Article

Synthesis, Characterization and Enzyme activity of Cobalt Complex with New 2- Thioxoimidazolidine-4-one Ligand Derived from Schiff base

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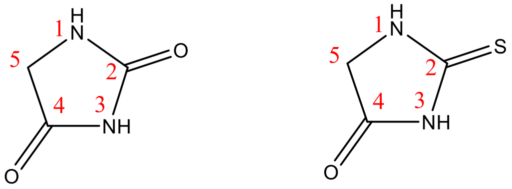
Abstract: In this work, a novel derivative of 2- thioxoimidazolidine-4-one (3-(((6-methoxynaphthalen-2-yl)methylene)amino)-2-thioxoimidazolidin-4-one) (L) has been synthesized by reacting 2-((6-methoxynaphthalen-2-yl)methylene)hydrazine-1-carbothioamide with ethylchloroacetate in the presence of sodium acetate, using 1,4-dioxane as the solvent. The chemical structures of the synthesized compound were determined using elemental analysis and a variety of physical and chemical techniques. Spectroscopic and magnetic moment studies were employed for this purpose. It was demonstrated that the metal complexes exhibited a geometric structure that exhibited an octahedral geometry. The synthesized complex was identified and their geometrical were suggested in solid state by using (FT-IR), (UV-Vis) spectroscopy, elemental analysis (C.H.N.S), flame atomic absorption technique, in addition to magnetic susceptibility and conductivity measurements. the inhibitory or activation effects of synthesized ligand and its metal complex on the activity of the alkaline phosphatase enzyme (ALP) at different concentrations.

Key words: Hydantoins, cobalt, 2-thioxo–imidazolin-4-one

1. Introduction: Hydantoins [imidazolidin-2,4–dione and 2-thioxo–imidazolin-4-one] are referred to heterocyclic compound, which has a wide variety of biological and pharmacological [1], 2-thioxoimidazolidin-4-one are the sulfur isotopes of hydantoins in which involve the substitution of either one or both carbonyl groups by thiocarbonyl groups [2].

Thiohydantoin heterocycles are found in a wide range of biologically active chemicals, including medicinal medications used for the treatment of anti-tumor substances. Thiohydantoins have also been utilized as medications for the treatment of cancer. Nevertheless, the primary focus of attention lies in the utilization of thiohydantoins for the therapeutic management of cancer. The structural characterisation of thiohydantoin is crucial for understanding their modes of action due to its significant biological effects [3]. 2-thiohydantoins are most notably known due of their wide applications as hypolipidemic[4], anticarcinogenic[5], antimutagenic[6], anti-fibrolytic [7] antiviral (e.g., against herpes simplex virus, HSV) [8], human immunodeficiency virus (HIV) [9]and antimicrobial (antifungal and antibacterial)[10], anti-inflammatory agents, [11] as well as antioxidant [12].



imidazolidine-2,4-dione **2-thioxoimidazolidin-4-one**

Figure 1. The molecular structures of imidazolidine-2,4-dione and 2-thioxoimidazolidin-4-one.

The compound possesses the ability to exist in a state of tautomeric thione-thiol isomerism, wherein equilibrium is maintained between the –NH-C=S and –N=C-SH functional groups. Ambidentate ligands exhibit the ability to coordinate with transition metal ions, hence playing a significant role in both industrial and medicinal applications [1]. 2-thioxoimidazolidin-4-one is a five-membered heterocyclic ring that possesses two nitrogen atoms. It has two functional groups, a thion group (C=S) at position 2 and a carbonyl group (C=O) at position 4. The coordination properties of this compound have showed potential in the development of biologically active substances [13]. In order to investigate the coordination behavior of the new ligand, 3-(((6-methoxynaphthalen-2-yl)methylene)amino)-2-thioxoimidazolidin-4-one [L], towards various divalent metal ions, we synthesized new metal complexes. The purpose of this work is to investigate the transition metal complex of a thioxoimidazolidine ligand.

