

Synthesis, Spectral and Biological Studies of Co (III), Ni (II), and Cu (II) Complexes with New Heterocyclic Ligand Derived from Azo dye

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ABSTRACT

Imidazole is an important type of aromatic compounds heterogeneous ring, as it enters in many areas, including chemical and biological, due to the high therapeutic property of imidazole and the wide range of its clinical drugs. As the researchers proceeded to prepare a large number of chemical treatments, including Antifungal, Antioxidant[1], anti-inflammatory[2], antimicrobial[3], anticancer[4] and anti-tumor agents[5]. The imidazole ring is present in many natural products and in the human body, among which are vitamin B7 or biotin[6], and there is an imidazole ring in medicines to treat certain diseases such as cimetidine [7] which is used. In the treatment of stomach and duodenal wounds and the drug Metronidazole, or as it is known commercially as Flagyl[8, 9]. In this study, we report the preparation, identification of new ligand 2-[2-(5-chloro carboxy phenyl) azo] 5-methyl imidazole (5-MeCPAI) and its complexes Co (III), Ni (II) and Cu (II) ions. The synthesis compounds were studied by various spectral analysis and screened for their biological activities against *Klebsilla pneumonia*, *streptococcus* as antibacterial and *Aspergillus Niger*. as antifungal. Moreover, the study of Cu (II) complex of prescription drug anticancer by using the lines of breast cancer cells and compared with line of the normal cells by MTT assay.

Keywords: Azo imidazole, Mass spectra, XRD diffraction studies, antibacterial, antifungal and anticancer.

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INTRODUCTION

Imidazole is an important type of aromatic compounds heterogeneous ring, as it enters in many areas, including chemical and biological, due to the high therapeutic property of imidazole and the wide range of its clinical drugs. As the researchers proceeded to prepare a large number of chemical treatments, including Antifungal, Antioxidant[1], anti-inflammatory[2], antimicrobial[3], anticancer[4] and anti-tumor agents[5]. The imidazole ring is present in many natural products and in the human body, among which are vitamin B7 or biotin[6], and there is an imidazole ring in medicines to treat certain diseases such as cimetidine [7] which is used. In the treatment of stomach and duodenal wounds and the drug Metronidazole, or as it is known commercially as Flagyl[8, 9]. In this study, we report the preparation, identification of new ligand 2-[2-(5-chloro carboxy phenyl) azo] 5-methyl imidazole (5-MeCPAI) and its complexes Co (III), Ni (II) and Cu (II) ions. The synthesis compounds were studied by various spectral analysis and screened for their biological activities against *Klebsilla pneumonia*, *streptococcus* as antibacterial and *Aspergillus Niger*. as antifungal. Moreover, the study of Cu (II) complex of prescription drug anticancer by using the lines of breast cancer cells and compared with line of the normal cells by MTT assay.

Experimental Part

Chemicals

The following chemicals were commercially available and were used without further purification: 2-amino-4-chloro benzoic acid (C₇H₆NO₂Cl) from Bidepharmatech, 5-methyl imidazole (C₄H₆N₂) Cheng Du Micxy Chemical, sodium nitrate (NaNO₂), Ethanol Absolute (CH₃CH₂OH) from Scharlau, CoCl₂.6H₂O, NiCl₂.6H₂O,

CuCl₂.2H₂O, Hydrochloric acid (HCl), Sodium hydroxide (NaOH) from B.D.H and Fluka. All the reagents used were analytical grade pure with no further purification, and all the solutions were prepared with deionized water.

