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# Synthesis and Bioactivity of new complexes containing multi-substituted aryl imidazole as ligands

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## Abstract

In this study. new series of complexes containing a multi-substituted arylimidazole ligand, 4-methoxy-2-(1H-phenanthro[9,10-d]imidazol-2-yl)phenol, were prepared. The imidazole ligand (L) was prepared through a condensation reaction between a diketone (9,10-phenathroquinone), an aromatic aldehyde ( 2-hydroxy-5-methoxybenzaldehyde ), and ammonium acetate, in the presence of glacial acetic acid as a catalyst and solvent . Metal complexes were then prepared by reacting this ligand with transition metal salts, including Fe(II), Co(II), Cu(II), and Ni(II), in appropriate solvent such as ethanol, under controlled temperature and stirring conditions to check the formation of stable complexes. The structures of the Imidazole ligands were determined by elemental analyses, IR, $^{1}$H-NMR and molar conductance measurements. It is observed that the synthesized complexes have tetragonal and octahedral geometrical structures. The imidazole ligand (L) and its derivations play important application for supramolecular assemblies due to they can also provide bidentate N-donor sites for chelating with metal ions to form bridge ligands. Antibacterial activity of the ligand and its metal complexes were tested against selected bacteria by disc diffusion method.

**Keywords:** 9,10phenathroquinone, imidazole ligand, complex, antibacterial activity**.**

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*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 Vol. 3, No.3, 2025
www.zjhms.alzahraa.edu.iq

## Introduction

Imidazoles is a five-membered heterocyclic compound consisting of 3 carbon atoms, 4 hydrogen atoms, 2 nitrogen atoms, and 2 double bonds [1]. It is also renowned as 1,3-diazole[2]. The name of imidazole was stated by Arthur Rudolf Hantzsch (1857–1935) in 1887[3]. which is the constituent of several natural products such as histamine, nucleic acids, some alkaloids, etc [4]. Current scientific studies provide valuable information about the structure, functions, physicochemical properties, and biological role of imidazole[5] . imidazole is a small chemical compound that exhibits unique structural complexity[6]. It is serves as a practical and versatile nucleus and a rich source of chemical reactions [7]. Imidazoles are vital compounds that are involved in many biological processes important for the survival and continuity of living organisms, especially through their participation in enzyme catalysis[8] . Furthermore, imidazole-based compounds exhibit a wide spectrum of pharmacological activities, such as anti-inflammatory [4], antimalarial [9], antidiabetic[10], antifungal[11], Anti-bacterial activity[12], antioxidant[13], antitumor activities[2], antiprotozoal[14], antihypertensive medications[3] , antimalarial[15] , antidepressant, [16]and diazole with anticancer properties[17] . Given the broad applications of imidazole derivatives, and our ongoing quest for a deeper understanding of the chemical [18] and physical properties of biologically important metal complexes [19] in this paper we report the synthesis and characterization of a multisubstituted aryl imidazole ligand and its corresponding complexes with Ni (II), Co (II), Cu (II) and Fe (II) ions [20]. Metal complexes have attracted important attention in the field of coordination chemistry because of their wide range of applications including ion exchange[21], hydrogen storage, catalysis[22] ,various biological and medicinal roles[23] .The findings of this study indicate that most likely metal complexes preferred the an octahedral geometry [24]and exhibit promising antimicrobial activities against abroad range of micro-organisms [25]. This current project aims to synthesize new imidazole -based ligand, with its corresponding metal complexes, and to screened their biological activities with other heterocyclic as ligand - transition metal complexes.

## EXPERIMENTAL

AL ZAHRAA UNIVERSITY JOURNAL
Health and Medical Science

*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 Vol. 3, No.3, 2025
www.zjhms.alzahraa.edu.iq

## Materials and Measurements

The solvents and chemicals which used in this research were obtained from various manufacturers, such as Aldrich, GCC and Hi-Media, and were used as is without further purification. All the reactions were monitored using (TLC), where spots were detected on plates after coating them with potassium permanganate ($KMnO_4$) dye and heating. FT-IR spectra were recorded using KBr discs on a SHIMADZU FTIR-8400S. Proton nuclear magnetic resonance ($^1$H NMR) spectra were measured in dehydrogenated dimethyl sulfoxide (DMSO-$d_6$) using a Bruker Bio Spin at 500 MHz. Melting points were recorded utilizing a Stuart (SMP30) capillary thermoelectric melting.

## Synthesis of imidazole (L) derivative

The diketone (9,10-phenanthroquinone) (1mmol) was mixed with the aromatic aldehyde (2-hydroxy-5-methoxybenzaldehyde) (1mmol) and ammonium acetate (4mmol) in a 100-mL round-bottom flask, using $CH_3COOH$ (20 mL) as a solvent. The mixture was heated under reflux at (110–120) °C for 5–6 hrs. until the reaction was complete. The reaction was monitored by thin-layer chromatography (TLC). A sufficient amount of cold water was then added to the reaction, followed by dropwise addition of $NH_4OH$ solution with stirring to obtain the solid precipitate. The resulting material was collected by filtration, washed thoroughly with deionized water to remove residual base and salts, dried, and recrystallized in hot ethanol[26].

## Synthesis of metal complexes

Each complex was synthesized by dissolving each salt ($FeCl_2.4H_2O$, $CoCl_2{\cdot}6H_2O$, $CuCl_2.2H_2O$, and $NiCl_2.6H_2O$) (1mmol) in ethanol, then (1mmol) of tri-substituted imidazole was mixed with 20 mL of ethanol at a (metal: reagent) (1:2). This solution was gradually added to each salt solution, stirring continuously. The mixture was then heated at 75°C with continuous shaking for 2 hours until the reaction was complete. The resulting precipitate was collected by filtration, washed with ethanol, allowed to dry, and subsequently purified using the recrystallization technique [27].

## Biological Activity Assay

### *In vitro* Antibacterial assay

Grow each bacterial strain overnight in nutrient broth at 37°C. Adjust the culture turbidity to a McFarland standard of 0.5 (~1.5 x $10^8$ CFU/mL) using sterile saline. Using a sterile swab, spread



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AL ZAHRAA UNIVERSITY JOURNAL
Health and Medical Science

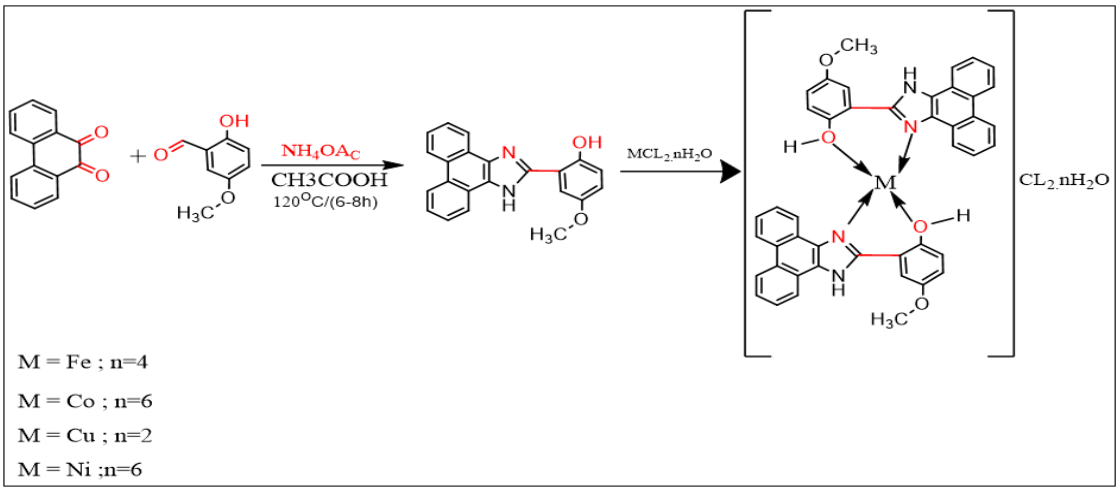
*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 **Vol. 3, No.3, 2025**
www.zjhms.alzahraa.edu.iq

the adjusted bacterial suspension evenly onto Mueller-Hinton agar (MHA) plates to obtain a homogeneous lawn. Allow the plates to dry for 5–10 minutes. Using a sterile cork punch, create three 6-mm-diameter wells in each plate. Place 100 µL of test samples at concentrations of 300, 200, and 100 ppm in each well. Place a Carbenicillin disk on the same plate to serve as a positive control. Incubate the plates at 37°C for 18–24 hours. After incubation, measure the zones of inhibition (in mm) around each well using a ruler, and record the diameter from edge to edge including the well[28, 29].

