through the carboxylic group upon complex formation. The molar conductance data reveals that the compounds exhibit non-electrolytic properties, primarily attributable to their notably low conductance values. A broad band in the FT-IR spectrum of copper complex represents the presence of water molecules. UV-visible spectral data proved that the synthesized compounds have shown $\pi-\pi^*$ and $n-\pi^*$ transitions, which indicated that all the compounds are aromatic. Various concentrations of the synthesized compounds were employed to investigate their inhibitory effect on the alkaline phosphatase (ALP) enzyme, conclusively demonstrating their ability to inhibit its activity.

Keywords: Metal carboxylates, FT-IR, UV-visible, ALP inhibition, Molar conductance

Introduction

In the last fifteen years, the area of inorganic chemistry has become one of the dominant research works. Metal carboxylates are very important and mainly inspiring because they not only form open-framework structures but also work as a linker between inorganic moieties [1]. The innovative designs of carboxylates can be

developed by changing the nature of reactants, and reaction conditions, and carrying out reactions in the presence of additives (organic amines) [2]. The significance of carboxylate ligands in coordination chemistry is widely recognized, as they fulfil pivotal roles in the synthesis of Metal-Organic Frameworks

[3]. Coordination polymers with specifically designed and controlled links are of great interest due to their multipurpose topologies, interesting designs possible applications in magnetism, bio-mimetic materials, and smart optoelectronics [4].

Moreover, metal-organic coordination networks (MOCNs) are recognized as substantial consideration attention due to their formation and design. MOCNs can formulate chiral pores and functionalities that can be used in enantioselective separations and asymmetric catalysis of heterogeneous compounds [5]. It is reported that 1D and 2D MOCNs can be prepared and examined by STM at well-defined surfaces [6]. The designs of MOCN complexes possess wide applications in catalysis, luminescence, drugs, etc. The main thing of these complexes is to study their structures [7].

The biological activities of metals are enhanced upon coordination with the ligands [8]. It is also proposed that the activities of enzymes have been inhibited by the presence of oxygen and nitrogen donor systems by reducing the polarity of metal ions. The change in polarity is due to the partial distribution of positive charge with the donor group in the chelating ring system [9]. Metal carboxylates show antibacterial, antifungal, and enzymatic activities. The exploration of antiferromagnetic interactions was made possible by investigating model compounds of metalloenzymes using metal carboxylates [10].

Experimental work

Chemicals and instrumentation

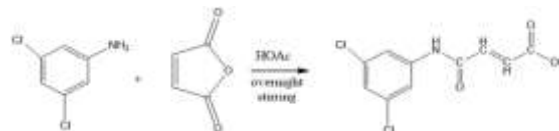
Glacial acetic acid (CH_3COOH), 3,5-dichloroaniline ($\text{C}_6\text{H}_3\text{Cl}_2\text{N}$), maleicanhydride ($\text{C}_4\text{H}_2\text{O}_3$), sodium hydroxide ethanol ($\text{C}_2\text{H}_5\text{OH}$), copper

sulphate ($\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$), zinc sulphate ($\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$), (NaOH), sodium bicarbonate (NaHCO_3), cadmium sulphate ($\text{CdSO}_4 \cdot \text{H}_2\text{O}$), and vanadyl sulphate ($\text{VOSO}_4 \cdot 5\text{H}_2\text{O}$) are used in the current work. All chemicals utilized in this study were of analytical grade and highly pure; therefore, they were employed as received without the need for further purification.

Gallenhamp capillary melting point apparatus (UK) was used for the determination of melting points. Conductance was noted on Elico conductivity Bridge Model No. CM-102 Kit, China. FT-IR spectra were recorded on FT-IR Nicolet using a KBr pallet method in the range from $4000\text{--}400\text{ cm}^{-1}$. UV-visible spectra were recorded on UV-visible spectrophotometer Jasco V-770 from 200 to 400 nm. The biological activities of compounds were checked by alkaline phosphatase inhibition at different concentrations.