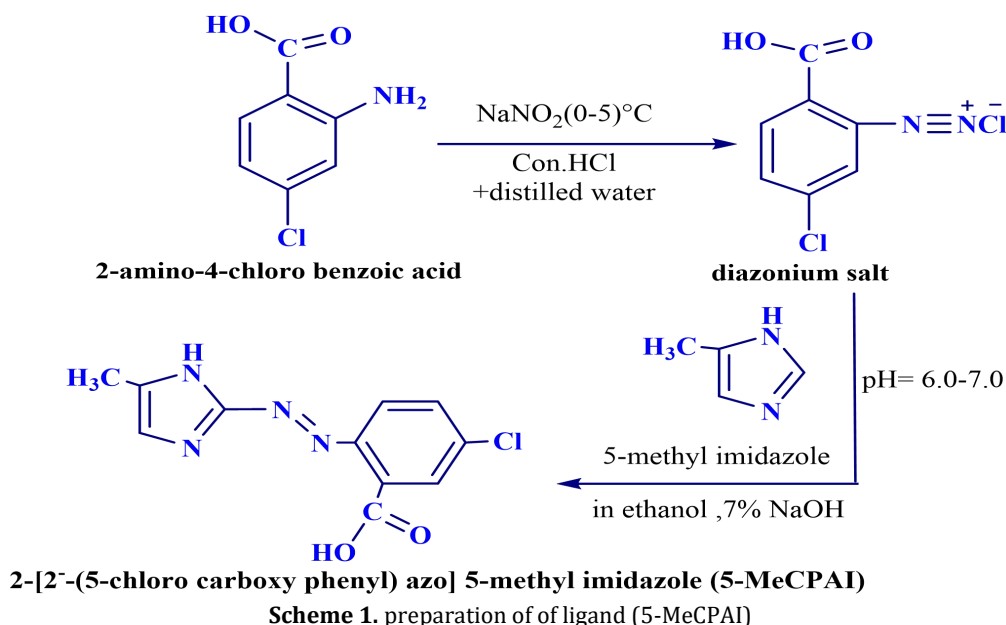
Instruments

UV-vis spectrophotometer double beam PC 1650 SHIMADZU, while FTIR Spectra in the range of 400-4000 cm⁻¹ were recorded using FTIR 8400S Shimadzu Spectrophotometer (Japan), Molar conductivity performed on 720(WTW) Mass spectra was taken by using direct probe electron impact agilent 5973 mass spectrometer, The element analysis was measured by Costech ECS Elemental 4010, while magnetic measurements of complexes carried out by Balance Magnetic Susceptibility Model -M.S.B Auto, ¹H-NMR were acquired with BRUKER-400 spectrometer in DMSO-d₆, The morphology of prepared materials was noted by field emission scanning electron microscopy (FESEM) with (MIRA3 TESCAN - Czech).

Synthesis of ligand 2-[2-(5-chloro carboxy phenyl) azo] 5-methyl imidazole (5-MeCPAI)

The azo reagent 5-MeCPAI Scheme (1) was Synthesis by dissolving 2-amino-4-chloro benzoic acid (1.7 gm) in (40 ml) Ethanol and in a mixture consisting of (4 ml) of HCl and (16 ml) distilled water, and it was cooled to (3) C°. Added this mixture to solution of (0.75gm) of NaNO₂ in (35 ml) of distilled water was added dropwise at 0-5 C° and the mixture was stirred for 30 min. This diazonium chloride solution was added dropwise in 250 ml beaker containing (0.9 gm) of 5-methyl-imidazole with stirring dissolved in a mixture of (30) cm³ of Ethanol and (20 ml) of NaOH 7% and cooled to 0-5 C°. The structure was verified by ¹H-NMR, mass spectrum, Infrared spectra and UV-Vis spectrometry.

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General procedure for synthesis of complexes

The complexes were prepared by adding (0.302 g) from ligand (5-MeCPAI) dissolved in hot ethanol (40 ml) and added dropwise with stirring stoichiometric amount of (1:2) for Co (III), Ni (II), Cu (II) chloride salt dissolved in 30 ml hot buffer solution (ammonium acetate) at pH = 7.

The mixture was heated to 50°C for (60 min), then left over night. The solid product thus formed was filtered off, washed with ethanol and dried in a desiccator over anhydrous CaCl_2 [10]. Elemental analysis is in agreement with formula of the ligand (5-MeCPAI) and its complexes given in (Table 1).

Table 1. Elemental analysis and physical properties of the ligand (5-MeCPAI) and its complexes.

compound	color	m.p °C	Yield %	Molecular Formula (Mol.wt)	(calc.) Found %			
					C	H	N	M
Ligand (5-MeCPAI)	Dark red	180	76	$\text{C}_{11}\text{H}_9\text{ClN}_4\text{O}_2$ (264.66)	(49.92) 49.11	(3.43) 3.01	(221.17) 0.99	-----
Co (III)- Complex	purple	193	68	$\text{C}_{22}\text{H}_{16}\text{Cl}_3\text{CoN}_8\text{O}_4$ (621.71)	(42.50) 42.13	(2.59) 2.39	(18.02) 17.98	(9.48) 9.32
Ni (II)- Complex	Greenish Olive	197	61	$\text{C}_{22}\text{H}_{16}\text{Cl}_2\text{NiN}_8\text{O}_4$ (586.01)	(45.09) 44.97	(2.75) 2.65	(19.02) 18.95	(10.01) 9.93
Cu (II)- Complex	greenish dark	208	83	$\text{C}_{22}\text{H}_{16}\text{Cl}_2\text{CuN}_8\text{O}_4$ (590.87)	(44.72) 44.27	(2.73) 2.78	(18.96) 18.54	(10.74) 10.61

RESULTS AND DISCUSSION

The infrared spectrum data of ligand (5-MeCPAI) showed band at 1717 cm^{-1} for (C=O), 3047 cm^{-1} for (Ar-H), 3271 cm^{-1} for (OH), 3107 cm^{-1} for (N-H) imidazole, 1678 cm^{-1} for (C=N) inside imidazole ring, 2977 cm^{-1} for (C-H) for (CH_3), 1476 cm^{-1} for (N=N) and 1587 cm^{-1} due to aromatic (C=C). FT-IR spectra have proved to be the most appropriate technique to give sufficient information to elucidate the nature of the bonding between bonding and complexes. The numerous shifts in the position or change in the shape of the bands of the complexes compared to the absorption bands of free bonding due to the formation of the bands of the mineral complexes as in Table (2) and Fig.1.FT-IR spectra for all complexes, there are new bands observed that were never observed in the ligand spectrum (5-MeCPAI), and this may be attributed to $\nu(\text{M}-\text{O})$ and $\nu(\text{M}-\text{N})$. Thus, the infrared spectroscopy data lead to an indication that (5-MeCPAI) bonding behaves as a three-dimensional chelating agent coordinated with metal ions.[11-13].