## Results and Discussion

### 1. Preparation of ligand and its metal complexes

"An imidazole-based ligand was efficiently synthesized via a condensation reaction, affording a (yellow to pale yellow) crystalline solid in high yield (85-90%). Subsequent complexation with transition metal salts of Co(II), Fe(II), Ni(II), and Cu(II) resulted in the formation of distinctively colored precipitates. The observed color changes serve as a clear indication of successful metal–ligand coordination and the formation of stable complexes, as illustrated in Scheme 1.




Scheme 1 . Reaction scheme of Imidazole ligand and its complexes (A1-A4).

### 2. Elemental analyses

Table 1 summarizes the elemental analysis, and physical properties of synthesized ligand and complexes. The obtained elemental analysis values show excellent agreement with the calculated values, confirming the accuracy of the proposed molecular and geometric formulas. The validity of the structures was supported by spectroscopic measurements and magnetic moment data.



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Health and Medical Science

*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 **Vol. 3, No.3, 2025**
www.zjhms.alzahraa.edu.iq

Furthermore, the newly synthesized complexes exhibited excellent solubility in common organic solvents like ETOH, DMF, and DMSO, with little or no solubility in $H_2O$. All complexes exhibited very high melting points, which can be attributed to their somewhat ionic nature, resulting in high thermal stability. The data of elemental analysis (C. H. N) for both the ligand and complexes were in good agreement with the calculated values, as shown in Table (1). Furthermore, the microanalytically analysis results indicated that the molar ratio between the metal ion and the ligand in all complexes was 1:2, supporting the proposed structure of these new complexes.

**Table 1. The elemental analysis and physical characteristics of the synthesized complexes.**

| Comp | Standard Formula | M.Wt (g/mol) | Color | M:L | Elemental content analysis (calc.) found C% | H% | N% | Λ ($Ohm^{-1}.cm^2mol^{-1}$) | Yield% | M.P $^0C$ |
|---|---|---|---|---|---|---|---|---|---|---|
| L | $C_{22}H_{16}N_2O_2$ | 340.38 | Light-Yellow | | (77.63) 77.61 | (4.74) 4.71 | (8.23) 8.21 | | 87 | 135-137 |
| A1 | $[Fe(L)_2]Cl_2$ | 807.51 | Brown | 1:2 | (71.75) 71.73 | (4.38) 4.36 | (7.61) 7.62 | 104.01 | 65 | 153-154 |
| A2 | $[Co(L)_2.Cl_2]$ | 810.59 | Light-Blue | 1:2 | (69.32) 69.35 | (4.14) 4.12 | (7.19) 7.15 | 21.43 | 85 | 148-149 |
| A3 | $[Cu(L)_2.Cl_2]$ | 815.21 | Light-Green | 1:2 | (71.00) 71.02 | (4.33) 4.31 | (7.53) 7.52 | 18.54 | 80 | 192-194 |
| A4 | $[Ni(L)_2.Cl_2]$ | 810.36 | Light-Brown | 1:2 | (65.22) 65.20 | (3.98) 3.95 | ( 6.91) 6.90 | 25.19 | 69 | 163-165 |





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Health and Medical Science

*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 Vol. 3, No.3, 2025
www.zjhms.alzahraa.edu.iq

### 3.Infrared spectroscopy of synthesized ligand (L) and its complexes

The spectrum of synthesized ligand appeared and (3234) $cm^{-1}$ and ( 3414) $cm^{-1}$ related to υ(O-H) and υ (N-H) groups, this band shifted almost by (10-20) $cm^{-1}$ to low frequency in all IR-spectra of metal ion complexes, this indicating the participates of the (O) atom of ligand in the coordination process, respectively and absorption bands around 1616 $cm^{-1}$which related to C=N stretching inside imidazole ring. After that the metal complexes the C=N band shifted to lower wavenumbers, that is indicating for coordination of the imidazole nitrogen to the metal center. Also appear new bands in the range of 500–600 $cm^{-1}$, attributed to (M–O) and (M–N) vibrations, confirming metal-ligand coordination[30].

**Table 2. Electronic spectral data of the free ligand and its metal complexes**

| Compounds | N-H | O-H | C=N | C-N | M-O | M-N |
|---|---|---|---|---|---|---|
| L | 3414 | 3234 | 1614 | 1279 | - | - |
| A1 | 3360 | 3059 | 1649 | 1276 | 516 | 435 |
| A2 | 3446 | 3289 | 1600 | 1230 | 503 | 412 |
| A3 | 3317 | 3210 | 1649 | 1282 | 576 | 430 |
| A4 | 3429 | 3182 | 1608 | 1230 | 478 | 432 |

