



Synthesis, coordination study, and anti-microbial ability of new mixed-ligand complexes derivatized from azo imidazole, and 1, 10-phenanthroline

Israa N. Witwit*, Husham M. Mubarak, Razzaq A. Ibrahim, Mohauman M. Al-Rufaie

Department of Chemistry, College of Science, University of Kufa, Najaf, Iraq.



ARTICLE INFO

Article history

Received 17 May 2024

Received revised 18 June 2024

Accepted 12 July 2024

Available online 20 December 2024

Corresponding Editors

Alfayyadh I. H.

Aboulthana W.

Keywords

Bacteria,

divalent biological,

Imidazole azo,

Mixed-ligand complexes

ABSTRACT

A series of mixed-ligand complexes were prepared by reacting by a new primary imidazole azo ligand (4MCFD) and 1,10-phenanthroline as a secondary ligand with divalent ions of cobalt, nickel, mercury, and cadmium, yielding complexes with the general formula $[M(4MCFD)(1,10\text{-phen})Cl_2]$. The synthesized complexes were characterized using C.H.N. analysis, atomic absorption, FT-IR, UV-Vis, magnetic susceptibility, and molar conductivity. The stability constant ($K_{\text{Stability}}$) values and the effect of time at 25°C indicated high stability for these complexes. An octahedral geometry was suggested for all complexes, with both primary and secondary ligands acting as bidentate. The inhibition ability of the free ligands at 50 mg/mL in DMSO was found to be lower than that of the complexes against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.

Introduction

Mixed-ligand complexes are compounds that contain more than one ligand coordinated with the same central atom or ion within the coordination sphere. The combination of multiple ligands in a single complex can enhance its stability (Nesa et al. 2020; Majeed et al. 2020; Smolková et al. 2023; Talib et al. 2023). These complexes can exhibit selectivity in their interactions with other molecules. For instance, in biological systems, they can selectively bind to specific biomolecules or ions, which is crucial for various biological processes and drug design (Di Santo et al. 2005; Darawsheh et al. 2014; Slassi et al. 2019;).

The study of mixed-ligand complexes provides valuable insights into the fundamental principles of coordination chemistry, including factors that influence ligand substitution reactions, isomerism, and electronic

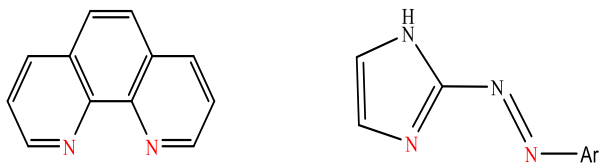
structure. Furthermore, these complexes have applications in the development of chemical sensors and chelation therapy, where they are used to remove toxic metal ions from the body (Yousif 2019; Ismael et al. 2020; El-Sonbati et al. 2022).

Imidazole azo and 1, 10-phenanthroline ligands are significant in coordination chemistry (Chin et al. 2011; Wang et al. 2022). They are widely used in various applications due to their unique characteristics and coordination abilities. Both ligands can coordinate to a metal center in their low oxidation states within the coordination sphere using two nitrogen atoms from different positions. This chelating ability is important for the formation of a stable five-membered ring with the metal center. Additionally, imidazole azo can act as a multi-dentate ligand depending on the type of substituted functional groups in the ortho position of the aromatic rings

*Corresponding author Email address: israa.witwit@uokufa.edu.iq



attached to the azo group. The general structures of these ligands are shown in Figure 1.



1,10-phenanthroline ligand General structure of imidazole azo ligands

Fig 1. General structure of Imidazole azo and 1, 10-phenanthroline ligands.

The primary aim of this study is to prepare and characterize a series of mixed ligand complexes using the new imidazole azo compound (E)-2-((3-chloro-4-fluorophenyl)diazinyl)-4-methyl-1H-imidazole (4MCFD) as the primary ligand and 1, 10-phenanthroline as the secondary ligand. These complexes were synthesized with six divalent ions from the first series of transition elements. The study also investigates their antimicrobial activity and their ability to inhibit the growth of three resistant bacterial species.

Materials and methods

Chemicals

All liquid and solid chemicals used were supplied by B.H.D., Merck, CDH, AK Scientific, and Sigma Aldrich companies, with high purity.

Instrumentation

The magnetic susceptibility measurements of the solid prepared complexes were recorded using an Msb-Mki Balance. The mass spectrum for the Co (II) complex was recorded using a SCIEX 3200 Mass Analyzer. Electronic spectrum measurements were performed using a Shimadzu 1800 UV spectrophotometer. FT-IR measurements were obtained using a Shimadzu FT-IR-8400S spectrometer, and molar conductivity values were measured using a WTW inoLab pH/Cond 720. Elemental analysis (C.H.N) was conducted using a Perkin Elmer 2400 Elemental Analyzer.

Preparation of solid mixed ligand Complexes

The primary ligand (4MCFD) was prepared by a coupling reaction between the diazonium salt of 10 mmol 3-chloro-4-fluoroaniline and 10 mmol of the coupling base 4-methyl imidazole (Witwit, I.N. et. al.2023) as shown in Figure 1. The solid complexes were prepared at a molar ratio of 1:1:1 [M:4MCFD:1,10-phen] by mixing 0.238 g of the primary ligand (4MCFD) with 0.179 mL of the

secondary ligand 1,10-phenanthroline and 1 mmol each of $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, CdCl_2 , and HgCl_2 salts in 30 mL of ethanol.

The mixture of each complex was refluxed for 2 hours, followed by Thin Paper Layer Chromatography until the precipitation appeared, then filtered, dried, and recrystallized from hot ethanol. The percentage yield of the complexes was calculated as shown in Table 1 and Figures 2 and 3 illustrate the chemical reactions involved in the preparation of the new complexes.

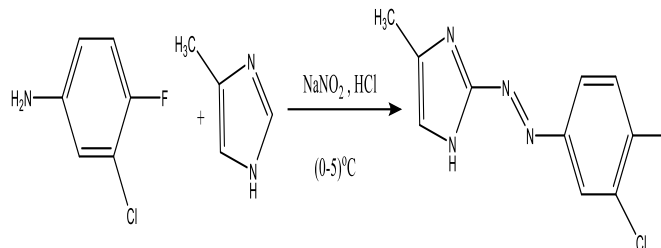


Fig 2. General reaction for the preparation of the primary ligand (4MCFD).