$^1\text{H-NMR}$ (DMSO-d_6) spectrum Fig.2.data of ligand(5-MeCPAI) show: 13.303 (s, 1H, OH), 9.134 (s, 1H, NH), 7.568– 7.982 (d, 3H, Ar-H), 3.437 (s, 3H, CH_3), 2.523-2.535 (DMSO). The $^1\text{H-NMR}$ (DMSO-d_6) spectrum Fig.3.data of Cu (II) complex show: 7.924-7.952 (d, 2H, NH), 7.495 – 7.522 (s, 6H, Ar-H), 3.764 - 4.510 (s, 6H, CH_3), 2.504 (DMSO) [14-17] . These compounds have been listed in Table (3).

The mass spectrum of the ligand (5-MeCPAI) (Scheme 2, Fig.4.) assigned to molecular peak ion $[\text{M}]^+$ at $m/z^+ = 264.6$. The mass spectrum of azo imidazole ligand showed several peaks attributed to the molecular ions at (m/z^+) 250 , 216, 173, 156 ,139, 111, 98, 85,76,66,50 and 44 were due to various fragments ions $[\text{C}_{10}\text{H}_7\text{ClN}_4\text{O}_2]^+$, $[\text{C}_{10}\text{H}_8\text{N}_4\text{O}_2]^+$, $[\text{C}_9\text{H}_7\text{N}_4]^+$, $[\text{C}_7\text{H}_5\text{ClO}_2]^+$, $[\text{C}_6\text{H}_4\text{ClN}_2]^+$, $[\text{C}_6\text{H}_4\text{Cl}]^+$, $[\text{C}_3\text{H}_6\text{ClN}_4]^+$, $[\text{C}_3\text{H}_8\text{N}_3]^+$, $[\text{C}_6\text{H}_4]^+$, $[\text{C}_5\text{H}_6]^+$, $[\text{C}_4\text{H}_2]^+$, and $[\text{C}_3\text{H}_8]^+$ respectively[18, 19].

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Table 2. The FT-IR (in cm^{-1}) data of ligand (5-MeCPAI) and its complexes.

Group	(5-MeCPAI)	Co (III)- Complex	Ni (II)- Complex	Cu (II)- Complex
ν - (OH)	3271 m.	----	----	----
ν - (NH)	3107m.	3093w.	3093w.	3093w.
ν - (C-H)	3047 m.	3012 w.	3010 w.	3042 w.
ν - (CH ₃)	2977 w.	2968w.	2967 w.	2974 w.
ν - (C=O)	1717m.	1702 m.	1700 m.	1700 m.
ν - (C=N)	1678 s.	1550 s.	1550 s.	1547 s.
ν (COO-) asym.	----	1609 s.	1596 s.	1617 s.
ν (COO-) sym.	----	1319 m.	1309 m.	1311 m.
ν - (N=N)	1476 m.	1404 s.	1417 s.	1404 s.
ν - (C=C)	1587 m.	1488 w.	1488 w.	1450 w.
ν - (M - O)	----	540 w.	540 w.	570 w.
ν - (M - N)	----	462 w.	470 w.	472 w.