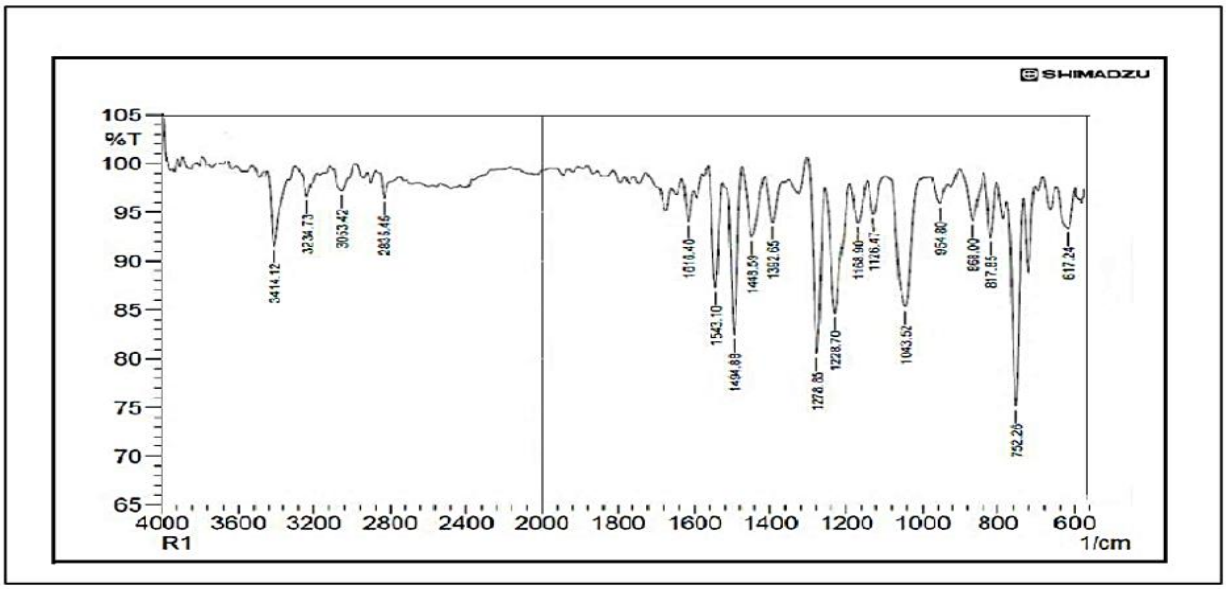


**Fig1**.FT-IR for L





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Health and Medical Science

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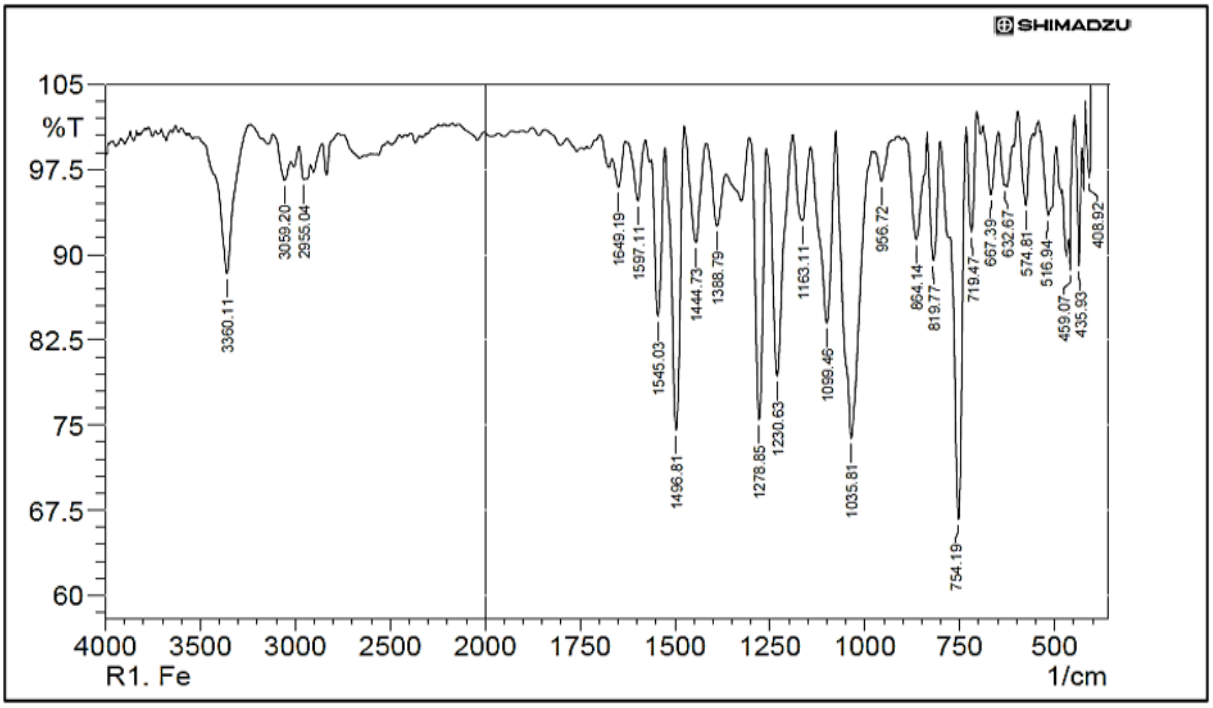


**Fig 2.** FT-IR for A1

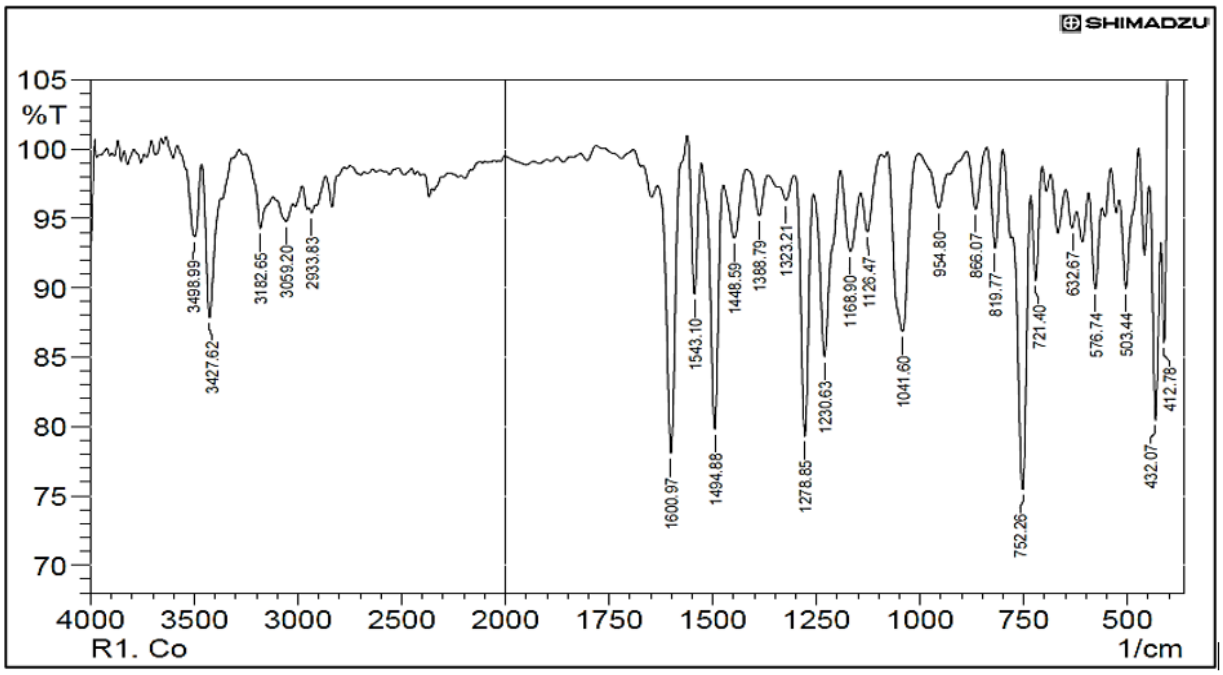


**Fig3.** FT-IR for A2





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*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 **Vol. 3, No.3, 2025**
www.zjhms.alzahraa.edu.iq

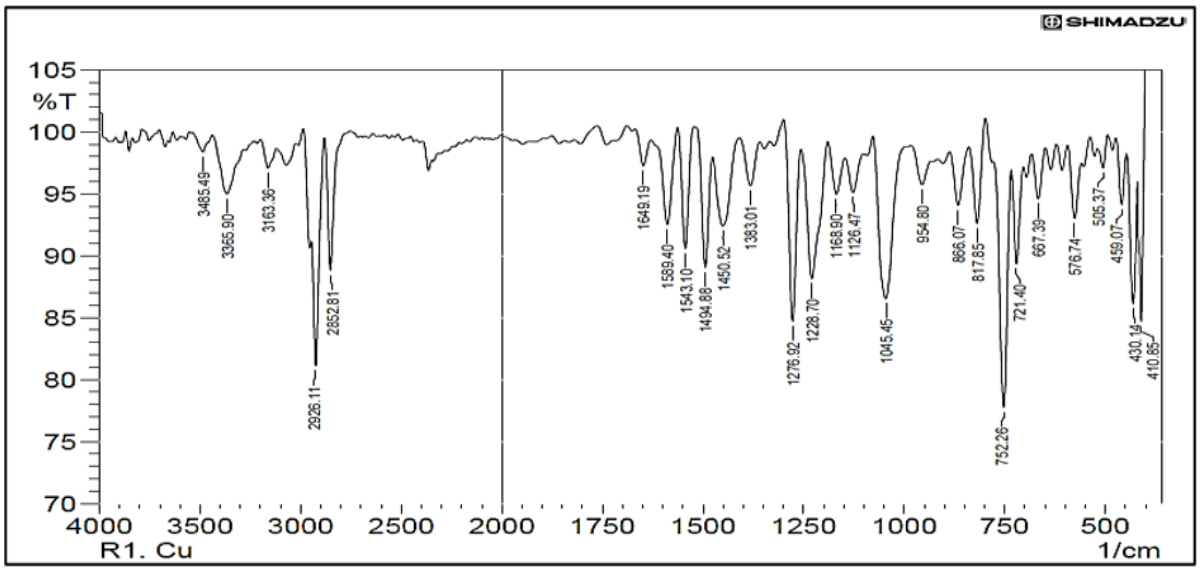


**Fig4.** FT-IR for A3

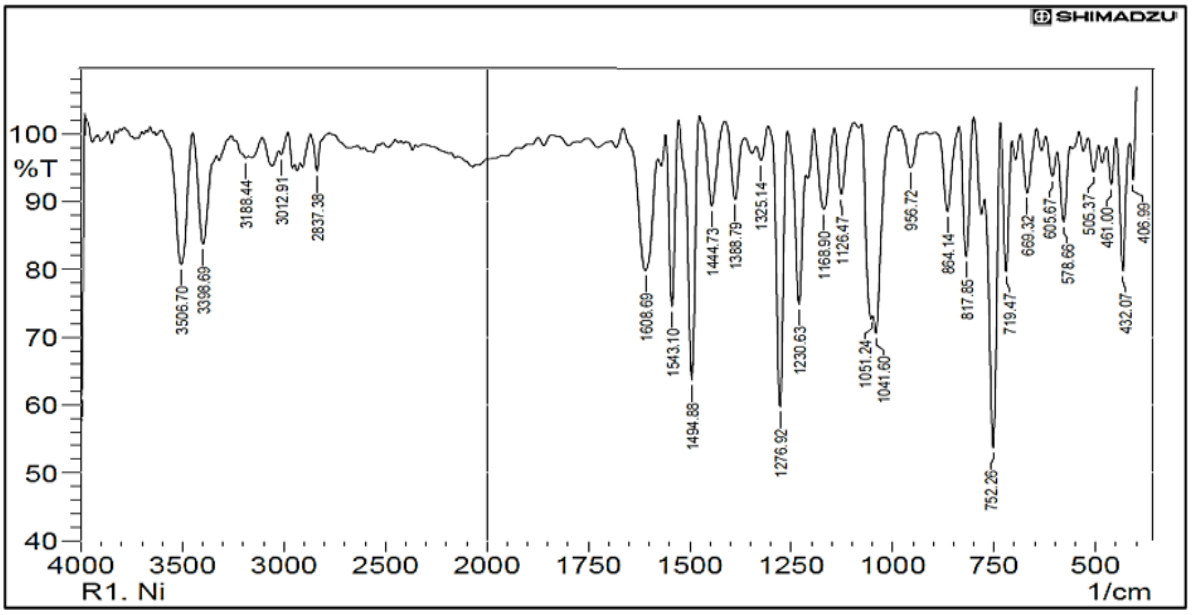


**Fig5**. FT-IR for A4

## 4. Electronic transition spectra

The electronic spectral data of the free ligand and its complexes are showed in (Table 3), and the corresponding spectra are presented in Figure 2, The free ligand showed characteristic π→π* and n→π* transitions in the UV region (typically 230–290 nm). Upon complexation, additional



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AL ZAHRAA UNIVERSITY JOURNAL
Health and Medical Science

*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 Vol. 3, No.3, 2025
www.zjhms.alzahraa.edu.iq

absorption bands were observed in the visible region (~400–700 nm), attributed to d–d transitions specific to each metal ion, confirming the formation of coordination complexes[31].