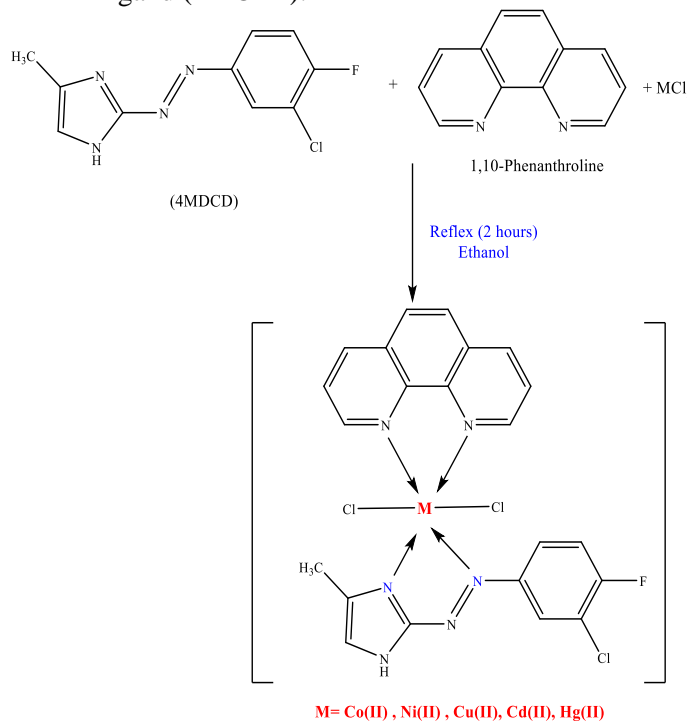


Fig 3. Chemical reaction for the preparation of the new mixed ligand complexes.

Calculation of the values of Stability Constants for the complexes

The following equations at 10^{-4} M for the prepared complexes were used to calculate the stability constants at mole ratio [M (4MCFD) (1, 10-phen)], [1:1:1]:



$$\alpha = \frac{n\alpha C}{1 - \alpha}$$

M: Central metal ion; L: Ligand; C: Ligand concentration; n: The total number for ligands; α : Dissociation degree.

$$K_{Stability} = \frac{[ML_n]}{[M][L]^n} \quad (2)$$

$$K = \frac{1 - \alpha}{4\alpha^2 C^3} \quad (3)$$

$$\alpha = \frac{A_m - A_s}{A_s} \quad (4)$$

Where A_m is complex absorption value at the mole ratio; A_s is value of the complex absorption with increasing ligand.

Study the stability of the complexes in time

The Absorption of the complexes in ethanol at the concentration (10^{-4}) M was measured in UV-Vis. Region, between 0- 240 minuet under the (λ max) of each one, the time curve was plotted by making the absorption values on the Y-axis and the wavelength values in the X-axis.

Study the antibacterial activity of the complexes

The antibacterial activity was examined by the agar well diffusion technique (Wang et al. 2022). For the in-vitro antibacterial testing of the newly synthesized compounds versus *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*, Mueller-Hinton agar (MHA) was used as the testing medium. Bacterial isolate suspensions were created using a micropipette to correspond to the 0.5 McFarland standard, and they were sterilized for 1.5 hours at 121 °C. Each complex was prepared at a 50 mg/mL concentration by dissolving it in DMSO solvent. The solutions were then applied to the culture's wall and incubated for 24 hours at 37°C. The inhibition zone, which measures the efficacy of the inhibition, measured in millimeters using DMSO as a reference.

Results and Discussion

Elemental analysis, and Molar Conductivity

Elemental analysis plays an important role in coordination chemistry by identifying coordination compounds' composition, structure, and purity. It serves as a critical tool for characterizing these complex compounds. Elemental analysis of the prepared complexes revealed close agreement between calculated and practical values, supporting their molecular structures. Conductivity measurements at 10^{-3} M in ethanol and

dimethylsulfoxide solvents indicated that the complexes were non-electrolytes. The absence of AgCl precursors during the drop addition of AgNO₃ solution further confirmed the presence of chloride ions within the coordination sphere as detailed in Table 1.

Mass Spectrum

In coordination chemistry, mass spectrometry is a useful technique that provides insights on the molecular mass determination, analyzing fragmentation patterns, and studying the behavior of the coordination compounds. The mass spectra of Co (II) showed a molecular peak at $m/z = 548$, confirming the molecular structure of this complex. Additionally, the base peak is due to $[C_6H_7N]^+$ fragment is illustrated in Figures 4 and 5.

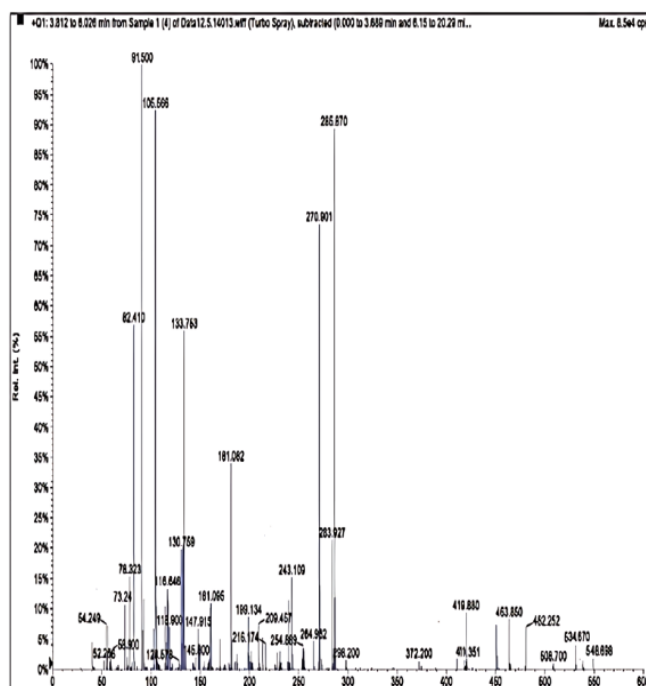


Fig 4. Mass spectrum of $[Co (4MCFD) (1, 10\text{-phen}) Cl_2]$ complex.

FT-IR of the ligands and their solid complexes

Infrared spectroscopy is considered an essential tool in coordination chemistry due to its ability to track changes in functional groups between free ligands compared to their complexes. The $\nu(N-H)$ vibration of the imidazole ring at 3417 cm^{-1} in the spectrum of the primary ligand (4MCFD) showed no clear change in the spectra of the complexes, indicating that the (N1) nitrogen of the of the imidazole ring does not participate in coordination.