S = strong, m= medium, w = weak

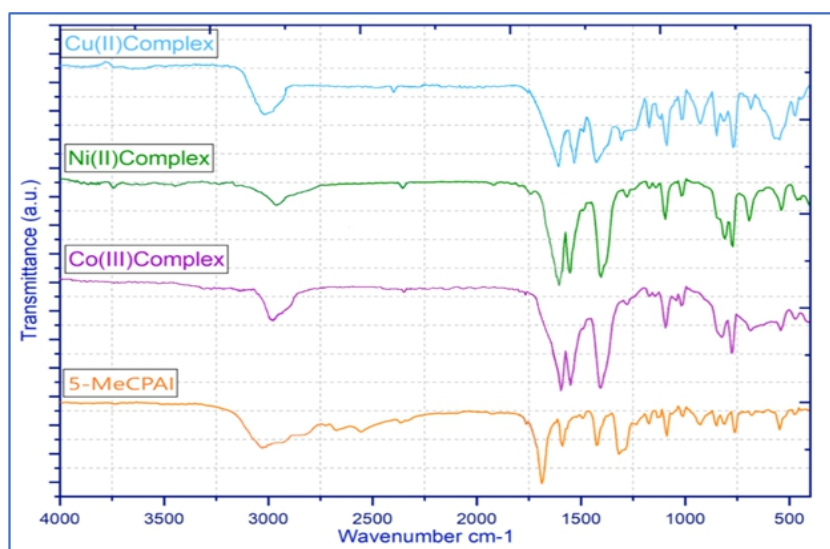


Fig.1. FT-IR Spectrum of ligand (5-MeCPAI) and its metal complexes

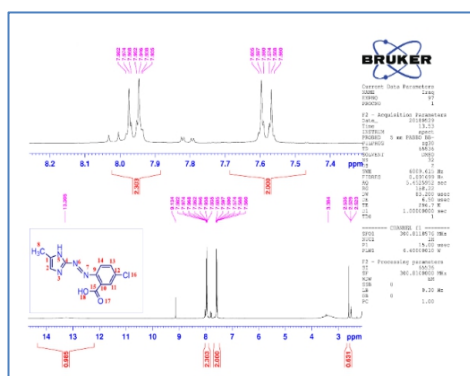


Fig 2. ^1H -NMR of ligand (5-MeCPAI).

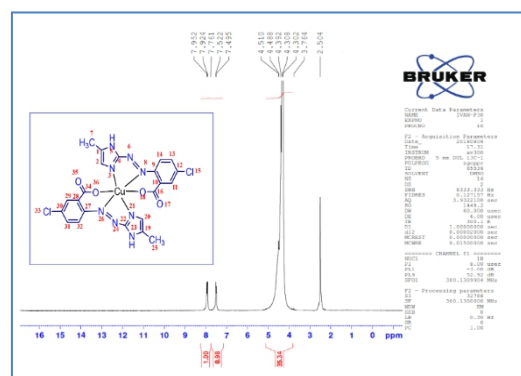


Fig.3. ^1H -NMR of Cu (II)-Complex.

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Table 3. The ^1H NMR spectra of ligand (5-MeCPAI) and Cu (II) complex

(5-MeCPAI) δ , ppm (H atoms, peak, assignment)	J-J coupling	Cu (II) complex δ , ppm (H atoms, peak, assignment)	J-J coupling
2.523-2.535 (DMSO- d_6)	0.631	2.504 (DMSO- d_6)	-----
3.437 (3H, S, 8)	-----	3.764-4.510 (6H, S, 2, 25)	35.34
7.568-7.982 (3H, d, 13, 11, 14)	2.000	7.495-7.522 (6H, S, 13, 31, 11, 29, 14, 32)	0.98
9.134 (1H, S, 5)	2.303	7.924-7.952 (2H, d, 5, 23)	1.00
13.305 (1H, S, 18)	0.985		

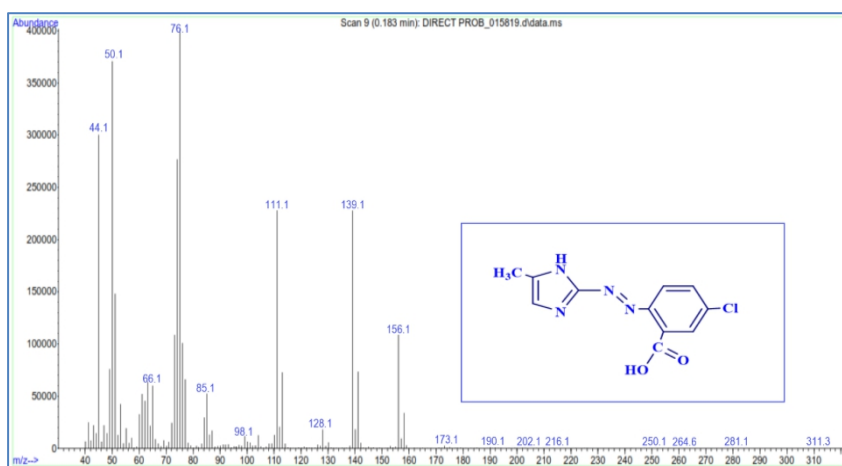
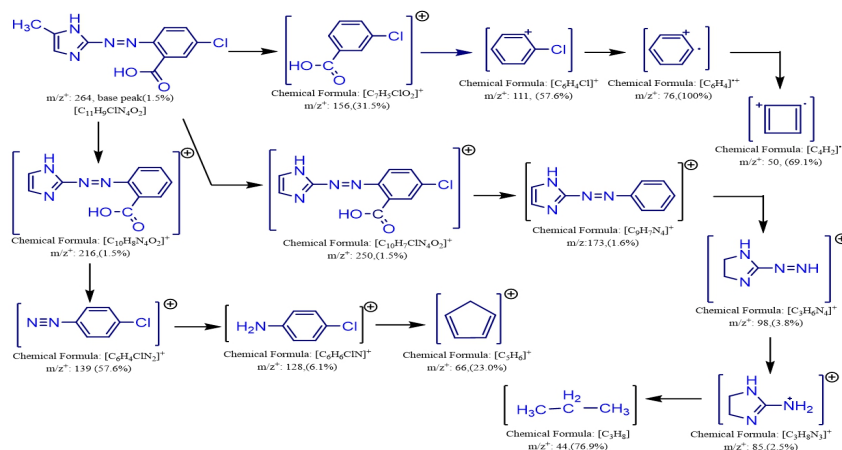