**Table 3 . Electronic data of ligand (L) and its complexes (A1-A4).**

| Compounds | $\lambda_{max}$ nm | Band Assignment |
|---|---|---|
| L | 220,322 | π→π*, n→π* |
| A1 | 597 | d-d / LMCT transition |
| A2 | 495 | d-d / LMCT transition |
| A3 | 510 | d-d / LMCT transition |
| A4 | 500 | d-d / LMCT transition |

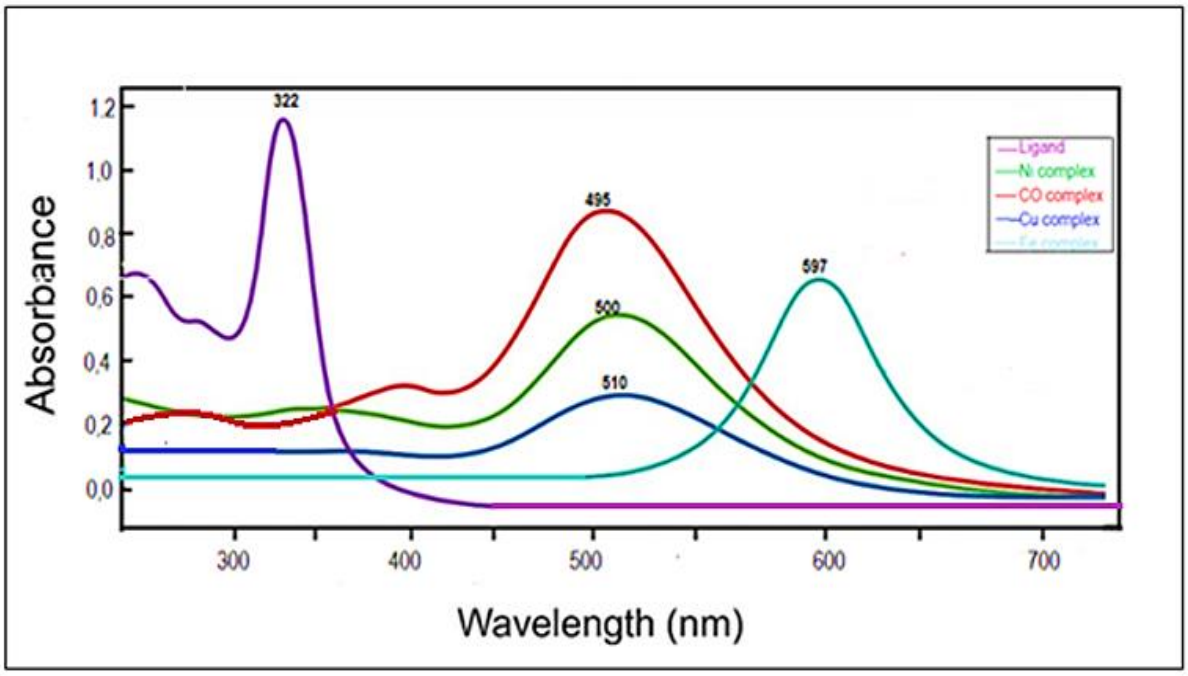


**Fig.6.** UV-Visble spectra for the imidazole and its complexes

**5. $^{1}$H NMR Spectroscopy of the imidazole ligand and its complexes**

$^{1}$H NMR spectrum of imidazole are shown in Figures (7). The imidazole showed a signal at δ12.48 ppm is attributed to the NH proton in the imidazole ring. The appearance of a singlet signal at δ 10.77 ppm indicates the presence of (OH) a hydroxyl proton in an aromatic



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Health and Medical Science

*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 **Vol. 3, No.3, 2025**
www.zjhms.alzahraa.edu.iq

environment with strong internal hydrogen bonding. The multiple signal range between δ 8.99–7.10 ppm is attributed to the aromatic protons of naphthalene and the benzyl ring. The singlet signal at δ 3.98 ppm is due to a methoxy group attached to an aromatic ring. In the $^{1}$H NMR spectra of the complexes (A1,A2, A3 and A4) in Figures (7-11). The coordination of the ligand to the metal ion caused most change and shift during complexation.