2. Experimental

Material and Methodlogies

all the reagents and starting materials were purchased from commercial chemical, and used 6-methoxy-2-naphthaldehyde, thiosemicarbazide, ethanol, 1,4-dioxane, ethylchloroacetate were either energy chemicals or BDH and were used without further purification, The melting points of the synthesized compounds was accomplished by using a (digital Stuart scientific SMP30) and were uncorrected, The infrared spectrum (FTIR) was measured using a (SHIMADZU FT-IR 8400S spectrophotometer) in the range (4000-600cm^{-1}) at Mustansiriyah University / College of Science / Department of Chemistry / Iraq, The ^{1}H- NMR is recorded utilizing a (Bruker DMX-500 NMR spectrophotometer) at a frequency of (400, 600µHZ) using (DMSO-d^{6}) as solvents with TMS as an internal reference, At the (BrukerBioSpin GmbH) at (400µHZ) in Gazi Osman PaŞa (GOP) University / Turkey, GC-Mass were recorded using (Shimadzu model GCMS-QP 2010 PLUS) at university of Samarra /College of Education for

Pure science / Samarra / Iraq, The magnetic susceptibility values of the prepared complex were obtained at room temperature using Magnetic Susceptibility Balance - Sherwood Scientific Ltd (UK) at Chemistry department / College of Science / Al-Mustansiriyah University, Conductivity measurements were carried out using WTW conductivity meter, with using DMF as a solvent ($1*10^{-3}$ M), Chemistry department/ College of Science / Al-Mustansiriyah University.

Synthesis of 2-((6-methoxynaphthalen-2-yl)methylene)hydrazine-1-carbothioamide:

To solution of naphthaldehyde (0.01 mol, 0.18 g) in (30 mL) of ethanol, thiosemicarbazide (0.01 mol, 0.09 g) was added, The reaction mixture was refluxed for 4 hrs, then cooled and added to crushed ice, the precipitate formed was filtered, washed with distilled water and recrystallized from (ethanol)[16], M.Wt=186.21, M.P=212-214°C, Yield=75%, yellow precipitate.

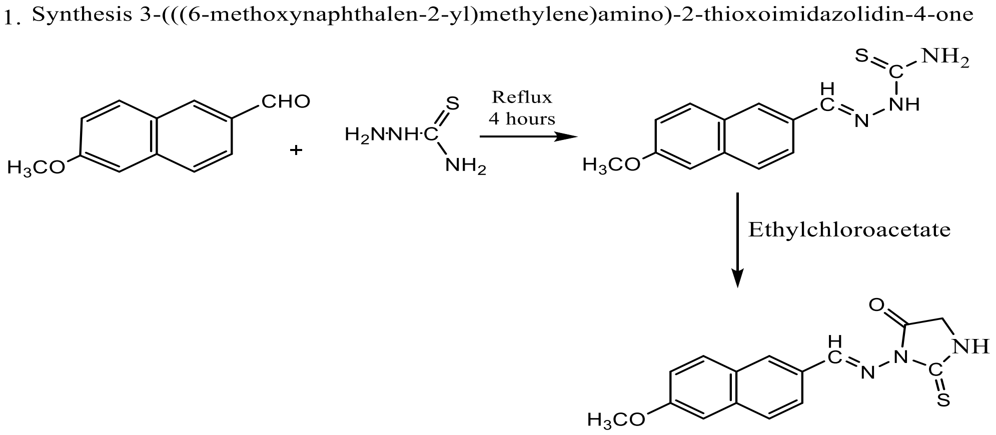
Synthesis of (E)-3-(((6-methoxynaphthalen-2-yl)methylene)amino)-2-thioxoimidazolidin-4-one:

0.26 grams (equivalent to 0.01 moles) of 2-((6 methoxynaphthalen-2-yl)methylene)hydrazine-1-carbothioamide was dissolved in 30 mL of 1,4-dioxane with continuous stirring for a duration of 10 minutes. Then, 0.82 grams (equivalent to 0.01 moles) of sodium acetate was added to the solution. Ethylchloroacetate was then added drop by drop with constant stirring. The resulting mixture was refluxed for 8 hours, followed by cooling and addition to crushed ice. The formed precipitate was filtered, washed with distilled water, and recrystallized from a mixture of ethanol and 1,4-dioxane in a ratio of 3:7.