Fig 4. Mass spectrum of azo imidazole ligand (5-MeCPAI)



Scheme 2. Mass spectrum fragmentation of azo imidazole ligand (5-MeCPAI)

Electronic spectra studies

The UV-Vis spectra of ligand (1-MeCPAI) shows three bands in UV- visible region the first band at 469 nm (21321cm^{-1}) attributed to a $n \rightarrow \pi^*$ transition of the azo ($-\text{N}=\text{N}-$) group, this band showed a red shift coordinated between the metal ion and the nitrogen atom of azo group. The second band located at 331 nm (30211cm^{-1}) corresponds to the $\pi \rightarrow \pi^*$ transition of the $(\text{C}=\text{N})$ group of imidazole. While the third band observed at 245 nm (40816cm^{-1}) is assigned to the $\pi \rightarrow \pi^*$ transition of the $(\text{C}-\text{C})$ of imidazole and phenyl rings. Electronic spectrum of the Co (III)- complex displays bands at 297nm (33670cm^{-1}) 474 nm (21097cm^{-1}) and 959 nm (10427cm^{-1}). may be assigned to Center Ligand , $^1\text{A}_{1g} \rightarrow ^1\text{T}_{2g(\text{F})}$ and

$^1\text{A}_{1g} \rightarrow ^1\text{T}_{1g(\text{F})}$ transitions respectively in an octahedral. The electronic spectrum of Nickle (II) complex exhibits three absorption bands at about 528nm (18939cm^{-1}), 309nm (32362cm^{-1}) and 241 nm (41493cm^{-1}) which may be attributed to $^3\text{A}_{2g} \rightarrow ^3\text{T}_{2g(\text{F})}$, $^3\text{A}_{2g} \rightarrow ^3\text{T}_{1g(\text{F})}$ and $^3\text{A}_{2g} \rightarrow ^3\text{T}_{1g(\text{p})}$, the magnetic moment of Nickle (II) complex was found at 2.81 B.M which was very close to the octahedral environment. The Copper (II) complex displays a broad asymmetric band around at 480 nm (20833cm^{-1}). The broadness band indicates the three transitions $^2\text{B}_{1g} \rightarrow ^2\text{A}_{1g}$ ($\text{dx}^2\text{-y}^2 \rightarrow \text{dz}^2$) (ν_1), $2\text{B}_{1g} \rightarrow ^2\text{B}_{2g}$ ($\text{dx}^2\text{-y}^2 \rightarrow \text{dyz}$) (ν_2), and $^2\text{B}_{1g} \rightarrow ^2\text{Eg}(\nu_3)$, (charge transfer), which are of similar energy and gives rise to only one broad absorption band ($^2\text{B}_{1g} \rightarrow ^2\text{Eg}$), the magnetic moment of Copper (II) complex

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was found at 1.75 B.M and the structure of this complex distorted octahedral geometry (Z- in or Z- out) according to Jahn – teller effect. The electronic spectra of azo imidazole ligand(5-MeCPAI) and complexes were studied

in absolute ethanol (10^{-3} M) as a solvent at room temperature. The characteristics of these bands are summarized in Table (4)[20-24]

Table 4. Electronic spectra, magnetic moments, geometry, Conductivity and hybridization

Compounds	λ_{max} (nm)	Absorption Bands (cm ⁻¹)	Transitions	μ_{eff} (B.M)	Geometry	Λ_m S.mol ⁻¹ .cm ²	Hybridization
5-MeCPAI	245	40816	$\pi \rightarrow \pi^*$	-----	-----	-----	-----
	331	30211	$\pi \rightarrow \pi^*$				
	469	21321	$n \rightarrow \pi^*$				
Co (III)- Complex	297	33670	Center Ligand	0.08	Octahedral regular	77	d^2sp^3 (Low spin)
	474	21097	$^1A_1g \rightarrow ^1T_{2g(F)}$				
	959	10427	$^1A_1g \rightarrow ^1T_{1g(F)}$				
Ni (II)- Complex	241	41493	$^3A_{2g} \rightarrow ^3T_{1g(P)}$	2.81	Octahedral regular	73	Sp^3d^2 (high spin)
	309	32362	$^3A_{2g} \rightarrow ^3T_{1g(F)}$				
	528	18939	$^3A_{2g} \rightarrow ^3T_{1g(F)}$				
Cu (II)- Complex	571	17513	$^2B_{1g} \rightarrow ^2E_g$	1.75	distorted (Z-in or Z-out)	81	Sp^3d^2
B.M= Bohr magneton							