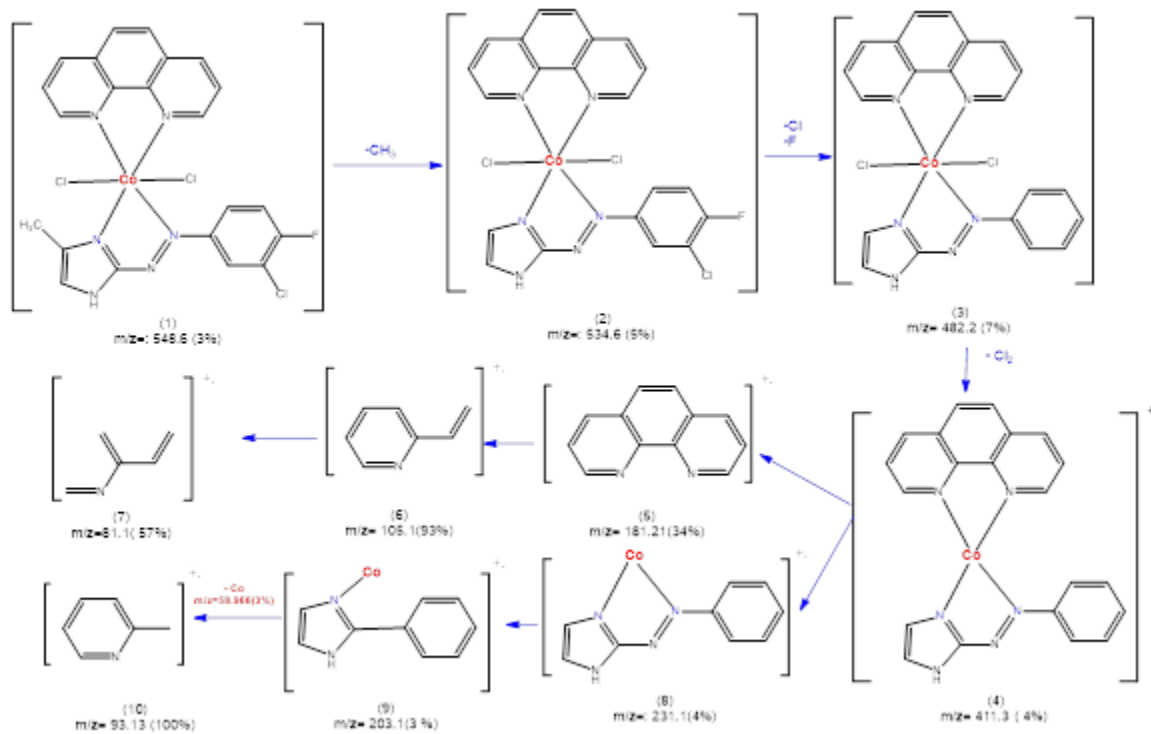


Fig 5. Mass fragmentation path of [Co(4MCFD)(1,10-phen)Cl₂] complex. (1) Chemical Formula: C₂₂H₁₆Cl₃CoFN₆, (2) [C₂₁H₁₄Cl₃CoFN₆]⁺, (3) [C₂₁H₁₆Cl₂CoN₆]⁺, (4) [C₂₁H₁₆CoN₆]⁺, (5) [C₁₂H₈N₂]⁺+H⁺, (6) [C₇H₇N]⁺, (7) [C₅H₇N]⁺, (8) [C₉H₈CoN₄]⁺, (9) [C₉H₈CoN₂]⁺, (10) [C₆H₇N]⁺ - 2H⁺.

Table 1: Molar conductivity, Elemental analysis, and some of chemo-physical properties of the primary ligand and the prepared complexes

Compound (Molecular formula) (MW)*	Molar conductivity S. Cm ² .mole ⁻¹ ethanol DMSO		M.P.*	Color	Elemental analysis calculated (Found)			
					C	H	N	M
(4MCFD) (C ₁₀ H ₈ ClFN ₄) (238.65)	-----	-----	171	-	50.33 (50.30)	3.38 (3.40)	23.48 (23.49)	-----
Co (II) Complex (C ₂₂ H ₁₆ Cl ₃ CoFN ₆) (248.69)	10.3	12.0	183	Orange	48.16 (48.20)	2.94 (2.90)	15.32 (15.34)	10.74 (10.76)
Ni (II) Complex (C ₂₂ H ₁₆ Cl ₃ NiFN ₆) (548.45)	11.4	14.6	189	Brown	48.18 (48.20)	2.94 (2.91)	15.32 (15.35)	10.70 (10.71)
Cu (II) Complex (C ₂₂ H ₁₆ Cl ₃ CuFN ₆) (553.31)	14.2	17.5	204	Purple	47.76 (47.80)	2.91 (2.90)	15.19 (15.21)	11.48 (11.49)
Cd (II) Complex (C ₂₂ H ₁₆ Cl ₃ CdFN ₆) (602.17)	12.5	15.8	215	Red	43.88 (43.90)	2.68 (2.65)	13.96 (13.70)	18.67 (18.69)
Hg (II) Complex (C ₂₂ H ₁₆ Cl ₃ HgFN ₆) (690.35)	12.00	15.1	222	Dark Red	38.28 (38.30)	2.34 (2.33)	12.17 (12.20)	29.06 -----

*Abbreviation: MW: molecular weight; M.P.: melting point.

In contrast, the $\nu(\text{C-N})$ of the imidazole ring at 1253 cm^{-1} exhibited a clear shift in the complexes, supporting coordination through the (N3) nitrogen of the imidazole ring (Ward et al. 2022). These values exhibited noticeable changes in the complex's spectra because of the formation of coordination bonds with metal ions through the nitrogen atom of the imidazole ring (Abdullah et al. 2020). The $\nu(\text{C=N})$ of the secondary ligand (1,10-phenanthroline) appeared at 1558 cm^{-1} (Eni et al. 2020, Türkel, N. 2012). this peak shifts to lower frequencies in the complexes due to the involvement of the nitrogen atoms of heterocyclic rings in coordination. New peaks for $\nu(\text{M-N})$ (Mahdy et al. 2022; Jawad & Al-Adilee 2022; Dahi & Jarad 2020) appeared in the spectra of the complexes between $400\text{-}500 \text{ cm}^{-1}$, indicating coordination of the primary and secondary ligands with the central metal ions as shown in Table 2 and Figures from (6-12).