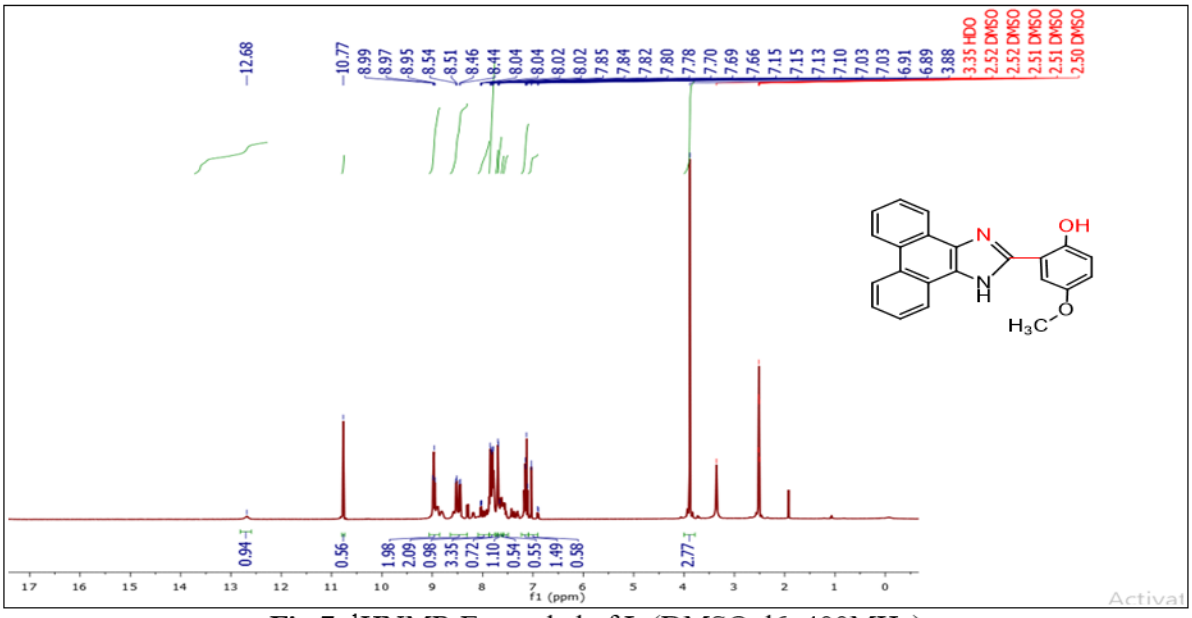


**Fig 7.** $^{1}$HNMR Expanded of L (DMSO d6 ,400MHz)

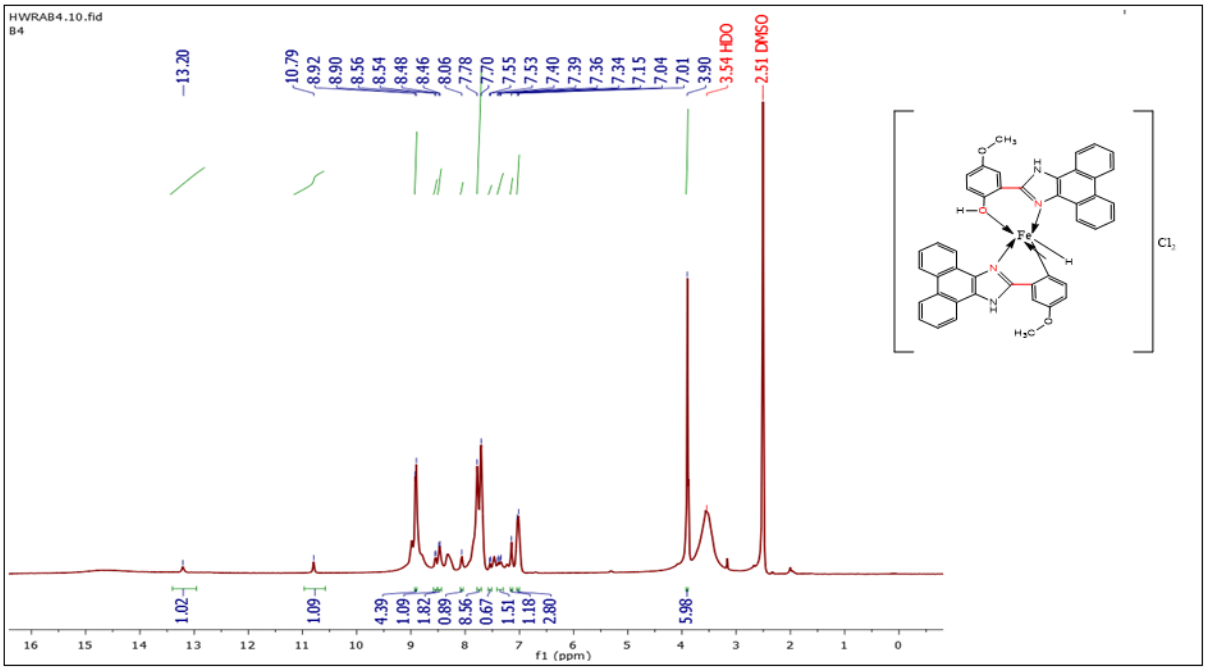


**Fig 8.** $^{1}$HNMR Expanded of A1 (DMSO d6 ,400MHz)





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*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 **Vol. 3, No.3, 2025**
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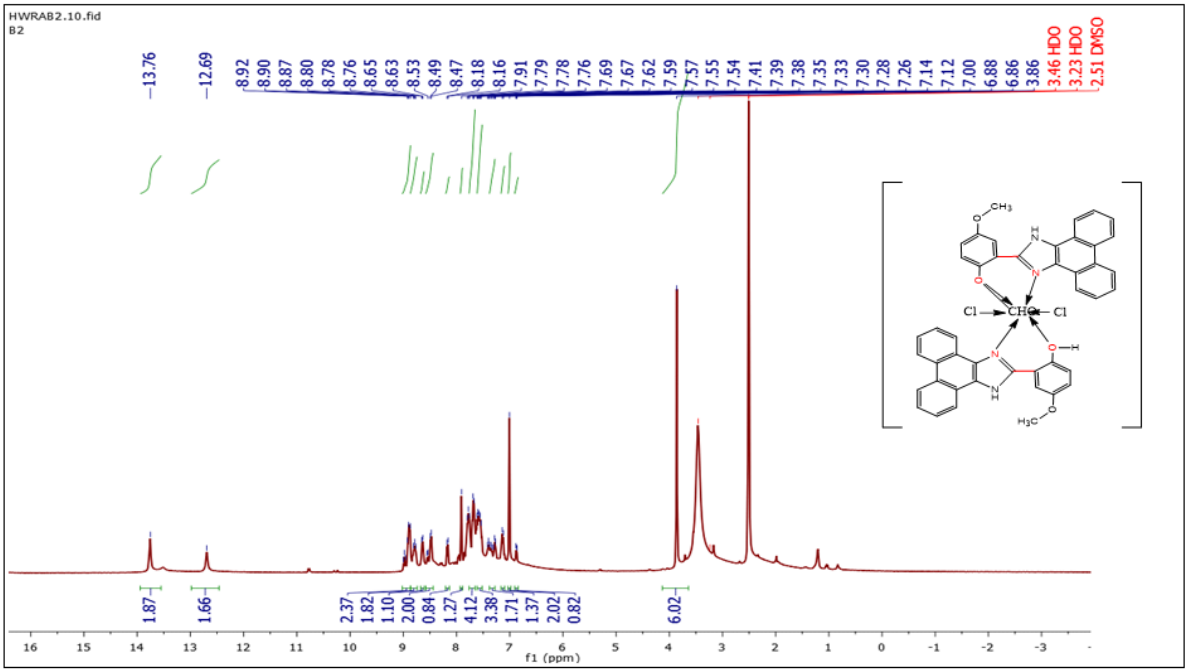