XRD Analysis

XRD diffraction patterns of the ligand (5-MeCPAI) and complexes were recorded in the range $2\theta = (5-80)^\circ$. The ligand has sharp diffraction peaks indicating crystalline nature. In order to determine some of the structural properties such as microstrains of crystalline size, crystalline structure, and dislocation density to record their purity and defects in crystalline structure while converting the ligand to metallic complex. The crystallite size is calculated by the Scherrer equation:

$D = \frac{k\lambda}{\beta \cos \theta}$

For calculating the dislocation density, the following equation was also used:

$$\delta = 1/D^2$$

Where D = crystallite average size, β = line broadening at half the maximum intensity in radians, k is Blanks constant (0.891), θ = Bragg angle, λ = X-ray wavelength and δ = Dislocation density[25-28]. The (XRD) patterns of ligand is illustrated in Fig.5. and the crystallographic data for ligand and complexes are summarized in Table (5).

Table 5. Crystal data of ligand (5-MeCPAI) and metal complexes

Compound	No.	2θ observed	d-spacing (Å°)	(I/I ₀) %	FWHM	Crystallite Size. (nm)	Lattice Strain	δ_{DX10}^{15} (lin m ⁻²)
5-MeCPAI	1	27.4306	3.24887	100	0.48750	17.53	0.0087	0.325
	2	24.5834	3.61832	66	0.49670	17.1	0.0099	0.341
	3	18.3742	4.82466	63	0.42670	19.71	0.0115	0.257
Co (III) -complex	1	18.3511	4.83068	100	0.33140	25.36	0.0090	1.554
	2	27.4691	3.24441	59	0.34450	24.81	0.0062	1.624
	3	24.5729	3.61984	34	0.33800	25.14	0.0068	1.582
Ni (II)- complex	1	18.4018	4.81749	100	0.33530	25.08	0.0090	1.589
	2	27.5692	3.23285	66	0.32500	26.3	0.0058	1.445
	3	24.6777	3.60471	38	0.34800	24.41	0.0069	1.678
Cu (II)-complex	1	18.4114	4.81500	100	0.26120	32.2	0.0070	0.964
	2	27.5174	3.23882	61	0.28940	29.53	0.0052	1.146
	3	24.5671	3.62068	37	0.27840	30.52	0.0056	1.073

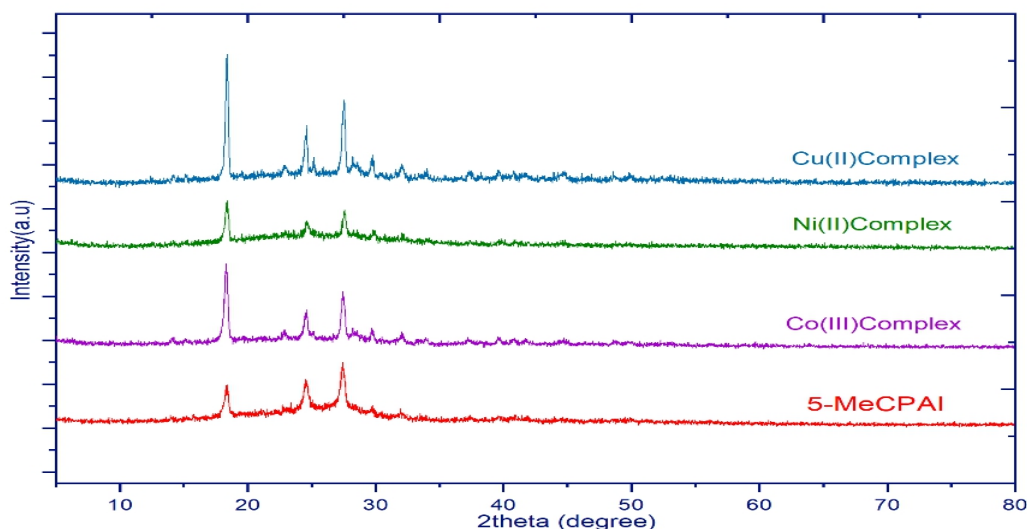


Fig 5. XRD patterns of ligand (5-MeCPAI) with some complexes.

FESEM characterizing of Ligands and complexes

The FESEM images of ligand (5-MeCPAI) and complexes of Co (III), Ni (II) and Cu (II) are shown in Fig 6. 5-MeCPAI was heterogeneous crystalline stones, however, after coordination, the Co (III) compound changes into lumpy

heterogeneous sheets with a smooth surface. Likewise, the FESEM images show that Ni (III) and Cu (II) were formed in a very uniform way in the form of cubes and few appeared spherical[29, 30].