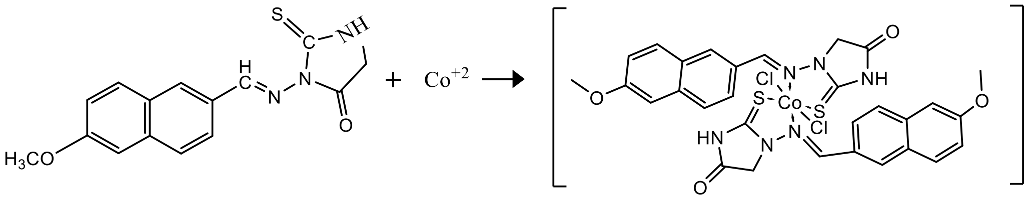
[1] M.Wt=299, M.P= 228-230 °C, Yield=75%, dark yellow precipitate..**خطأ! لم يتم العثور على مصدر المرجع**

Synthesis of metal complex

In the synthesis process, an ethanolic solution containing 0.05 mmol of the following metal ion salts, the cobalt(II) chloride hexahydrate salts (CoCl2·6H2O) was combined with an ethanolic solution containing 1 mmol of (E)-3-(((6-methoxynaphthalen-2-yl)methylene)amino)-2-thioxoimidazolidin-4-one (L), equivalent to 0.264 g. This reaction mixture was then subjected to reflux conditions for a duration of 2 to 3 hours, during which a precipitate was formed. Subsequently, the resulting product was filtered, washed with hot ethanol, followed by a wash with cold water, and finally, it was dried under vacuum conditions. The identification of the complex was carried out through elemental analysis, flame atomic absorption spectroscopy, FT-IR and UV-Vis spectrophotometry, magnetic properties susceptibility, and conductivity measurements [15].



Scheme 1: Synthesis of ligand.



Scheme 2: Synthesis of cobalt complex.

RESULTS AND DISCUSSION

The synthesis of Schiff's bases with ethylchloroacetate resulted in the formation of the target product, as depicted in Scheme 1. The compounds' structure was determined through the use of FTIR and ^{1}HNMR spectroscopy. The ^{1}HNMR spectrum displayed signals at certain chemical shifts: δ 3.93 (singlet, 3H, OCH_3); 7.91-7.39 (multiplet, 6H, ArH); 11.98 (singlet, 1H, NH); 7.92 (singlet, 1H, HC=N); and 2.5 (singlet, 2H, CH2). The 1H nuclear magnetic resonance (NMR) spectrum of the ligand is depicted in (Figure 2). Additionally, the mass fragmentation spectrum and pattern of the ligand can be observed at m/ z^+ 299.(Figure 3) The molar conductance values of all complexes have been determined in DMF as a solvent at concentration of (10^{-3} M) and at room temperature (25 ^{0}C), for establishing electrolytic or non-electrolytic nature of the complexes [16]. The conductance of the complexes in DMF solution was in the range of (13-18 $S.cm^2.mole^{-1}$), illustrating that they are non-electrolytic nature, in contrast to the other complexes. The molar conductivity of synthesized complexes are exhibited in tables [3-3]. The transition of metal electrons in these elements' partially filled d-orbitals is frequently studied using magnetic measurements. These measurements give a concept about the structure, in addition, it giving details about the type of bonding and strength of ligand field of complexes by providing information about the number of unpaired electrons.