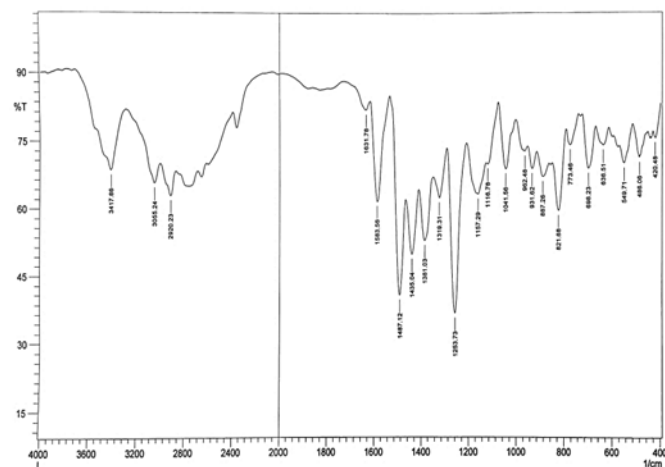


Fig 6. FT-IR spectra of the primary (4MCFD) ligand.

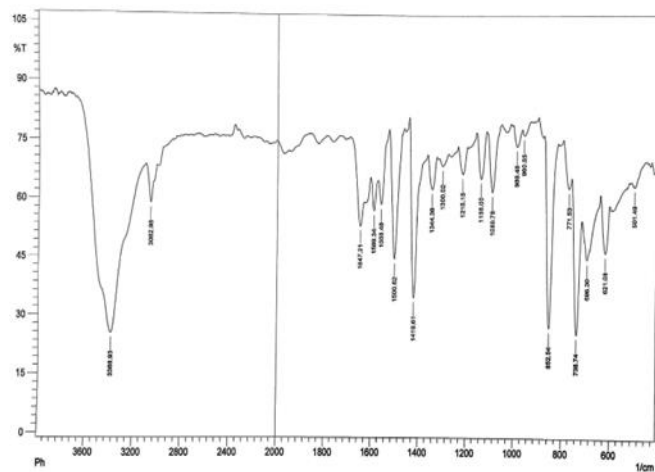


Fig 7. FT-IR of the secondary ligand (1, 10-phen).

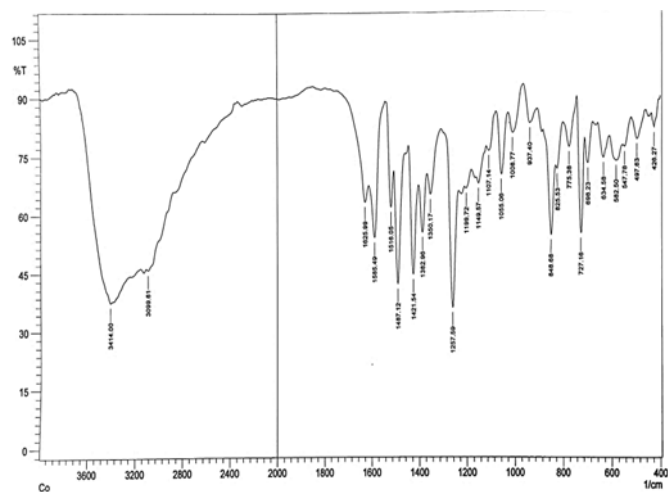


Fig 8. FT-IR for $[\text{Co} (4\text{MCFD}) (1, 10\text{-phen}) \text{Cl}_2]$.

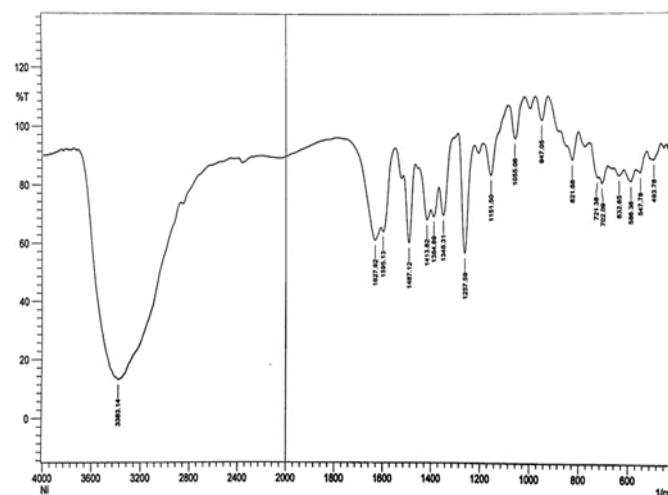


Fig 9. FT-IR for $[\text{Ni} (4\text{MCFD}) (1, 10\text{-phen}) \text{Cl}_2]$.

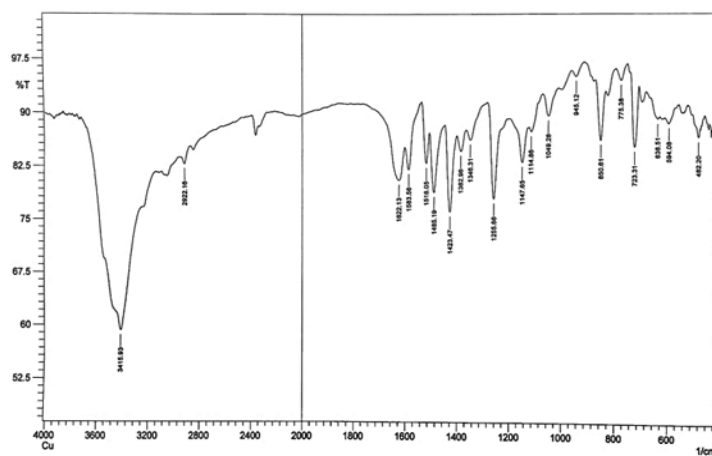


Fig 10. FT-IR for $[\text{Cu} (4\text{MCFD}) (1, 10\text{-phen}) \text{Cl}_2]$.