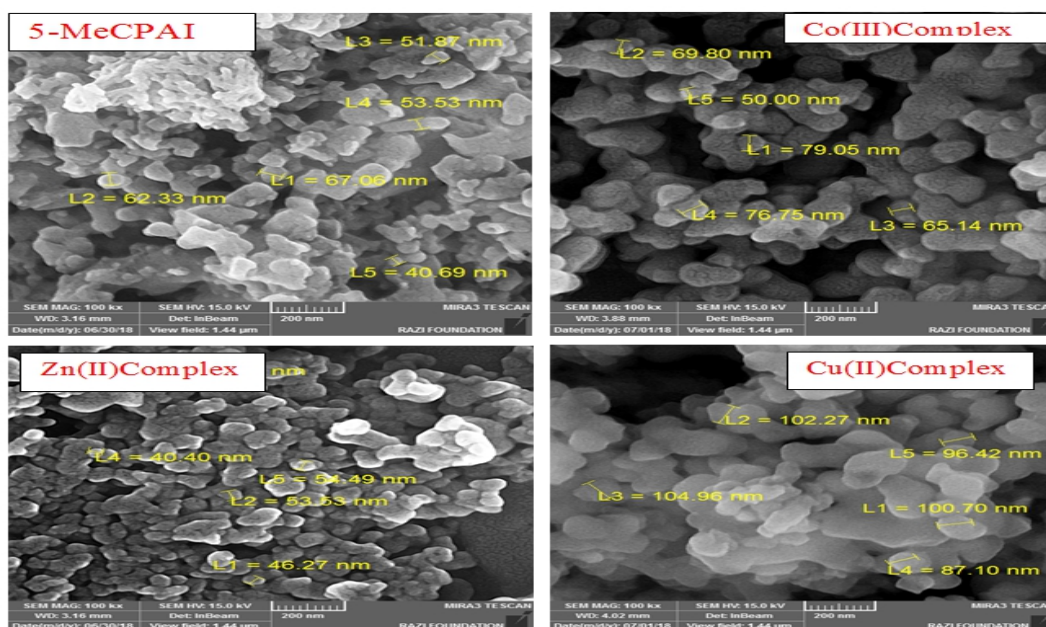


Fig 6. FESEM images of ligand (5-MeCPAI) with some complexes.

Biological Studies

The antibacterial activity of azo imidazole ligand (5-MeCPAI) and complexes were screened against two types of bacteria, *Staphylococcus* (Gram- positive Bacteria) and *pneumonia Klebsilla* (Gram Negative Bacteria) and it also evaluation of the antifungal activity by *Aspergillus*

Niger[31, 32]. These types were used to determine inhibiting effect on the growth of these organisms. The data of antimicrobial activates of the newly synthesized compounds are given in Table (6) and summarized in the Fig.7.

Table 6. Antimicrobial activity data of synthesis compounds

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Compound	Anti- bacterial Activity		Anti- fungal Activity
	<i>Staphylococcus</i>	<i>pneumonia Klebsilla</i>	<i>Aspergillus Niger</i>
Ligand =(5-MeCPAI)	++	+++	+
Co (III) -complex	++	-	-
Ni (II)- complex	++	-	-
Cu (II)-complex	+++	+++	++

(+++): high active—inhibition zone > 12 mm, (++): moderate active—inhibition zone = 9-12 mm, (+): slightly active—inhibition zone = 6-9 mm, (-): inactive

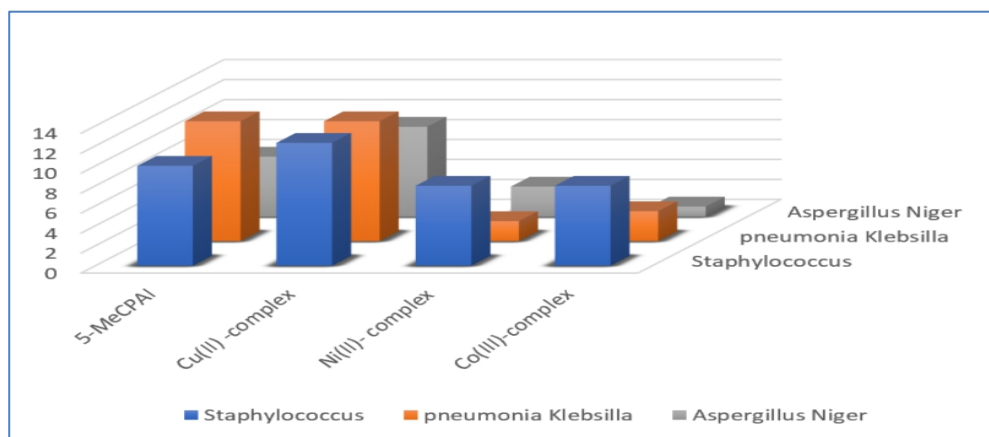


Fig 7. Statistical representation for biological activity of ligand (5-MeCPAI) with prepared complexes.