The effective magnetic spin of the complexes was determined in the solid state by Faraday's method [17] by using spin only magnetic moment corresponded to the equation, The magnetic susceptibility data at (298 K) for complexes is obtained by the following equation [18], [19]:

$X_m = X_g * Mwt$ …………………………..….(1)

$X_A = X_m - D$ ………………………….…......(2)

$\mu_{eff} = 2.83 \sqrt{X_A . T}$ B.M ………………… (3)

Where: T = Temperature (K)

X_A = Atomic susceptibility

X_g = Gram susceptibility

D = Diamagnetic correction factor, by using Pascal's constant

for ligand atoms and metal ions, we can estimate the value of this factor. The results obtained from Faraday's method were compared with those calculated from spin only ($\mu_{s.o}$), magnetic moment values obtained from the following equation [20] [21]:

$\mu_{S.O} = 2\sqrt{S(S+1)}$ B.M ……… (4)

Where S = n/2 (n = no. of unpaired electrons)

The FTIR spectrum displayed several characteristic bands. One such band appeared at 3049 cm^{-1}, which can be attributed to the C-H stretching vibrations of the aromatic ring. Another prominent band was observed at 1705 cm^{-1}, indicating a strong stretching vibration of the C=O bond in the imidazole ring. Additionally, the spectrum exhibited a band at 1623 cm^{-1}, corresponding to the stretching vibrations of the azomethine group (HC=N). Furthermore, two bands were observed at 1563 and 1504 cm^{-1}, indicating the presence of C=C bonds in the aromatic ring. Lastly, a stretching vibration band of the C=S bond was observed at 1036 cm^{-1}. Frequencies ranging from 850 to 1550 cm^{-1} have been attributed to the C=S stretching frequency in the literature [22]. (Figure 4)

The Co(II) complex exhibited a decrease in the absorption frequency of the (C=S) moiety, which was observed at a wavenumber of 1027 cm^{-1}. The observed transition suggests that the thiol group participated in coordination with the metal ion. The azomethine moiety exhibited a redshift, manifesting at a wavenumber of 1581 cm^{-1} in the coordination compound. The observed shift indicated that the nitrogen atom of the azomethine group exhibited coordination behavior. The absorption band due to the C=O stretching band was observed at 1708 cm^{-1}, which was in the same region as in the ligand, confirming that it was not involved in coordination.

Furthermore, the spectra of the metal complexes exhibited the presence of new vibration bands with reduced frequencies of 422 cm^{-1} and 552 cm^{-1}. The appearance of these new bands suggests the existence of metal-ligand interactions inside the complexes. The previously mentioned evidence demonstrates in (Figure 6).

In the DMF solvent, the UV-Vis. spectrum of the Ligand, as shown in Figure 5, revealed the presence of three absorption bands at wavelengths (267, 37453) and (287, 34843), which were attributed to the intraligand electronic transitions of π-π* nature. Additionally, an absorption band at wavelength (331, 30211) was observed and assigned to the n-π* transition. The Co(II)L complex, as illustrated in (Figure 7), demonstrated novel absorption bands within the visible region. These bands were observed at wavelengths of 850 nm (11764 cm^{-1}), 623 nm (16051 cm^{-1}), and 593 nm (16863 cm^{-1}). These transitions were assigned to ^4T_1g (F) → ^4T_2g(F) (ν1), ^4T_1g (F) → ^4A_2g (F) (ν2), and $^4T_1g_{(F)}$→ $^4T_1g_{(P)}$ (ν3), respectively. These transitions suggest that the complex has an octahedral geometry [23]. The parameters B^- and β, and Dq were determined to have values of 536, 0.68, and 1470 cm^{-1}, respectively. These values further support the conclusion of an octahedral geometry for the Co(II) complex. The β parameter value indicates a covalent character for the metal-ligand bonds. Molar conductivity and Magnetic susceptibility measurements demonstrated that the complex is paramagnetic with a magnetic moment of 4.2 B.M The magnetic measurement for Co(II) complexes exhibited magnetic moment values, which agree with octahedral [14] and exhibits nonelectrolytic behavior in DMF solvent, The result was further confirmed by the elemental analysis (C.H.N.S.) and FT-IR spectrum data. The complex's suggested structure can be proposed based on all of the evidence : see Figure 7

By refluxing the corresponding metal salt with the ligand, L, in a 1:2 molar ratio, the ligand and complex were created. A different melting point was observed in the complex, indicating the isolation of a relatively pure chemical. The complexes' measured molecular weights agreed with the complex's predicted formula. Micro-elemental analysis, the melting point, and m/z data (see Table 1).