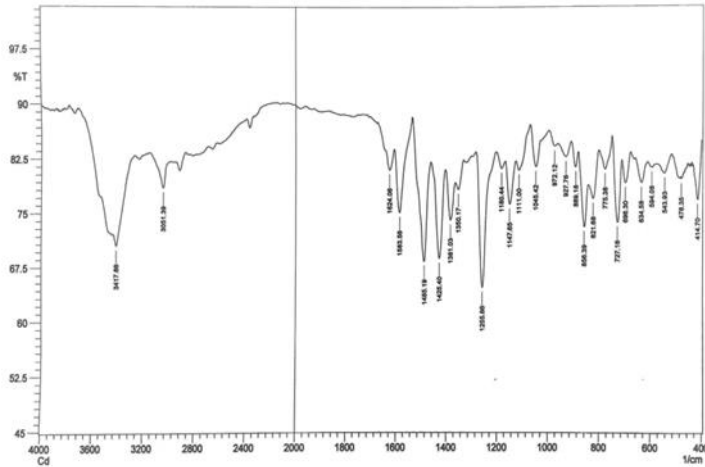


Fig 11. FT-IR for [Cd(4MCFD)(1,10-phen)Cl₂].

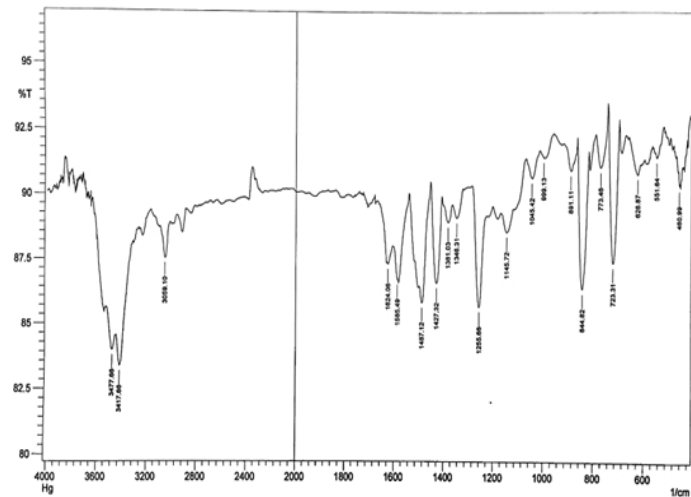
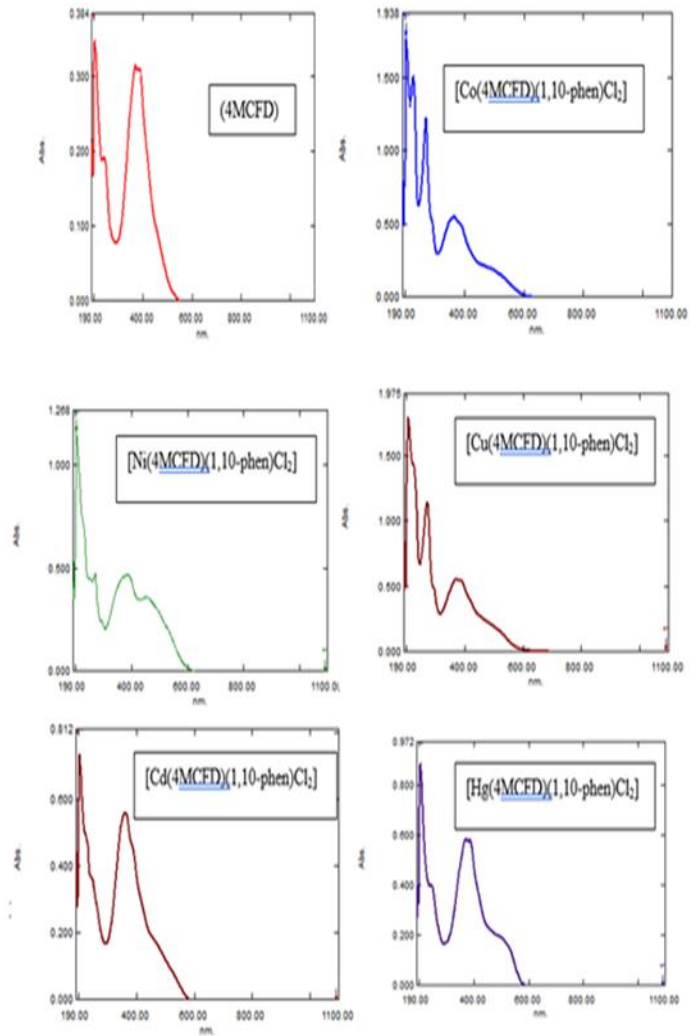


Fig 12. FT-IR for [Hg(4MCFD)(1,10-phen)Cl₂].

Electronic spectra of the ligands, and their complexes

Studying the electronic transitions in the UV-Vis region is an important tool in coordination chemistry. The electronic spectra of the free ligands and their complexes were measured at a concentration of 0.5×10^{-4} M using ethanol as a solvent. The primary ligand exhibited π - π^* and n - π^* transitions at 204 nm, 241 nm, and 370 nm, as well as an ILCT transition at 385 nm. All of these transition positions showed clear shifts in the spectra of the complexes due to the coordination process as illustrated in Figure 13 and Table 3.

Fig 13. Electronic spectra of the primary ligand, and



mixed ligand complexes in ethanol solvent.

Stability constant values for the prepared complexes

The stability constants were calculated according to the mole ratio (1:1:1) [M(4MCFD)(1,10-phen)] at 25°C, using a concentration of 10^{-4} M for the ligands and metal ions. Both A_m and A_s were measured at the λ_{max} for each complex. The resulting values, presented in Table 4, indicate high stability for the complexes and consistency with the Irving-Williams series for divalent ion complexes of the first transition period (Nickless, G. et al. 1967), as follows:



Magnetic susceptibility of the complexes

Magnetic susceptibility is a significant tool in coordination chemistry because it provides information on the electronic structure, bonding, and magnetic behavior of coordination complexes. It helps in the characterization, identification, and design of these compounds, leading to a better understanding of their properties and reactivity. The magnetic susceptibility results confirm the di-magnetic behavior for both [Cd (4MCFD) (1, 10-phen) Cl₂] complexes, indicating an electronic configuration of (nd¹⁰) for both Cd (II) and Hg (II) central metal ions.