Synthesis of (e)-4-((3,5-dichlorophenyl) amino)-4-oxobut-2-enoic acid (HL)

1 mmol solution of maleic anhydride ($\text{C}_4\text{H}_2\text{O}_3$) in 300 mL of glacial acetic acid (CH_3COOH) was added to the 1 mmol solution of 3,5-dichloroaniline ($\text{C}_6\text{H}_3\text{Cl}_2\text{N}$) in 150 mL glacial acetic acid and the mixture was stirred at room temperature overnight. The yellow-colored precipitates of (e)-4-((3,5-dichlorophenyl) amino)-4-oxobut-2-enoic acid (HL) were formed. These precipitates were subsequently filtered, washed with 200 mL of cold distilled water, and left to air dry. The overall reaction is shown in Scheme 1.

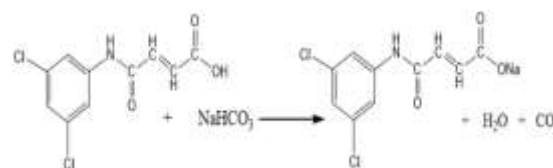


Scheme 1: Synthesis of ligand (HL)

Synthesis of sodium salt of ligand (NaL)

10 mmol aqueous suspension of ligand (HL) was prepared by mixing 2.60 g of ligand in 25 mL of water. To this 10 mmol aqueous solution (25 mL) of sodium bicarbonate (0.84 g) was added dropwise in suspension to make it a clear solution and then stirred for 10 to 15 minutes. A little bit of turbidity was found, which was removed by filtration. The filtrate was put in a water bath for two hours to reduce its volume. After that, the solution was dried in an oven at 100 °C for an hour resulting in sodium

salt of ligand in the form of white powdered form. The reaction is shown in Scheme 2 and the procedure is in Figure 1.



Scheme 2: Synthesis of sodium salt of ligand (NaL)

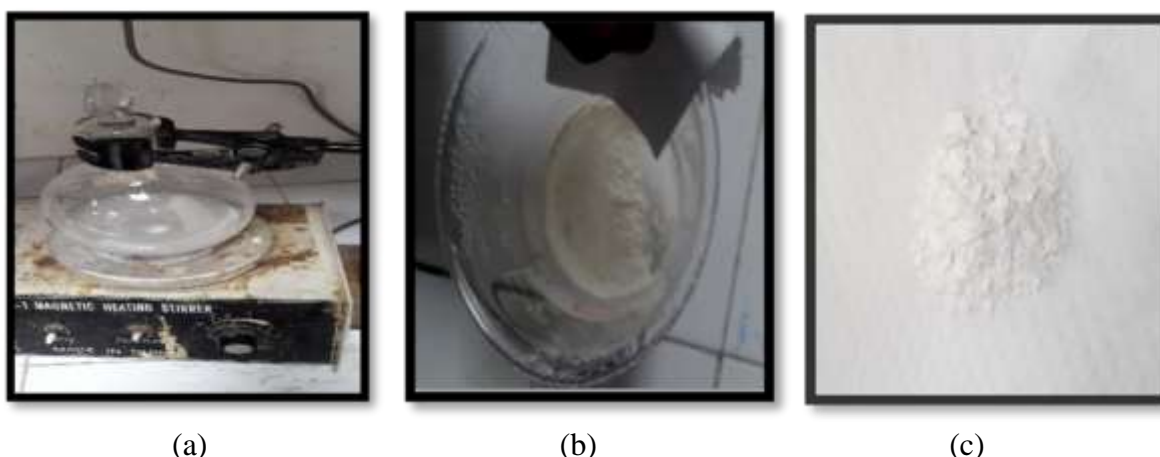
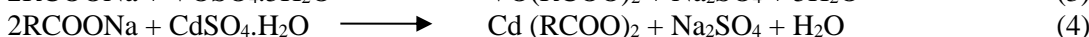
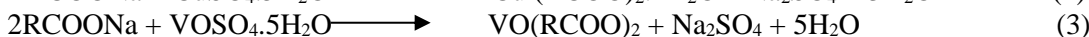
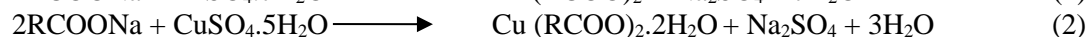
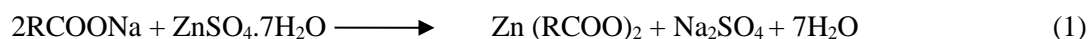


Figure 1: Synthesis of the sodium salt of ligand (a) Mixing of ligand and NaHCO₃, (b) Filtrate, and (c) Sodium salt of ligand