**Fig 9**. $^{1}$HNMR Expanded of A2 (DMSO d6 ,400MHz)

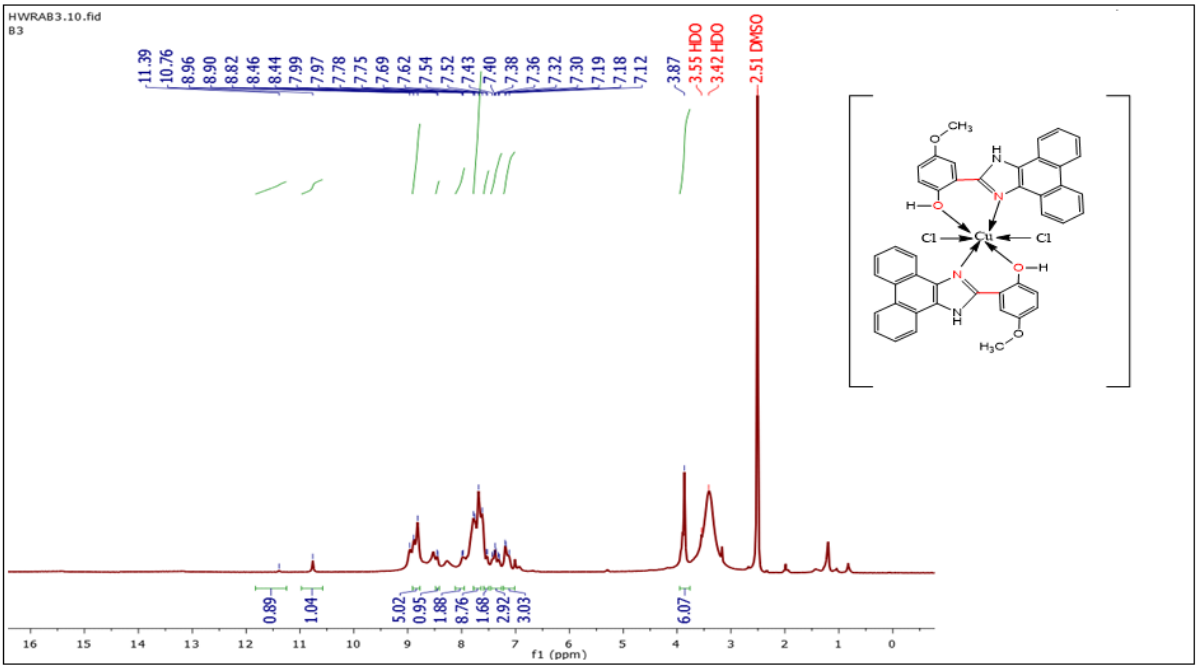


**Fig 10.** $^{1}$HNMR Expanded of A3 (DMSO d6 ,400MHz)





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*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 **Vol. 3, No.3, 2025**
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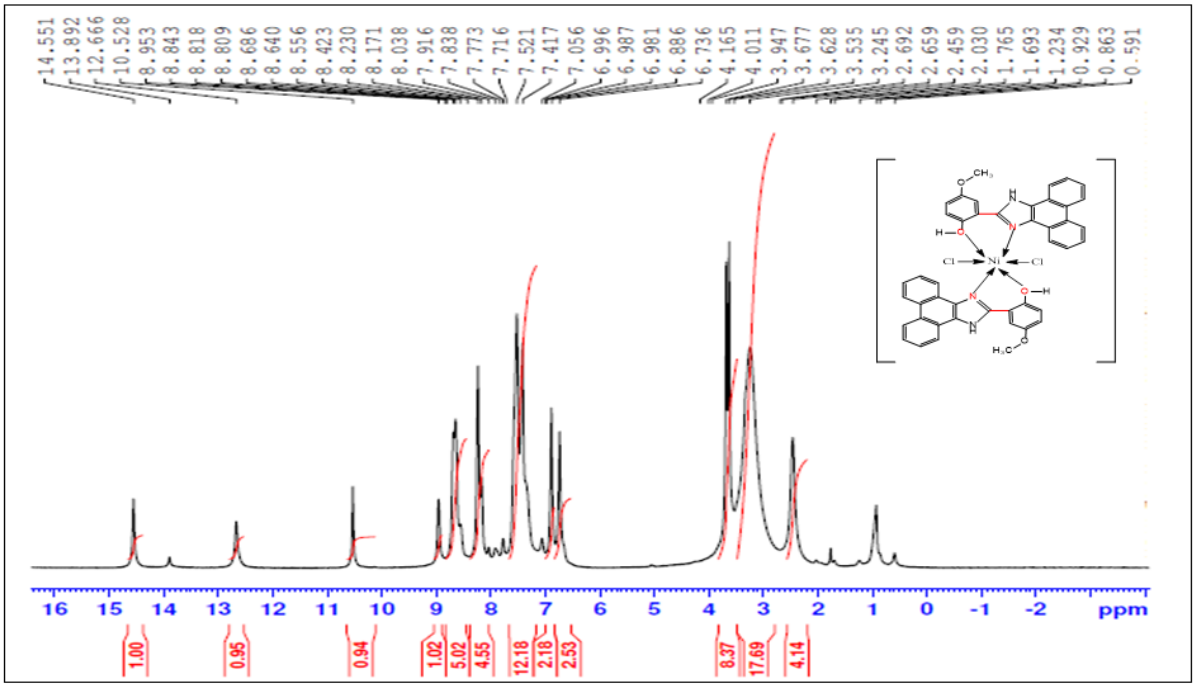


**Fig 11.** [1]HNMR Expanded of A4 (DMSO d6 ,400MHz)

## 6. Mass Spectra of the Metal Complexes

Mass spectrometry analysis of the synthesized metal complexes (A1-A4) provided conclusive evidence of their molecular structures. The spectra revealed distinct molecular ion peaks at m/z values of 807.51, 810.60, 815,21, and 810,38, attributed to $Fe^{2+}$, $Co^{2+}$, $Cu^{2+}$, and $Ni^{2+}$ complexes, respectively. These experimental values demonstrate excellent consistency with the theoretically calculated molecular weights derived from elemental analysis (Table 1).The appearance of well-defined molecular ion peaks with minimal fragmentation reflects the high stability of the complexes, while the regular variation in m/z values between the different metals demonstrates the successful coordination between the ligand and the transition metal ions. More importantly, the absence of any unexpected additional signals in the spectra confirms the purity of the prepared complexes and the reliability of the synthesis process. Thus, the close agreement between the experimental and theoretical data strongly supports the proposed molecular formulas and provides strong confirmation of the successful formation of the desired metal-ligand complexes.



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*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 **Vol. 3, No.3, 2025**
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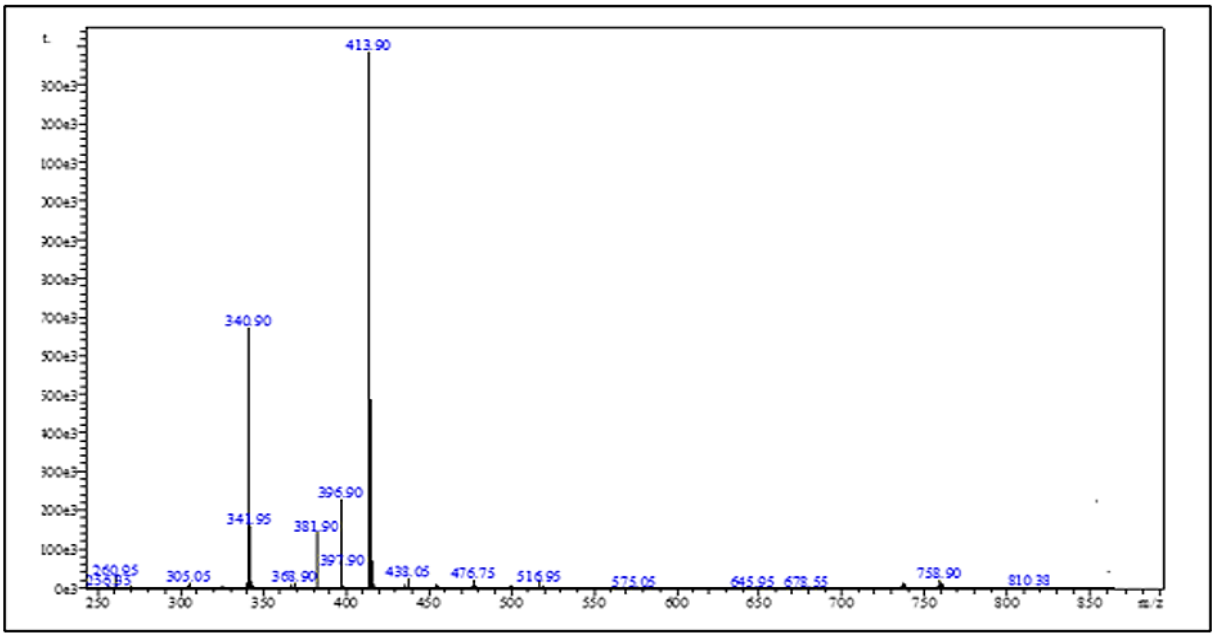


**Fig 12.** Mass Spectra of A1

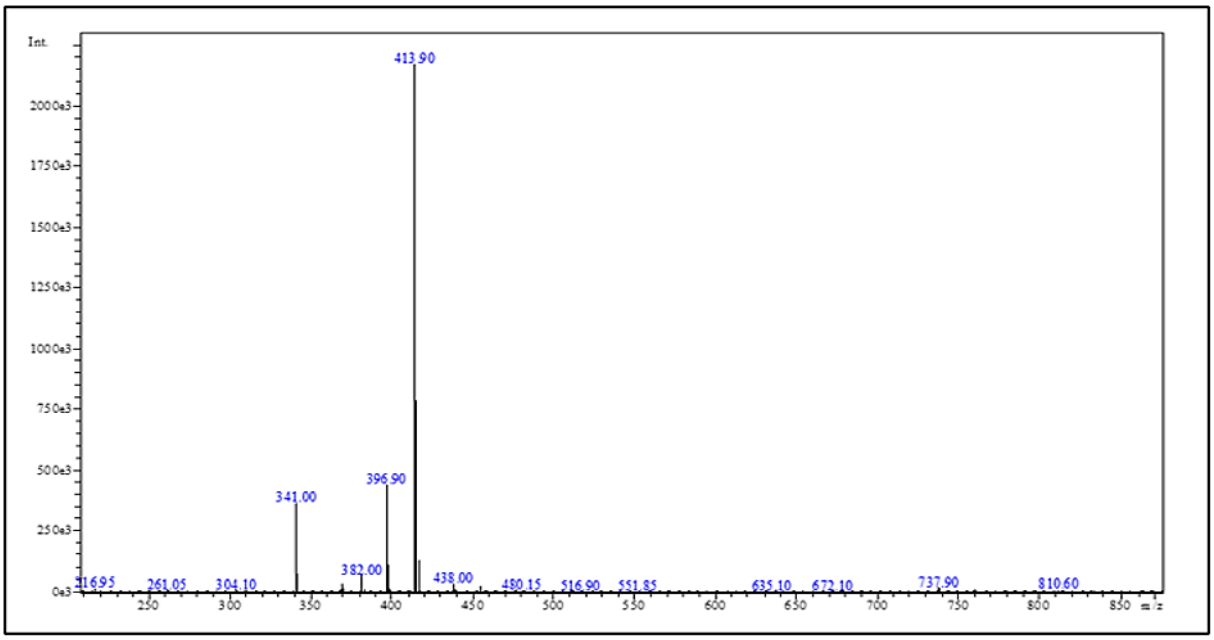


**Fig 13.** Mass Spectra of A2





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Health and Medical Science

*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 **Vol. 3, No.3, 2025**
www.zjhms.alzahraa.edu.iq