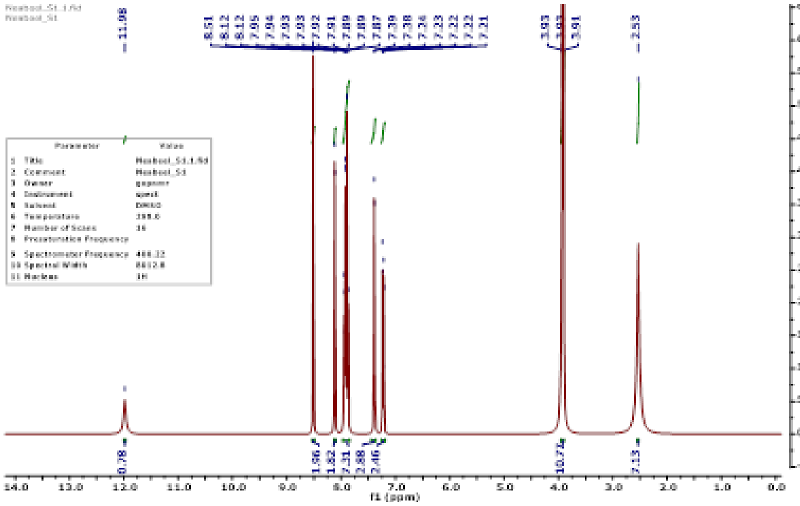


Figure 2: HNMR of ligand.