General procedure for the synthesis of metal carboxylates

Metal carboxylates were synthesized by mixing metal and ligand in a 1:2 ratio. For this, 0.5 mmol aqueous solution of metal salts (0.14 g of ZnSO₄·7H₂O, 0.13 g of CuSO₄·5H₂O, 0.13 g of VOSO₄·5H₂O, and 0.38 g of CdSO₄·H₂O) were dissolved in 20 mL of distilled water, separately, then added dropwise in 1 mmol aqueous solution

of sodium salt of ligand (0.28 g of HL in 15 mL of distilled water), while stirring continuously for 20 to 30 minutes and then filtered the above solutions to obtain metal carboxylates in the form of precipitates. These precipitates were washed with distilled water to remove contaminations provided by white, sky-blue, black, and off-white residues of ZnL₂, CuL₂·2H₂O, VO₂L₂ and CdL₂, respectively (Figure 2). The overall reactions are shown in Equations 1-4.



Where, R = C₉H₆Cl₂NO

Procedure for ALP inhibition studies

ALP inhibition was used to accomplish the biological activities of all the prepared compounds. ALP is a metalloenzyme and is

present in animals, plants, and bacteria. It plays an important role in mineralization, cardiovascular calcification, liver diseases like hepatitis and bone diseases like Paget's

disease [11]. The effects of the ligand, its sodium salt, and complexes were checked at room temperature by measuring the

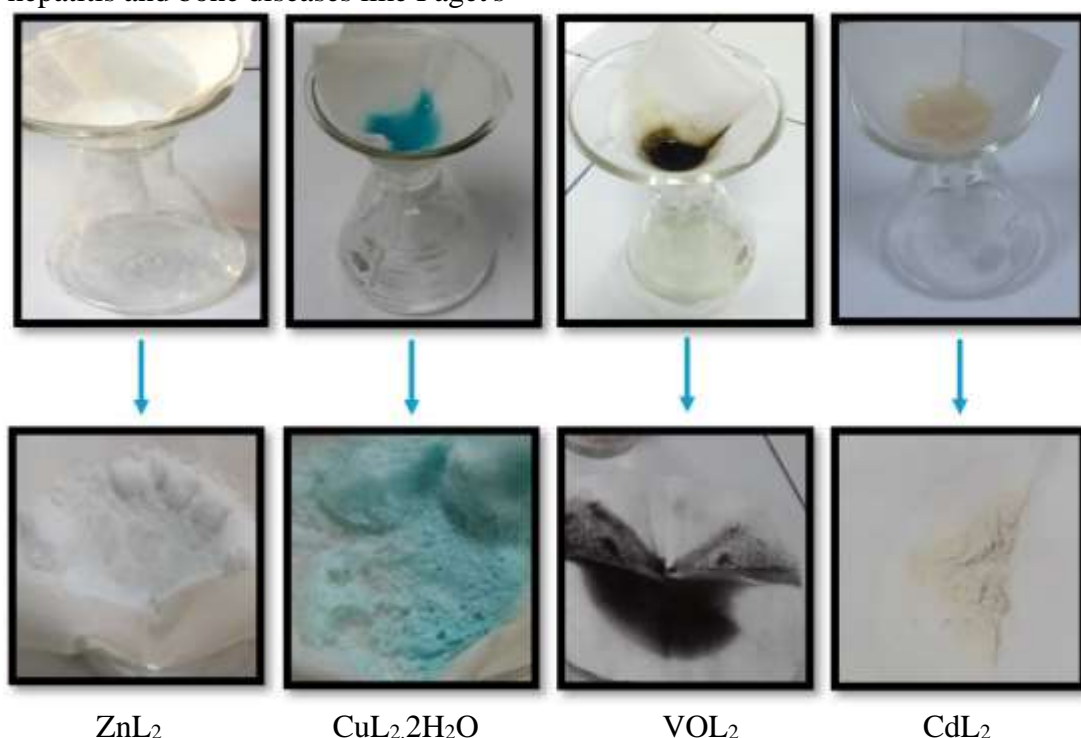
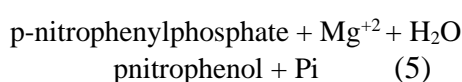


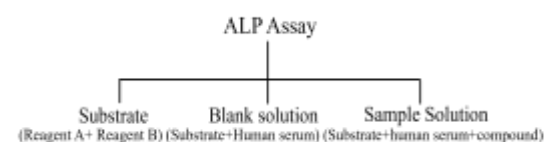
Figure 2: Synthesis of metal complexes

absorbance at 405 nm. In a typical procedure P-nitrophenol, a yellow-colored compound, was obtained by the hydrolysis of p-nitrophenylphosphate (Equation 5) [12].