The magnetic susceptibility value of Co (II) complex is 4.63 B.M., because of the presence of five electrons in t_{2g} orbitals and two electrons in e_g, orbitals, indicating a distorted octahedral shape of this complex. The value of 2.77 B.M. for the Ni (II) complex supports its octahedral geometry due to the equal electronic distribution in both t_{2g}⁶ and e_g² orbitals in the outer shell of the metal ions. Lastly, the value of 1.72 B.M. for the Cu (II) complex supports a distorted octahedral geometry due to the unequal electronic distribution in the splitting outer-shell (d) orbitals (t_{2g}⁶ e_g³) (Maliyappa & Keshavayya 2022; Obaid et al. 2020).

Study the stability of the complexes with time

The absorption value of 10⁻⁴ M for each complex solution in ethanol was measured from (0–160) min. at λ_{max} and the selected mole ratio, as shown in Figure 14. The results refer to the high stability of the colorimetric property in the room temperature during this period, and for repeating the measure of absorption after 24 hours, there was no noticeable change in their data.

Suggested structure [M (4MCFD) (1,10-phen) Cl₂] complexes

Based on the results of the previous analysis techniques, the octahedral geometrical shape of the complexes was suggested in which the primary and secondary ligands behave as a bidentate. The primary ligand (4MCFD) coordinated through (N) number (3) of imidazole ring and one of nitrogen atoms of azo functional group, while the secondary ligand coordinated through nitrogen atoms of heterocyclic rings as shown in the Figure 15.

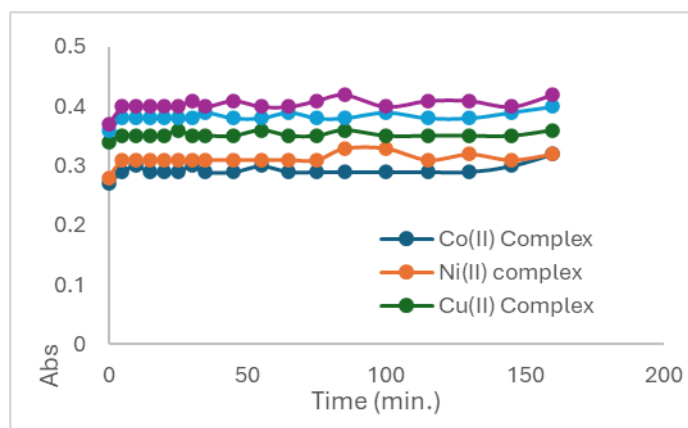


Fig 14. The stability of the complexes with time (minutes).

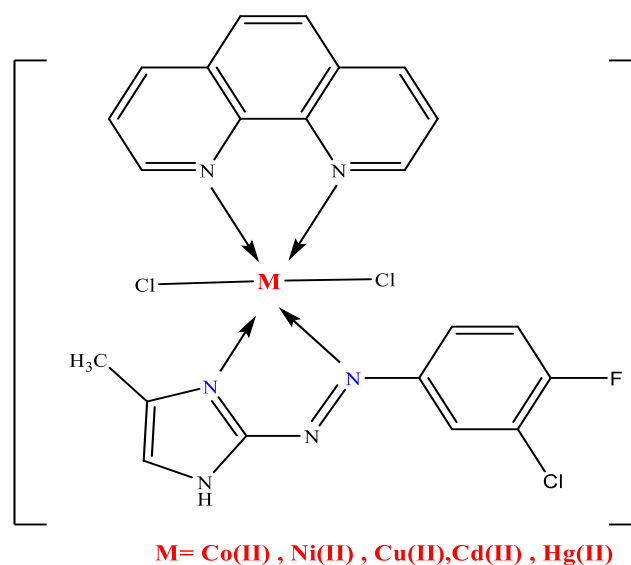


Fig 15. Suggested Structure of [M (4MCFD) (1, 10-phen) Cl₂] Complex.

The inhibition effect of the primary ligand and the complexes

The ability of the synthesized compounds to inhibit bacterial growth was tested on three different types of pathogenic microflora at a concentration of 50 mg/mL. The bacteria were chosen based on their abundance and resistance to antibiotics. *S. aureus* showed greater resistance because gram-positive bacteria are protected against antibiotics via a peptidoglycan layer, while *E. coli* and *P. aeruginosa* showed less resistance to the free primary ligand and the complexes because the Gram-negative bacteria

have a thinner peptidoglycan layer in their outer membrane (Li et al. 2023). According to the majority of research, metal complexes can become more active than free ligand. Chelation theory can be used to explain why metal complexes exhibit higher activity. Chelation reduces the polarity of the metal ion and may result in electron delocalization. As a result, the metal chelate has a stronger lipophilic nature, making it easier for it to penetrate the lipid layer surrounding the microbe and kill it as shown in Figures 16 and 17.

Conclusion

In this study, new mixed ligand complexes were prepared using an imidazole azo ligand (4MCFD) as the primary ligand and 1,10-phenanthroline as the secondary ligand with some transition divalent ions at molar ratio (M:L1:L2). The complexes were synthesized with a high yield and stability. All of these complexes exhibited octahedral geometry, and both ligands behaved as bidentate. The complexes showed notable inhibition towards *S. aureus*, *E. coli*, and *P. aeruginosa* bacteria compared to the free ligands.

Table 2. FT-IR frequencies values (cm^{-1}) for free ligands and their complexes

Compound	ν (C=N)	ν (N=N)	ν (C-N) 1,10-phen.	ν (C-N) imidazole	ν (M-N)
(4MCFD)	-	1487 s	-	1253 s	-
(1,10)-Phen.	1558 m	-	1419 m	-	-
Co Complex	1516 m	1421 m	1382 m	1257 s	426 w
Ni Complex	1413 m	1348 m	1384 m	1258 m	493 w
Cu Complex	1516 m	1423 m	1382 w	1255 m	482 w
Cd Complex	1425 m	1381 m	1381 w	1255 m	478 w
Hg Complex	1427 m	1381 m	1381 w	1255 m	460 w