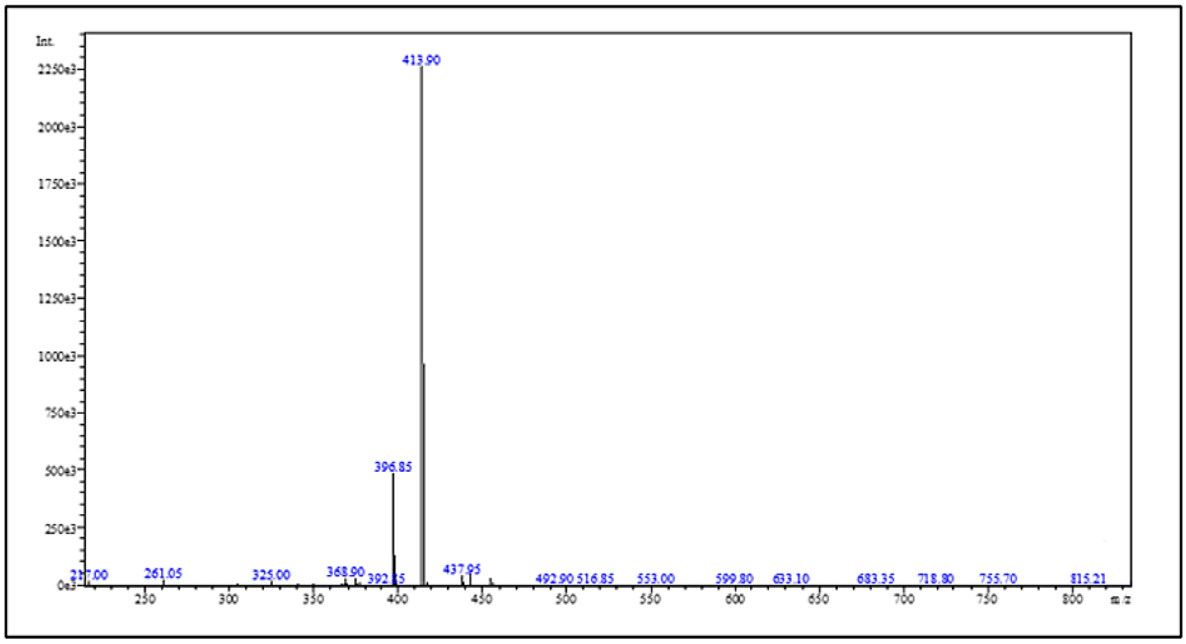


**Fig 14.** Mass Spectra of A3

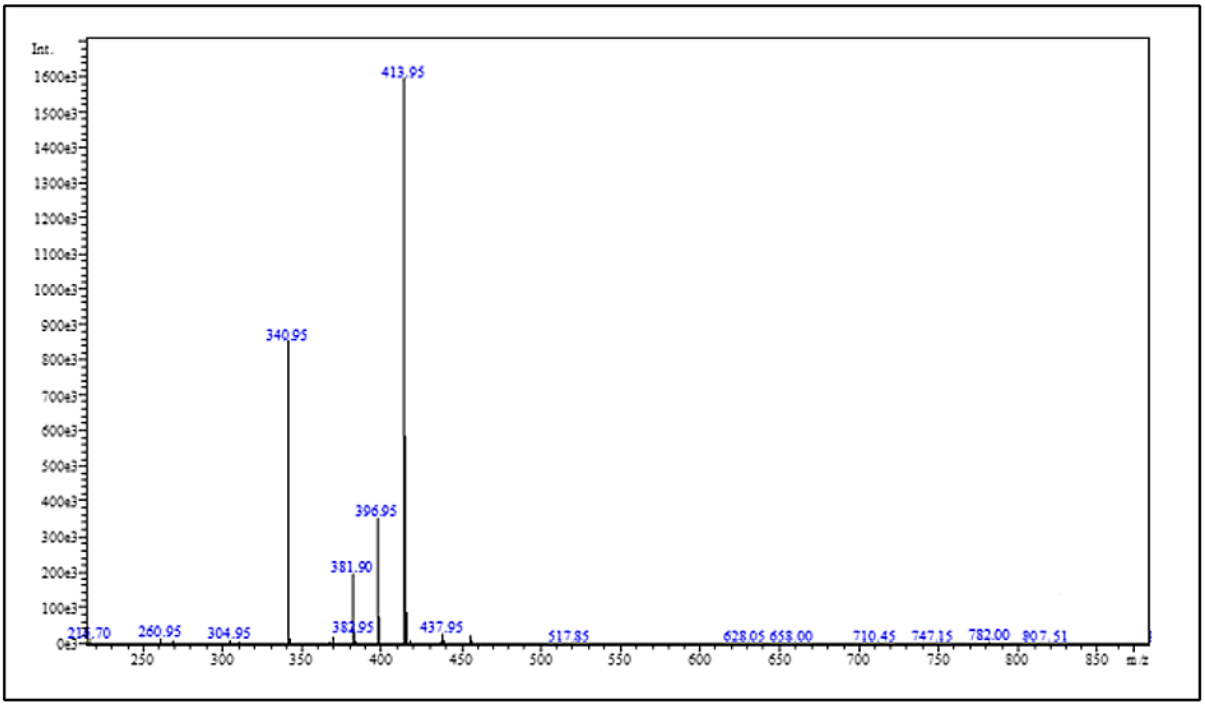


**Fig 15.** Mass Spectra of A4

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*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 **Vol. 3, No.3, 2025**
www.zjhms.alzahraa.edu.iq

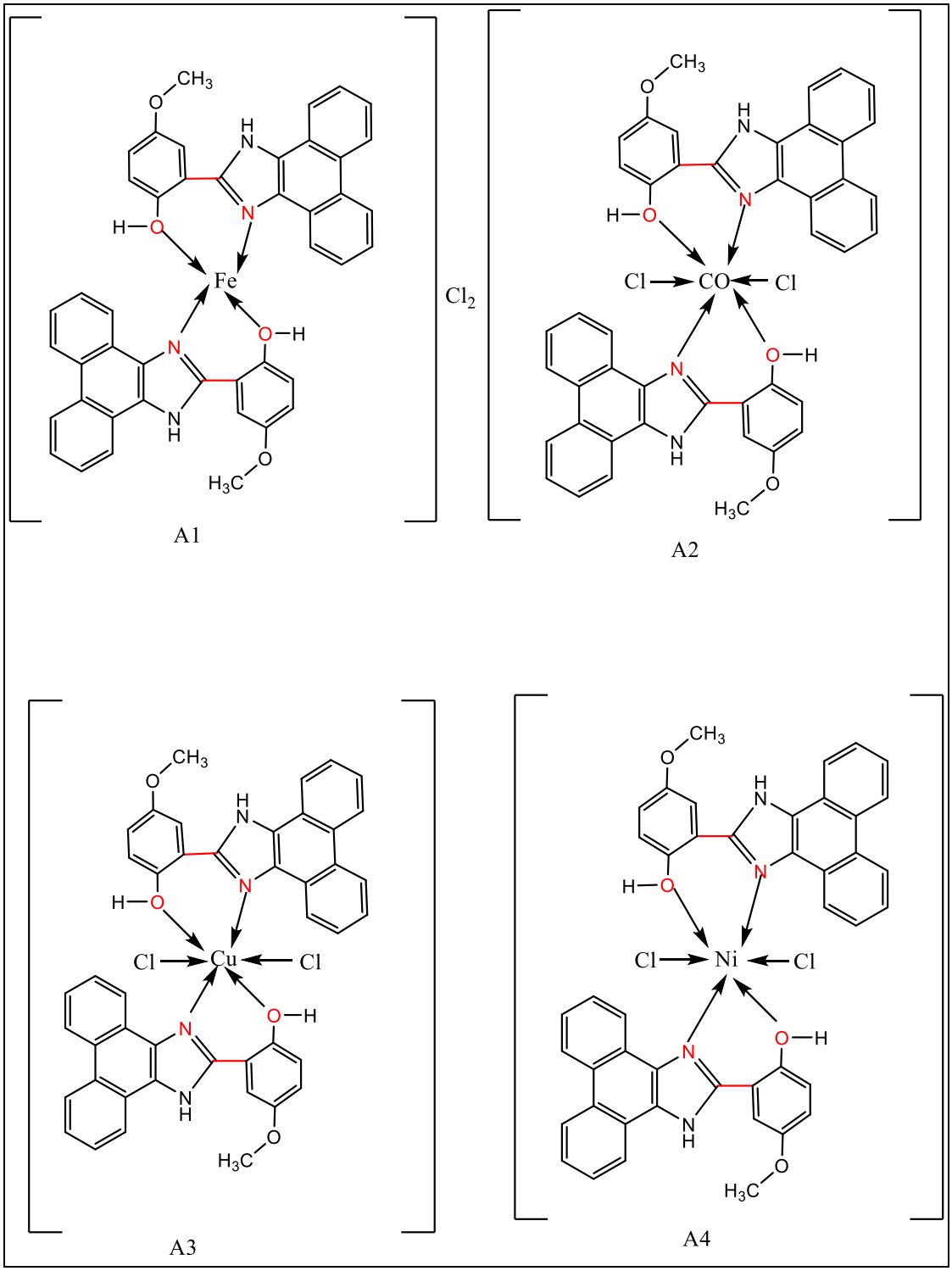


**Fig 16.** Proposed structure of the newly synthesized complexes.





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Health and Medical Science

*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 Vol. 3, No.3, 2025
www.zjhms.alzahraa.edu.iq

## Biological Activity (Antibacterial Activity)

The antibacterial activity of the synthesized metal complexes (A1-A4) was tested verses selected (Gram-positive) and (Gram-negative) bacterial strains, as table 4 and figures 17 and 18.