Outline of ALP Inhibition

The outline of the ALP assay is listed in Scheme 3 [13].



Scheme 3: Schematic diagram of ALP Assay.

Substrate Preparation

The substrate was prepared by using four parts of sodium buffer solution with pH 10 (Reagent A) and one part of p-nitrophenylphosphate 50 mMdm⁻³

(Reagent B). After that, it was incubated at room temperature for five minutes.

Blank solution preparation

A blank solution was prepared by taking human blood serum (100 µL) and reagent solution (2 mL) with the help of a micropipette in a cuvette. Reagents acted as a substrate while human blood serum has ALP enzyme. Blank reading was noted without having compounds that were compared with the absorbance of the sample solution.

Sample solution.

Compound solutions of different concentrations were added to the blank

solution. Reading of each compound solution was taken at 405 nm after one minute of incubation to confirm the alkaline phosphatase activity. Readings of average absorbance were noted to calculate the percentage of inhibition activity by using the formula given below in Equation 6.

$$\text{Percentage of inhibition} = \frac{A_c - A_s}{A_c} \times 100 \quad (6)$$

Equation 6: Percentage of inhibition rate

Whereas is the absorbance of the sample, A_c is the absorbance of the control reaction. The general method of ALP assay is shown below in Figure 3.

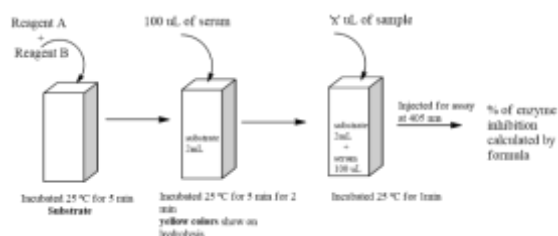


Figure 3: General method of ALP assay of compounds.

Results and discussion

All the compounds are soluble in dimethyl sulfoxide (DMSO) and have sharp melting points.

FT-IR Spectroscopy

FT-IR spectroscopy has been considered a valuable tool for attaining structural information about organic compounds [14]. Infrared spectra of all compounds were recorded using KBr pallets from 4000 to 400 cm^{-1} and the detail is summarized in Table 1 and spectra are shown in Figure 4.

Peaks that appeared in FT-IR spectra confirmed the synthesis of compounds. A peak appeared at 3259 cm^{-1} showing the stretching vibration of $\nu(\text{N-H})$ confirming

the synthesis of ligand. This peak remained in all metal carboxylates indicating that it was not involved in metal ions coordination [15]. The region between 1694-1744 cm^{-1} was assigned for $\nu(\text{COOH})$ vibrations of all compounds, which shifted to the higher wave number value of 19-50 cm^{-1} indicating COOH involvement in the coordination of metal ions [16]. The C=O peak of the carboxylic group shifted in the FT-IR spectra on complexation with metals providing important information about metal ions attachment with the ligand. In the copper complex ($\text{CuL}_2 \cdot 2\text{H}_2\text{O}$), the broadband at 3262 cm^{-1} indicated the presence of water molecules. This broad peak was not shown in other metal complexes (ZnL_2 , VOL_2 , and CdL_2) indicating that water molecules were not involved in metal-ligand attachment [17].

A characteristic vanadyl (V=O) peak appeared at 959 cm^{-1} , confirming the formation of VOL_2 . The peaks that appeared in the range 1043-1118 cm^{-1} have shown the presence of C-Cl in all compounds. Carbonyl (C=O) peaks appeared in the range 1622-1652 cm^{-1} proving that the carbonyl group did not participate in bonding.

UV-Visible Spectroscopy

In metal carboxylates, metals interact with the electronic cloud of ligand as a result their d orbitals become degenerate. Due to degeneracy, there was no visible absorption of light, so all the compounds appeared in the UV region (200-400 nm). By absorbing UV light compounds showed two types of transitions i.e., $\pi-\pi^*$ and $n-\pi^*$, which indicated that all the compounds were aromatic [18].