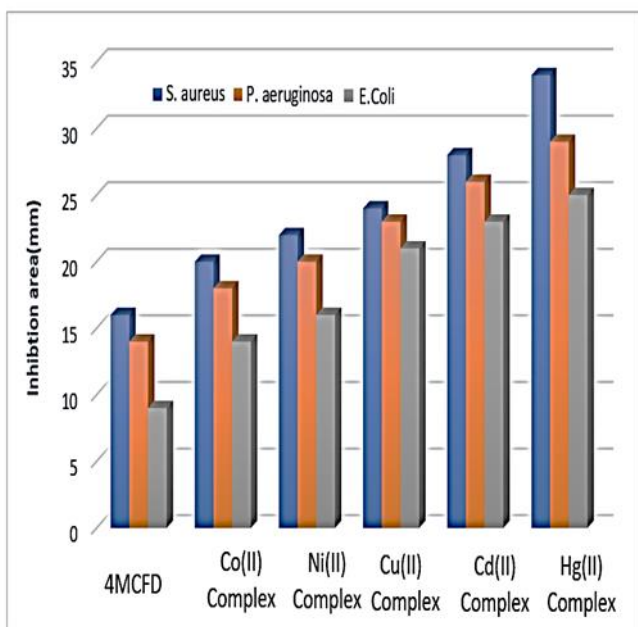
w: weak, m: medium, s: strong

Table 3. Types of electronic spectra for primary ligand and mixed ligand complexes

Compound	Wavelength (nm)	Type of Electronic Transition
(4MCFD)	204, 241	π - π^*
	370	n- π^*
	385	ILCT
[Co(4MCFD) (1,10-phen) Cl ₂]	203,227	π - π^*
	364	n- π^*
	451	MLCT
[Ni(4MCFD) (1,10-phen) Cl ₂]	203, 292	π - π^*
	392	n- π^*
	460	MLCT
[Cu(4MCFD) (1,10-phen) Cl ₂]	205, 272	π - π^*
	385	n- π^*
	482	MLCT
[Cd(4MCFD) (1,10-phen) Cl ₂]	203,227	π - π^*
	362	n- π^*
	481	MLCT
[Hg(4MCFD) (1,10-phen) Cl ₂]	204, 224	π - π^*
	370	n- π^*
	510	MLCT

Table 4. Values of A_m , A_s , α , and stability constants for the complexes at 25°C.

The Complex	A_m	A_s	α	$K_{\text{stability}}$	Log K stability
[Co (4MCFD) (1,10-phen) Cl ₂]	0.29	0.24	0.208	4.56×10^{12}	12.65
[Ni (4MCFD) (1,10-phen) Cl ₂]	0.31	0.28	0.107	1.94×10^{13}	13.28
[Cu (4MCFD) (1,10-phen) Cl ₂]	0.35	0.32	0.093	2.57×10^{13}	13.41
[Cd (4MCFD) (1,10-phen) Cl ₂]	0.38	0.35	0.085	3.11×10^{13}	13.49
[Hg (4MCFD) (1,10-phen) Cl ₂]	0.40	0.37	0.081	3.49×10^{13}	13.54

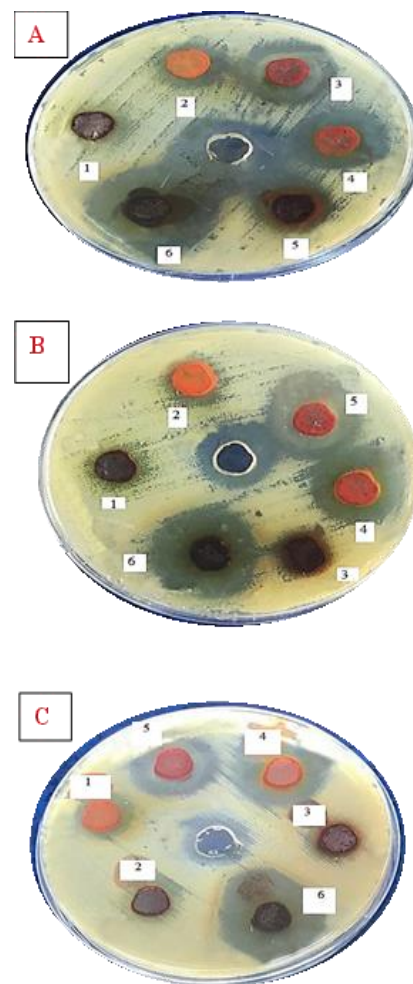
**Fig 16.** Inhibition area (mm) of the primary ligand (4MCFD), and mixed-ligand complexes against *S. aureus*, *E. coli*, and *P. aeruginosa*.

Ethics of Sampling

All the participants provided informed consent for inclusion in the study and were assured that all the information provided would be used solely for the purposes of this study and treated confidentially.

Conflict of interest

The authors declare that they have no conflict of interest.

**Fig 17.** Inhibition area (mm) against (A) *S. aureus*, (B) *P. aeruginosa* (C) *E. coli* at 50 mg /mL of: 1- 4MCFD; 2- [Co (4MCFD) (1, 10-phen) Cl₂]; 3- [Ni (4MCFD) (1, 10-phen) Cl₂]; 4- [Cu (4MCFD) (1, 10-phen) Cl₂]; 5- [Cd (4MCFD) (1, 10-phen) Cl₂]; 6- [Hg (4MCFD) (1, 10-phen) Cl₂].

Acknowledgements

We would like to thank College of Science, Department of Pathological Analysis, Clinical sites, and the patients for their participation.