**Table 4. Antibacterial activity of free ligand (L) and its complexes (A1-A4).**

| Sample | Zone of inhibition (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***Staphylococcus aureus*** | | | ***Streptococcus pyognenes*** | | | ***Pseudomonas aerogeza*** | | | ***Escherichia Coli*** | | |
| | Conc. µg/mL | | | Conc. µg/mL | | | Conc. µg/mL | | | Conc. µg/mL | | |
| | **100** | **200** | **300** | **100** | **200** | **300** | **100** | **200** | **300** | **100** | **200** | **300** |
| **L** | 21 | 27 | 38 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 20 | 21 | 23 |
| **Fe** | 19 | 24 | 30 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 14 | 17 | 19 |
| **Co** | 16 | 26 | 33 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 16 | 19 | 20 |
| **Cu** | 24 | 30 | 35 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 20 | 22 | 23 |
| **Ni** | 20 | 28 | 36 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 13 | 15 | 19 |
| **Carbenicillin** | 35 | 36 | 37 | 25 | 26 | 30 | 28 | 25 | 27 | 27 | 25 | 29 |
| **DMSO** | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

The results clearly demonstrated enhanced inhibitory effects compared to the free ligand. This improvement in biological activity is attributed to the chelation process, which led to reduces the polarity of the metal and increases the overall lipophilicity of the complexes. This property facilitates diffusion across the bacterial cell membrane and enhances interactions with intracellular biomolecular targets. A comparative analysis of the complexes revealed marked differences in their antibacterial efficacy. The Cu(II) complex exhibited the highest activity, which may be attributed to its high stability constant and stronger interaction with bacterial cell



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Health and Medical Science

*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 **Vol. 3, No.3, 2025**
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components. The Ni (II) and Co(II) complexes exhibited intermediate activity, while the Fe(II) complex exhibited relatively lower antibacterial effects compared to the other complexes. These differences can be caused by differences in the electronic configurations of the metals, their affinity for donor atoms, and the resulting geometry of the complexes, all of which influence their biological interactions. Overall, the results confirm that the metal coordination plays a key role in enhancing the antibacterial activity of the ligand, with Cu(II) complex emerging as the most promising candidates for antimicrobial applications.

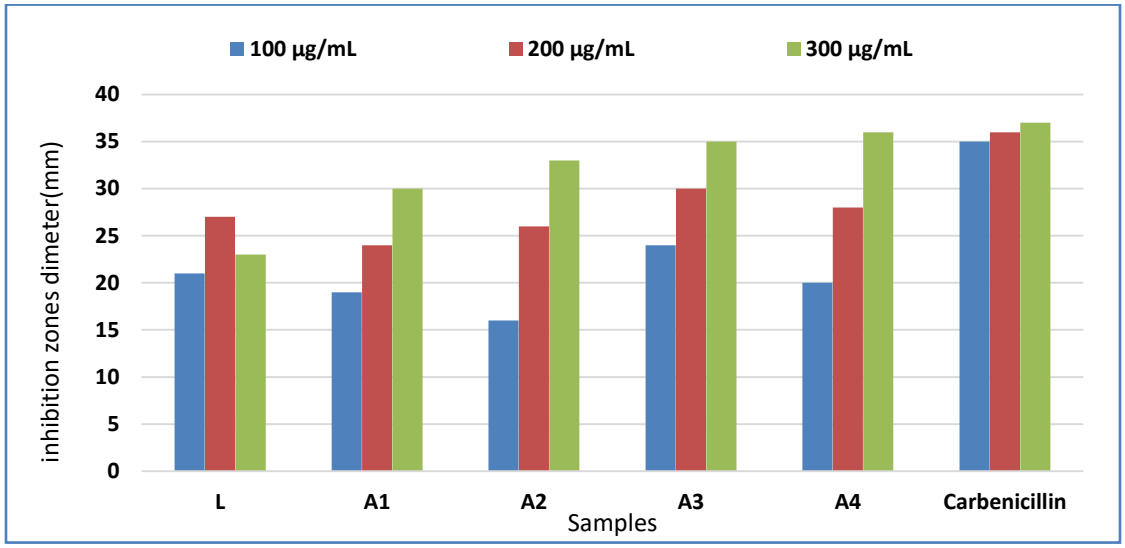


**Figure 17. The inhibitory activity of the synthesized compounds against Staphylococcus aureus.**

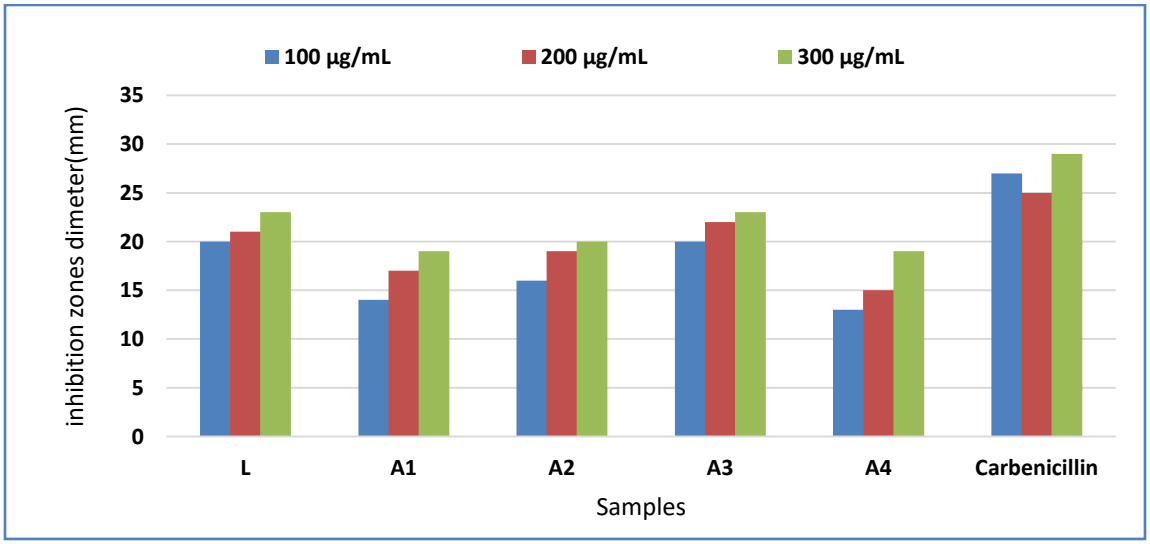


**Figure 18. The inhibitory activity of the synthesized compounds against Escherichia Coli.**





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Health and Medical Science

*Al-Zahraa Journal for Health and Medical Sciences 2025;* 3:140-161 Vol. 3, No.3, 2025
www.zjhms.alzahraa.edu.iq

## Conclusions

In this study, a new series of metal complexes were successfully prepared and characterized by coordinating a multi-substituted arylimidazole ligand, 4-methoxy-2-(1H-phenanthro[9,10-d]imidazol-2-yl)phenol, with some transition metal ions like Fe(II), Co(II), Cu(II), and Ni(II). The imidazole ligand was prepared via a condensation reaction. The coordination proses of metal ions salts with the ligand also led to the formation of stable metal complexes with tetrahedral or octahedral geometries, as identified through a variety of techniques, including elemental analysis, infrared spectroscopy, $^{1}$H-NMR, and molar conductivity measurements. These results confirmed the successful synthesis and stability of these complexes under controlled experimental conditions. The results of the bacterial tests displayed that the imidazole ligand and its complexes exhibit good antibacterial activity against the tested strains. Antibacterial activity was observed to significantly increase after complex formation compared to the free ligand, indicating that the complexation process contributes to enhancing the compounds' ability to interfere with the bacterial cell wall and affect its biological functions. The type of metal also appeared to offer a key role in determining the intensity of the activity, with some complexes exhibiting higher activity than others. Therefore, these complexes can be considered promising candidates for the development of antimicrobial agents.

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