The Ligand and the sodium salt of the ligand are different in the way that a single peak appeared in the ligand while two peaks appeared in sodium salt which was a clear indication of NaL formation Metal

carboxylates also showed different peaks as compared to ligand and its salt which confirmed the synthesis of metal carboxylates [19]. UV-visible spectra of all

compounds have been shown in Figure 5 λ_{\max} values of all compounds are listed in table 2.

Table 1: FT-IR data of the synthesized compounds.

Sr. No.	Compound s	OH	NH	COO H	C= O	CH=C H	C-H (Aromatic)	C=C(aromatic)		C- Cl	V= O	H ₂ O
								Stretchin g	Bendin g			
1	HL	3200	3259	1694	1622	3076	2997	1548	1399	1118	-	-
2	NaL	-	3271	1713	1646	-	-	1558	1347	1111	-	-
3	ZnL ₂	-	-	-	1652	-	-	1505	-	1043	-	-
4	CuL ₂ .2H ₂ O	-	-	-	1634	-	-	1550	1373	-	-	3262
5	VOL ₂	-	3227	-	1628	-	-	-	-	-	959	-
6	CdL ₂	-	3382	1744	1633	3173	2922	1555	1387	1115	-	-

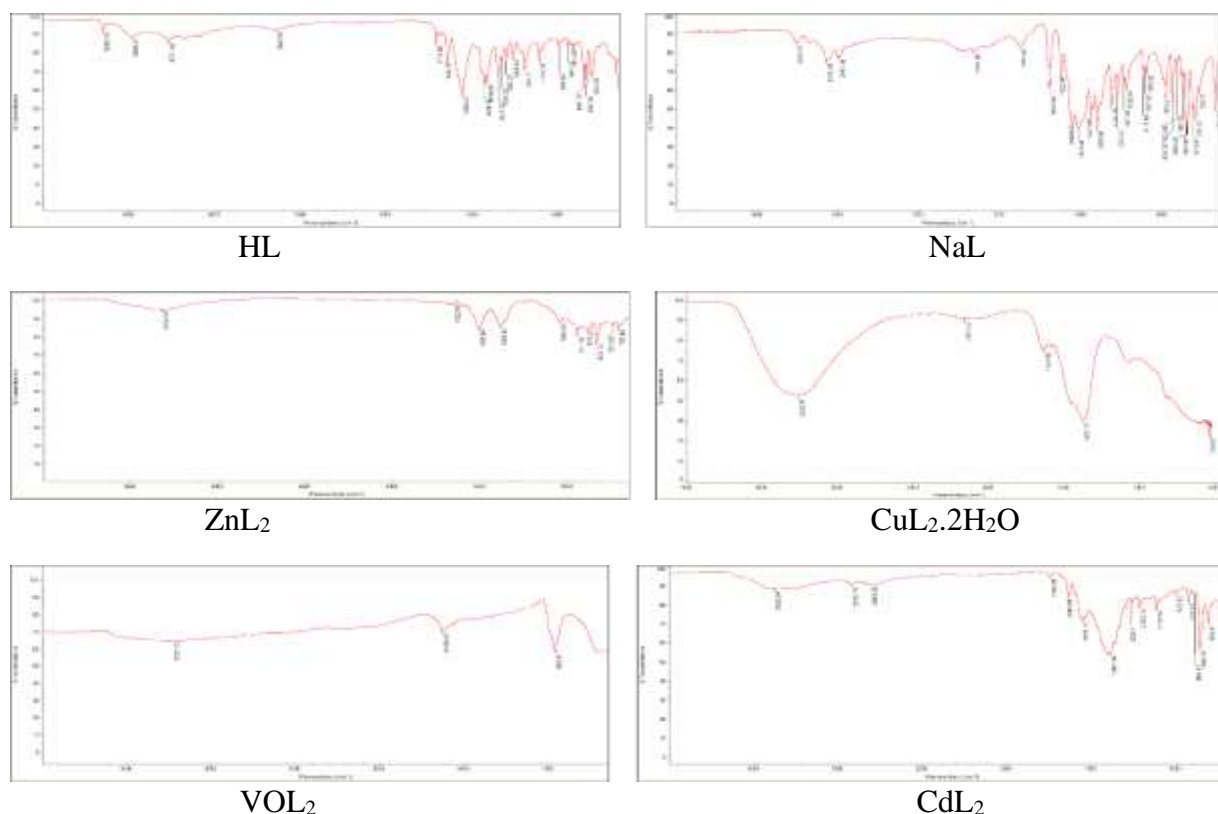


Figure 4: FT-IR spectra of synthesized compounds.