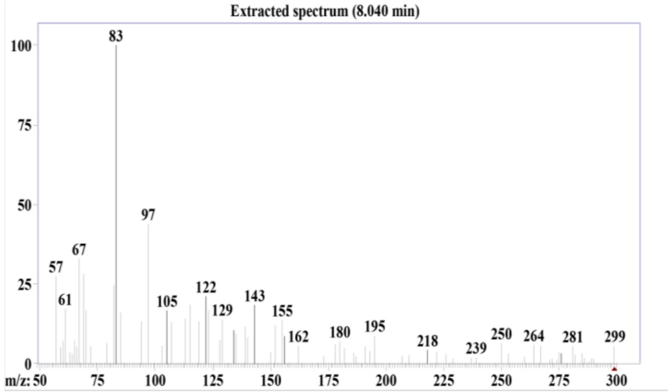


Figure 3: Mass spectra of ligand

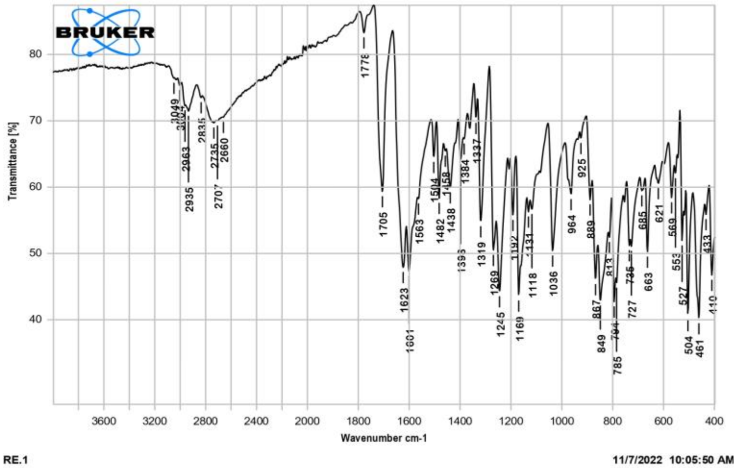


Figure 4: FTIR of ligand

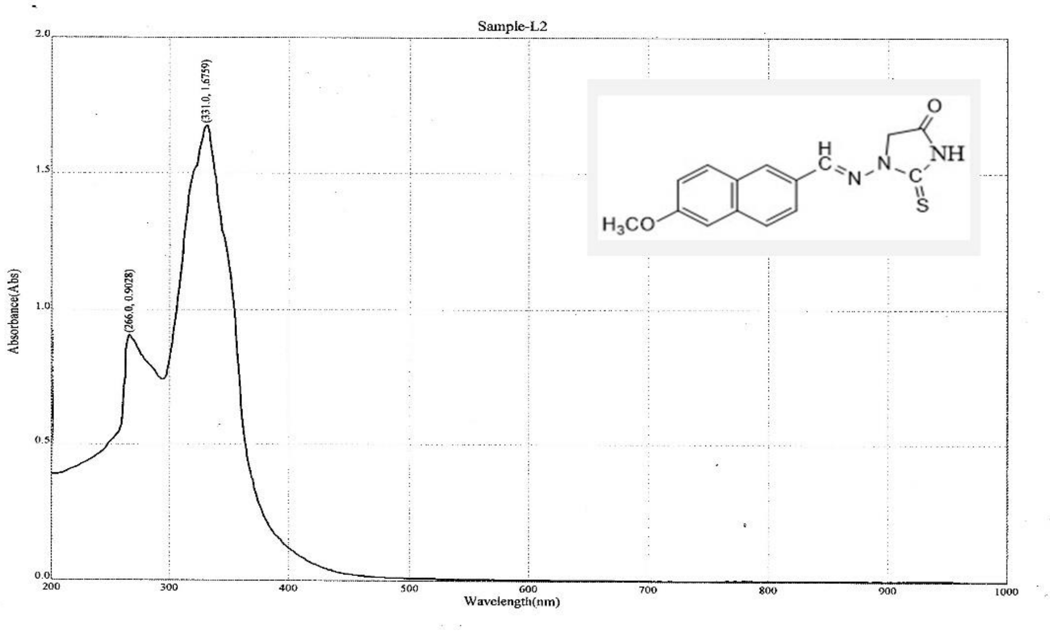


Figure 4: UV-Vis. spectra of ligand

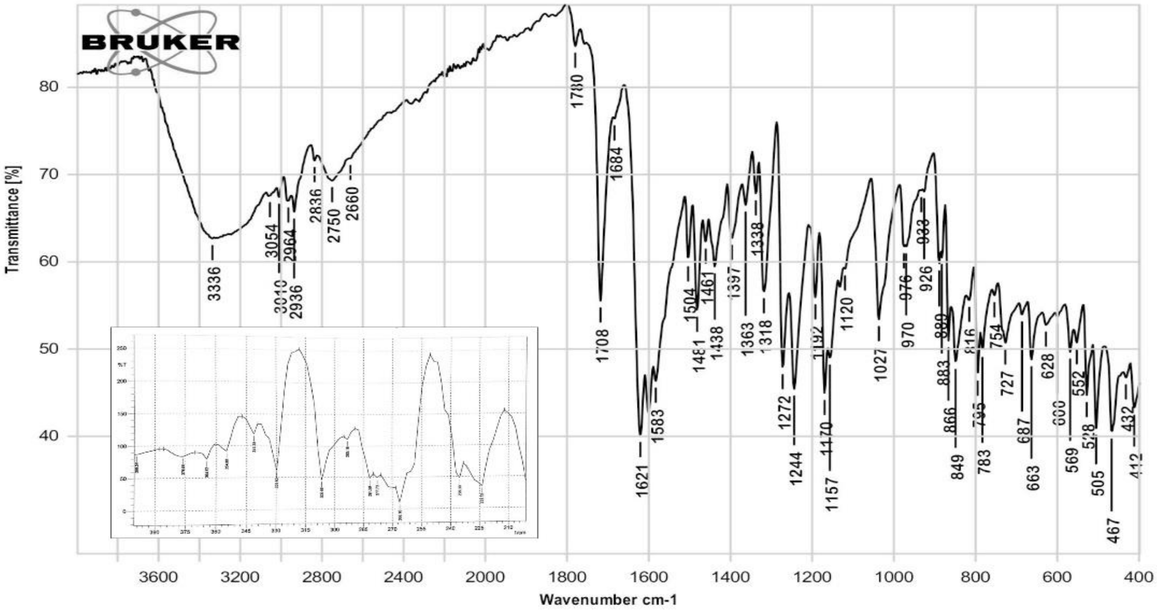


Figure 5: FTIR spectra of Co(II) complex

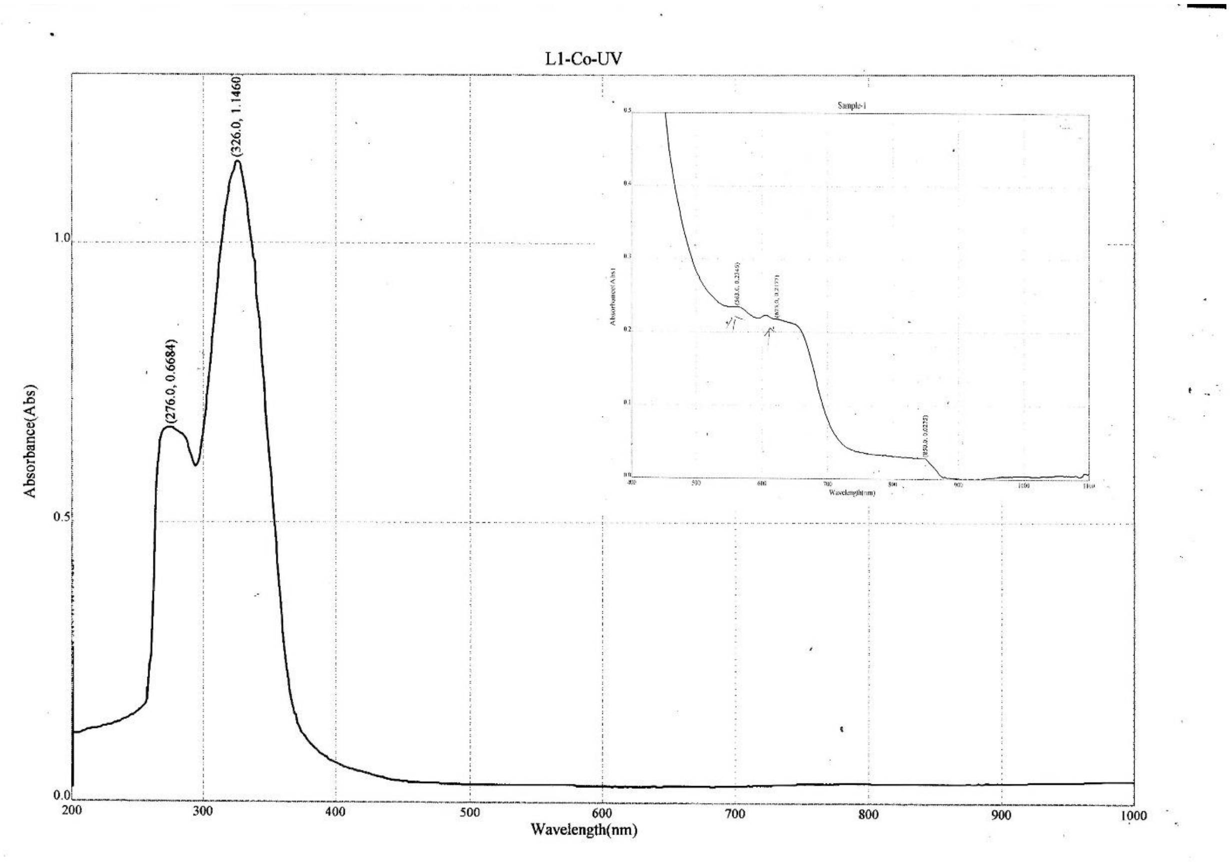


Figure 6: UV-Vis. spectra of Co(II)L complex

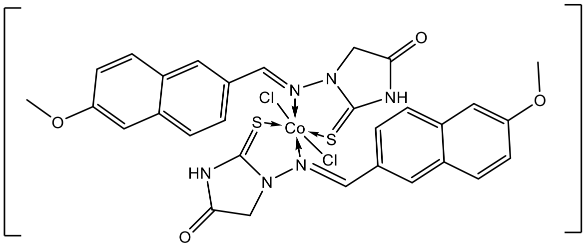


Figure 7: Proposed structure of Co(II) complex

Table 1: Many physical characteristics of the metal complex and ligand.

Comp.	color	M.p. ^{0}C	M.wt g.mol^{-1}	Yield(%)
$C_{15}H_{13}N_3O_2S$	yellow	230	299	75%
$C_{30}H_{26}Cl_2CoN_6O_4S_2$	brown	235	728.5	72%

Table 2: several elemental analysis of Ligand and its metal complex.

Comp.	Elemental analysis theoretical (experimental)%						Found (calc)
	C	H	N	O	S	Co	
$C_{15}H_{13}N_3O_2S$	60.19 (60.24)	4.38 (4.41)	14.04 (14.08)	10.69 (10.72)	10.71 (10.75)	------	299 (299)
$C_{30}H_{26}Cl_2CoN_6O_4S_2$	49.46 (49.49)	3.60 (3.65)	11.54 (11.57)	8.78 (8.80)	8.80 (8.79)	8.09 (8.11)	728 (728)