References

- Abdullah, E.S., Al-lami, M.R., Al-Khafaji, Y. and Al-Mamory, N., (2020). Preparation, Characterization and Biological Activity of new Transitions Metals Complexes with Benzoic Acid AZO-Derivative. *Test Engineering and Management*, 83(5-6), pp12899-12904.
- Chin, L.F., Kong, S.M., Seng, H.L., Khoo, K.S., Vikneswaran, R., Teoh, S.G., Ahmad, M., Khoo, S.B.A., Maah, M.J. and Ng, C.H., (2011). Synthesis, characterization and biological properties of cobalt (II) complexes of 1, 10-phenanthroline and maltol. *Journal of Inorganic Biochemistry*, 105(3), pp.339-347.
- Dahi, M.A. and Jarad, A.J., (2020). Synthesis, characterization and biological evaluation of thiazolyl azo ligand complexes with some metal ions. In *Journal of Physics: Conference Series* (Vol. 1664, No. 1, p. 012090). IOP Publishing.
- Darawsheh, M., Ali, H.A., Abuhijleh, A.L., Rappocciolo, E., Akkawi, M., Jaber, S., Maloul, S. and Hussein, Y., (2014). New mixed ligand zinc (II) complexes based on the antiepileptic drug sodium valproate and bioactive nitrogen-donor ligands. Synthesis, structure and biological properties. *European Journal of Medicinal Chemistry*, 82, pp.152-163.
- Di Santo, R., Tafi, A., Costi, R., Botta, M., Artico, M., Corelli, F., Forte, M., Caporuscio, F., Angiolella, L. and Palamara, A.T., (2005). Antifungal agents. 11. N-substituted derivatives of 1-[(aryl)(4-aryl-1 H-pyrrol-3-yl) methyl]-1 H-imidazole: synthesis, anti-candida activity, and QSAR studies. *Journal of Medicinal Chemistry*, 48(16), pp.5140-5153.
- El-Sonbati, A.Z., El-Mogazy, M.A., Nozha, S.G., Diab, M.A., Abou-Dobara, M.I., Eldesoky, A.M. and Morgan, S.M., (2022). Mixed ligand transition metal (II) complexes: Characterization, spectral, electrochemical studies, molecular docking and bacteriological application. *Journal of Molecular Structure*, 1248, p.131498.
- Eni, D.B., Yufanyi, D.M., Nono, J.H., Tabong, C.D. and Agwara, M.O., (2020). Synthesis, characterization and thermal properties of 1, 10-phenanthroline mixed-ligand complexes of cobalt (II) and copper (II): metal-mediated transformations of the dicyanamide ion. *Chemical Papers*, 74, pp.3003-3016.
- Ismael, M., Abdel-Mawgoud, A.M.M., Rabia, M.K. and Abdou, A., (2020). Design and synthesis of three Fe (III) mixed-ligand complexes: Exploration of their biological and phenoxazinone synthase-like activities. *Inorganica Chimica Acta*, 505, p.119443.
- Jawad, S.H. and Al-Adilee, K.J., (2023). Synthesis, spectroscopic characterization and biological activities as an anticancer and antioxidant of the Pd (II) and Pt (IV) complexes with a new azo dye ligand derived from 5-methyl imidazole. *Journal of Molecular Structure*, 1277, p.134846.
- Li, M., Zeng, M., Zhang, H., Chen, H. and Guan, L., (2023). Biological activity predictions of ligands based on hybrid molecular fingerprinting and ensemble learning. *ACS omega*, 8(6), pp.5561-5570.
- Mahdy, A.R., Ali, O.A.A., Serag, W.M., Fayad, E., Elshaarawy, R.F. and Gad, E.M., (2022). Synthesis, characterization, and biological activity of Co (II) and Zn (II) complexes of imidazoles-based azo-functionalized Schiff bases. *Journal of Molecular Structure*, 1259, p.132726.
- Majeed, S.R., Muhammed, N.T. and Al-Rawi, A.S., (2020). Study Spectroscopic and Characterization of Mixed Ligand Complexes of Ni (II) with New Azo-Schiff-Base Ligand and Its Activity as Antibacterial. *Journal of University of Shanghai for Science and Technology*. 22(10), 1894-1910.
- Maliyappa, M.R. and Keshavayya, J., (2022). Cu (II), Co (II), Ni (II), Zn (II) and Cd (II) complexes of novel azo ligand 6-hydroxy-4 methyl-2 oxo-5-[(4, 5, 6, 7-tetrahydro-1, 3-benzothiazol-2-yl) diazenyl]-1, 2-dihydropyridine 3-carbonitrile as potential biological agents: synthesis and spectroscopic characterization. *Chemical Papers*, 76(6), pp.3485-3498.
- Nesa, S., Hossain, S., Nasira, S., Uddin, N., Ashrafuzzaman, M., Habib, A., Rashid, A. and Haque, M., (2020). Mixed ligand complexes: Synthesis, characterization and antibacterial activity investigation. *International Journal of Chemical Studies*, 8, pp.306-312.
- Nickless, G., Pollard, F.H. and Samuelson, T.J., (1967). Thiazolylazo dyestuffs as coordinating ligands: Part I. the stability constants for 4-(2-thiazolylazo)-resorcinol and related compounds. *Analytica Chimica Acta*, 39, pp.37-46.
- Obaid, S.M., Jarad, A.J. and Al-Hamdani, A.A.S., (2020). Synthesis, characterization and biological activity of mixed ligand metal salts complexes with various

- ligands. In *Journal of Physics: Conference Series* (Vol. 1660, No. 1, p. 012028). IOP Publishing.
- Slassi, S., Fix-Tailler, A., Larcher, G., Amine, A. and El-Ghayoury, A., (2019). Imidazole and Azo-Based Schiff Bases Ligands as Highly Active Antifungal and Antioxidant Components. *Heteroatom Chemistry*, 2019(1), p.6862170.
- Smolková, R., Smolko, L., Poupon, M. and Samol'ová, E., (2023). Pentacoordinate Mixed-Ligand Zn (II) Complexes with Flufenamato and Niflumato Ligands: Crystal Structure, Spectral Characterization and Biological Properties. *Crystals*, 13(10), p.1460.
- Talib, A.A., Kadhium, A.J. and Mohammed, M.S., (2023). Preparation, Characterization and Biological Study of Complexes of Co (II) and Cu (II) with Mixed ligands of Azo naphthol compounds. *Journal of Chemical Health Risks*, 13(4), 133-141.
- Türkel, N. (2012). Study of Metal-1,10-Phenanthroline Complex Equilibria by Potentiometric Measurements. *ISRN Analytical Chemistry*. (1), 1– 5.
- Yousif, E., (2019). New Mixed Ligand Complexes; Synthesis, Spectral Analysis and Biological Activity, *Journal of Global Pharma Technology*. 11(02):196-203.
- Wang, C.L., Du, J., Yang, H. and Zhan, S.Z., (2022). A Water-Soluble Cobalt (II) Complex with 1, 10-Phenanthroline, a Catalyst for Visible-Light-Driven Reduction of CO₂ to CO with High Selectivity. *Catalysis Letters*, pp.1-8.
- Ward, H.A., Musa, T.M. and Nasif, Z.N., (2022). Synthesis and Characterization of some transition metals complexes with new ligand azo imidazole derivative. *Al-Mustansiriyah Journal of Science*, 33(2), pp.31-38.
- Witwit, I.N., Mubark, H. M., V, Zahraa Y., and Al Rufaie, M. M., (2023). Synthesis, bacterial inhibition, and coordination behavior study of new azoimidazole ligand with some of first series transition ions. *ACTA CHEMICA IASI*.31(2), 129-146.