Table 2: UV-visible spectral studies of compounds

Sr. No.	Compound	λ_{\max} (nm)	λ_{\max} (cm ⁻¹)	Evaluated Transitions
1	HL	277.5	36036	$\pi-\pi^*$
2	NaL	266	37594	$\pi-\pi^*$
		220	45455	$n-\pi^*$
3	ZnL ₂	287	34843	$\pi-\pi^*$
4	CuL ₂ .2H ₂ O	289.5	34542	$\pi-\pi^*$
5	VOL ₂	281	35587	$\pi-\pi^*$

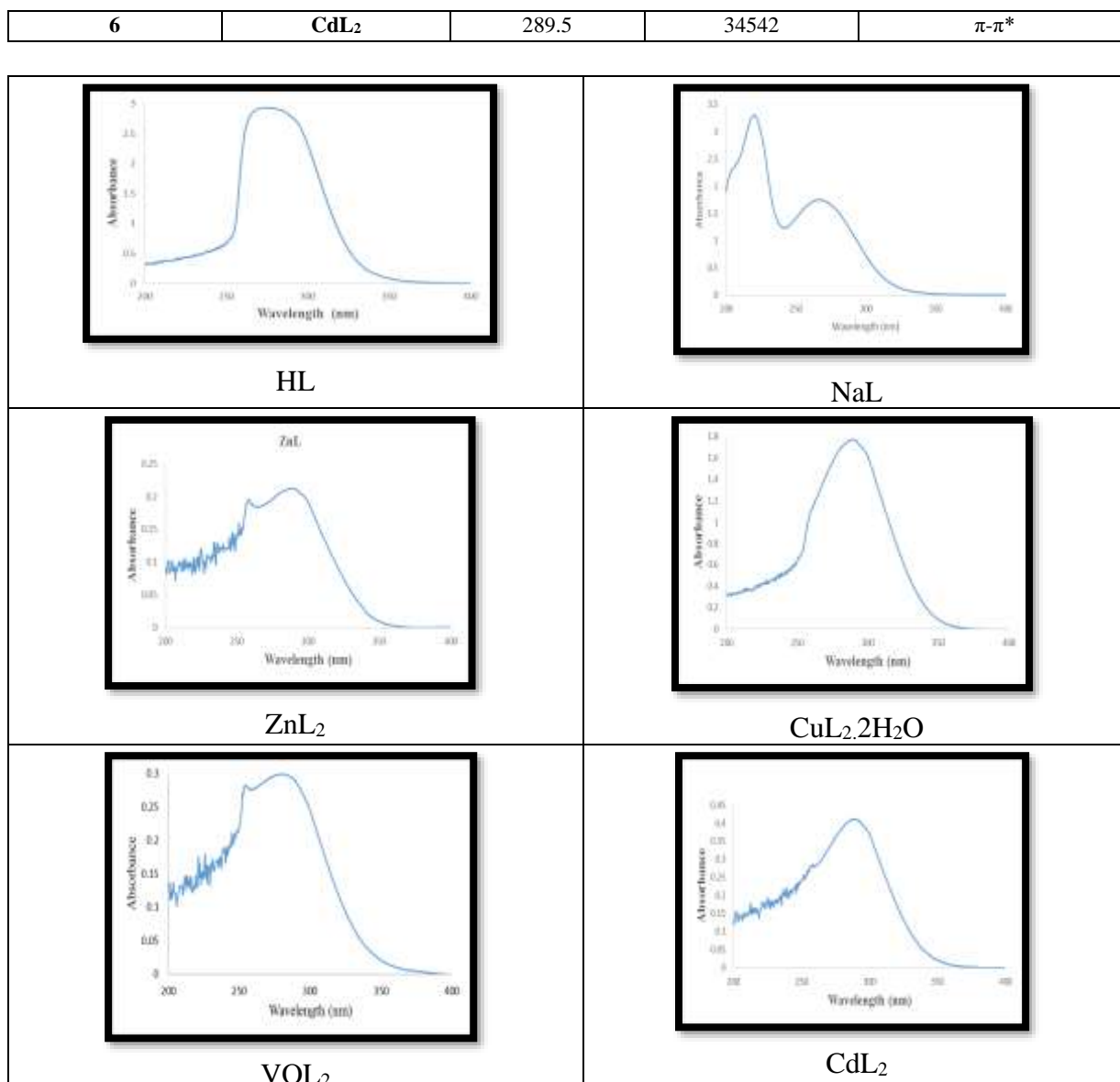


Figure 5: UV-visible spectra of synthesized compounds.

Conductometry

The conductance of the ligand and metal carboxylates has been measured in DMSO, while the conductance of sodium salt of the ligand was measured in distilled water. Dilute solutions were used for conductance measurement because there is an increase in molar conductivity by decreasing the concentration of different solutions. Constant readings were acquired in dilute solutions [20]. The conductance was measured in milli Siemens per mole at 0, 24, and 72 hours and is shown in Table 3. The compounds possess low conductance

values within the range of 0.01-2.72 mS/mol, which indicates that they have a non-electrolytic nature [21]. The conductivity of the ligand was very low, but the conductivity of its sodium salt was 1.44 mS/mol. The conductivity of metal carboxylates was in the range of 0.09-0.26 mS/mol indicating that the conductivity of sodium salt (NaL) was decreased by attaching the metal [22].

The order of conductivity of the compounds is as follows.

NaL > ZnL₂ > CuL₂.2H₂O > VOL₂ > CdL₂ > HL

Table 3: Conductance (mS/mol) of the synthesized compounds

Sr. No.	Compounds	Conductance
1	DMSO	0
2	Distilled water	0.02
3	HL	0.01
4	NaL	1.44
5	ZnL ₂	0.26
6	CuL ₂ .2H ₂ O	0.25
7	VOL ₂	0.21
8	CdL ₂	0.09

Biological activity