The influence of compounds on the activities of ALP enzymes in human serum

The activity of alkaline phosphatase (ALP) was assessed in comparison to a reagent blank using a Mindary Rs 203 instrument. To quantify the ALP enzyme activities, 160 µL of serum was mixed with 40 µL of enzyme-containing compounds, followed by the addition of 100 µL of dimethyl sulfoxide (DMSO). The outcomes of these compounds are presented in a summarized. (As Table 2)

Table 3: Activity of ALP Enzyme in presence and absence of Ligand (L1) and its metal complex)

Sample	Conc.	without	with	%inhibition	%activation
L	1.00E-01	87.2	66	24.31	
	1.00E-02		70	19.72	
	1.00E-03		90.1		3.3
	1.00E-04		48	44.95	
	1.00E-05		55.4	36.47	
CoL	1.00E-01	87.2	88		1.9
	1.00E-02		89		2.1
	1.00E-03		89.7		2.9
	1.00E-04		88.8		1.8

	1.00E-05		85.2	5.2	

In general, the study emphasizes that both ligands and their metal complex exhibit the capacity to impede the action of the ALP enzyme. The observed inhibitory effects exhibit a concentration-dependent relationship, wherein larger concentrations of the metal complex correspond to more pronounced inhibition. The inhibitory effects of these metal complexes are attributed to the coordination of the complexes with the active sites of the enzyme, as well as the possible displacement of crucial ions. The decrease in the production of p-nitrophenolate ions resulted in a corresponding reduction in absorbance at 510 nm, as examined by the results of experiments [25].

CONCLUSIONS

The synthesis and characterization of the recently developed 2-Thioxoimidazoline (L) ligand and the corresponding metal complex were carried out. cobalt(II) complex with molecular formula $[M(L)_2Cl_2]$ (where M = Co(II), L = thioxoimidazolidine derivative ligand), have been prepared and well-characterized using microanalytical, conductivity measurements, magnetic, spectroscopic, and physical analyses. Upon the outcome results of analyses, the stoichiometry of the synthesized complexes is 1:2 (M:L). The physical and chemical properties of the Cobalt complex were determined by using a variety of spectroscopic techniques. Both the free ligand (L) and its metal complex exhibit significant enzymatic activity.

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