Cytotoxic activity

One important topic of the current study is assessing anticancer activities of Cu complex against breast cancer cell lines MCF-7 and natural cell. The test (MTT) was used to examine the viability of cells[33, 34]. The ligand was observed that the best rate of inhibition of breast cancer (MCF-7) was found (48.69%) at 400 µg/mL while the rate of normal cell (WRL-68) - inhibition was observed with the same concentration of 73.03%. The table (7) are

illustrated effect the Cu (II) complex on breast cancer cell lines MCF-7 and compared with the normal cell line (WRL-68) of the same concentration using a 24-hour MTT test at 37 ° C. The Cu (II) complex in Fig.8. also showed selective cytotoxicity against the cancer cell line with IC₅₀ = 116.5 µg/mL: WRL-68 IC₅₀ = 209.2 µg/mL. The results showed that the Cu (II)-complex plays an important role in the rate of cancer cell growth inhibition and normal lines.

Table 7. Effect Cu (II) complex on MCF-7 cells and compared with the normal cell line of the same concentration using a 24-hour MTT test at 37 °

Test samples	IC ₅₀ (µg/ml)		
	Carcinoma Cell Lines		Normal Cell Line
Cu (II)-complex	116.5		209.2
	Mean Percentage (%) for each cell line		
	Conc. (µg/ml)	Carcinoma Cell Lines	Normal Cell Line
		Cell Viability	Cell Viability
	400	48.69	73.03
	200	57.95	84.45
	100	75.58	94.21
	50	88.62	95.18
	25	97.69	94.83
	12.5	96.37	95.60
	6.25	96.26	95.72

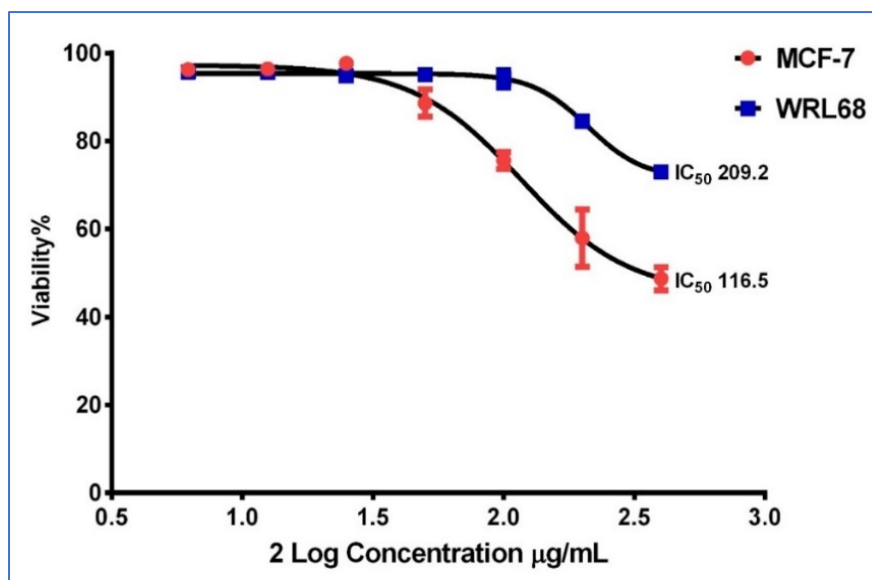


Fig 8. IC₅₀ (g/ml) values of the carcinoma cell lines and normal cell Line of Cu (II)-complex

CONCLUSION

In this paper, we reported the synthesis and spectral characterization of new azo dye ligand derived from 2-amino-4-chloro benzoic acid with 5-methyl imidazole (5-MeCPAI) and its metal complexes with Co (III), Ni (II) and Cu (II) ions. Further, a series of metal complexes comprising the ligand have been prepared and characterized by FT-IR, ¹H- NMR, mass and UV-Visb spectral studies. One the basis of their analytical and spectral data, the geometry proposed for all metal complexes is octahedral structure Fig.9. The ligand and

prepared complexes had different morphologies as appeared in XRD and FESEM studies. Additionally, the complexes are non-ion character, and some synthesis compounds have high biological activitie toward antibacterial and antifungal. The biological activity studied in cells viability and cytotoxicity assays on Cu (II)-complex by using the lines of breast cancer cell lines MCF-7 and compared with line of the nature cells. Therefore, through tests conducted to identify the possibility of using theCu(II)-complex as anticancer drugs development in future.

M= Cu (II); n=0
M= Ni (II); n=0
M= Co (III); n=1

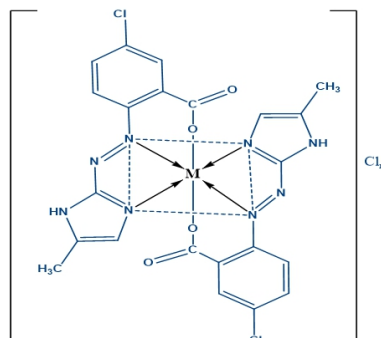
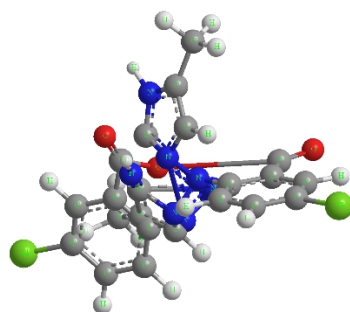


Fig 9. Proposed structural formula of metal complexes

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