The biological activity of all compounds was measured against the alkaline phosphatase (ALP) enzyme. Different concentrations i.e., 0.247 mM, 0.49 mM, 0.728 mM, and 0.961 mM were used to obtain ALP inhibition data of all compounds. It has been shown from the graph that HL, NaL, ZnL₂, CuL₂.2H₂O, and VOL₂ inhibited the activity of enzymes. [23] It is proposed that the increase in concentrations of compounds causes a decrease in the activity of enzymes until they are completely deactivated. A comparison of all compounds in Figure 6 has shown that ZnL₂ and VOL₂ were more active as compared to the ligand and its sodium salt, while CdL₂ and CuL₂.2H₂O were moderately active [24]. The order of ALP assay against the prepared compounds is shown below to check the inhibition rate of enzymes.

ZnL₂ > VOL₂ > CuL₂.2H₂O > CdL₂ > NaL > HL

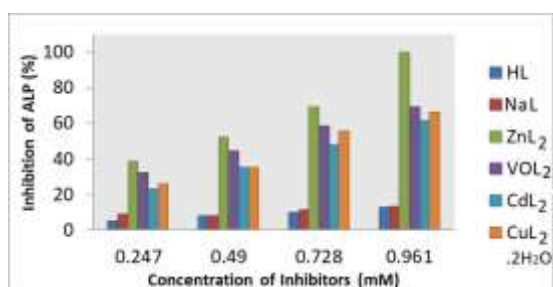


Figure 6: Inhibition of ALP by the synthesized compounds.S

Conclusion

Ligand (HL), its sodium salt (NaL), and metal carboxylates (ZnL₂, CuL₂.2H₂O, VOL₂, CdL₂) have been synthesized and characterized by physical parameters, conductance, FT-IR, UV-Visible spectroscopy. All the compounds were obtained in good yield and have sharp melting points. FT-IR spectra of all the compounds have confirmed that the carbonyl group and amine group were not involved in coordination, only the carboxylic group was involved in the complexation. Conductance measurement showed that all the compounds have very low conductance, so they are non-electrolyte. In UV-Vis spectra, π - π^* and n - π^* transitions were observed which indicated that all the compounds were aromatic. ALP inhibition provided the information that all the synthesized compounds inhibit the ALP activity due to its strong affinity to bind with the active sites of the enzyme.

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