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Synthesis And Anticancer Activity Evaluation Of Novel Ligand 2- [2 - (5-Chloro Carboxy Phenyl) Azo] 1-Methyl Imidazole (1-Mecpai) With Some Metal Complexes

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ABSTRACT

This work included synthesis of novel ligand 2- [2 - (5-Chloro carboxy phenyl) azo] 1-methyl imidazole (1-MeCPAI) was prepared by reacting a diazonium salt solution of 2-amino-4-chloro benzoic acid with 1-methyl imidazole. Also, preparation of a new series of chelate complexes of Nickel (II), Copper (II) and Zinc (II). Compounds have been characterized by UV-visible, FT-IR, 1H-NMR, 13C-NMR, magnetic susceptibility, Mass spectra, FE-SEM and XRD diffraction studies. We then studied biological activity in two groups of bacteria for all prepared compounds; Klebsilla pneumonia, Staphylococcus and Alternaria alternata as antifungal. The ligand (1-MeCPAI) also performed anticancer activity, Nickel complex and Copper complex by cells cytotoxicity and screen for in vitro antitumor activity against cancer of prostate line PC3. were evaluated using a colorimetric (MTT) test to determine their cellular viability. Copper complexes have especially shown selective cytotoxicity, destroying the cancer cell lines while not affecting the normal cells that are considered the essential for cytotoxic therapy. Tests have therefore carried out, which are designed to decide if this compound can be used to generate anti-cancer medicines in the future.

Keywords: synthesis, azo imidazole ,antibacterial and anticancer.

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INTRODUCTION

Recently, coordination chemistry have attracted considerable research attention because of their variety in coordination geometry, exquisite colors , and spectroscopic properties and their biochemical significance and their antitumor activity[1, 2] .It is known that the consistency of the metal ions with the ligands acts synergistically to increase their biological activities[3]. Researchers rely on this spectrum of chemical compounds in the medical field to be the most recent chemical treatments in this field .In addition, azo imidazole compounds have a lot of scientific attention because of their applications in different of applied and academic fields such as Analytical reagents[4], antioxidants[5], antifungal [6], anticancer[7]. azo imidazole derivatives along with biological activities also showed a variety of pharmacological activities such as antidepressant[8], antiviral[9] and against COVID-19 main protease (M^{pro}: 6LU7)[10]. In this study,the preparation of novel ligand 2-[2--(5-chloro carboxy phenyl) azo] 1-methyl imidazole (1-MeCPAI) and its complexes Nickel, Copper and zinc ions. The synthesis compounds were studied by various spectral analysis and screened for their biological activities. In addition, the analysis with the lines of prostate cancer cells for some prescription drug anti-cancer compound and with the MTT test lines of the regular cells.

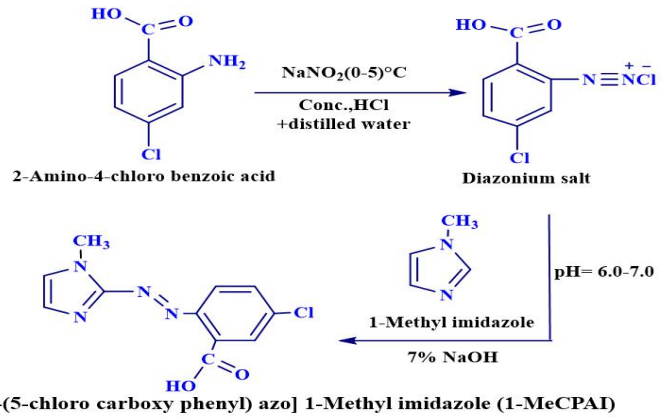
EXPERIMENTAL

Chemicals

The following chemicals were produced and used without further purification: 2-amino-4-chloro benzoic acid($C_7H_6NO_2Cl$) from Bidepharmatech, 1-methyl imidazole ($C_4H_6N_2$) Cheng Du Micxy Chemical, sodium nitrate ($NaNO_2$), Ethanol Absolute (CH_3CH_2OH) from Scharlau, $NiCl_2.6H_2O$, $CuCl_2.2H_2O$, $ZnCl_2.H_2O$, 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.

Preparation of 1-MeCPAI

The azo reagent 1-MeCPAI Scheme (1)was Synthesis by dissolving 2-amino-4-chloro benzoic acid (1.7 g) in (40ml) Ethanol and in In a mixture consisting of (4ml) of HCl and (16 ml) distilled water, and its was cooled to (3) C^o. Added this mixture to solution of (0.9 g) of $NaNO_2$ in (35) cm^3 of distilled water was added dropwise at 0-5 C^0 and the mixture was stirred for 30 min . This diazonium chloride solution was added dropwise in 250 ml beaker containing (1.5 ml) of 1-methyl-imidazole with stirring dissolved in (20 ml) of NaOH 7% and cooled to 0 -5C^0.The ligand (1-MeCPAI) 2-[2-(5-chloro carboxy phenyl) azo]1-methyl imidazole was obtained as Orange solid yield 87% , m.p (192 C^0). The structure was verified by UV-Vis., FT-IR spectra, ^{1}H-NMR, ^{13}C-NMR and mass spectrum.



Scheme (1):- preparation of of ligand (1-MeCPAI)

General procedure for synthesis of complexes

The complexes were prepared by adding (0.302 g) from ligand (1-MeCPAI) dissolved in hot ethanol (40 ml) and added dropwise with stirring stoichiometric of (1:2) for Ni(II), Cu(II) and Zn(II) chloride salt dissolved in (30 ml) hot buffer solution (ammonium acetate) at pH =6-7 . The mixture was heated to 50C° for (60 min), then left over night. Elemental analysis are in agreement with formula of the ligand (1-MeCPAI) and its complexes given in Table (1).

Table (1):- Elemental analysis and ligand physical characteristics (1-MeCPAI) and its complexes

Compound	color	M . P ° C	Yield %	Molecular Formula (M. wt)	(Calculate) Found %			
					C.	H.	N.	M.
Ligand =(1-MeCPAI)	Orange	192	87%	$C_{11}H_9ClN_4O_2$ (264.66)	(49.92) 49.11	(3.43) 3.01	(21.17) 20.99	---------
Ni(II) Complex	dark green	213	73%	$C_{22}H_{16}Cl_3CoN_8O_4$ (621.71)	(42.50) 42.13	(2.59) 2.39	(18.02) 17.98	(9.48) 9.32
Cu(II) Complex	dark brown	209	74%	$C_{22}H_{16}Cl_2N_8NiO_4$ (586.01)	(45.09) 44.97	(2.75) 2.65	(19.02) 18.95	(10.01) 9.93
Zn(II) Complex	light brown	198	67%	$C_{22}H_{16}Cl_2CuN_8O_4$ (590.87)	(44.72) 44.27	(2.73) 2.78	(18.96) 18.54	(10.74) 10.61

RESULTS AND DISCUSSION

The additional data Figure (1).and Table (2) shows FT-IR spectrums of ligand 1 MeCPAI) and their complexes. IR spectra of the ligand and it nickel, copper and zinc complexes exhibit various bands in the 4000 400 cm-1 region. The FT-IR data of ligand (1-MeCPAI) showed band at (1717) cm-1 for (C=O), 3101 cm-1 for (Ar-H), 3277 cm-1 for (OH), 1589 cm-1 for (C=N) inside imidazole ring, 2977 cm-1 for (C-H) for (CH_3), 1427 cm-1 for (N=N) and 1488 cm-1 due to aromatic (C=C)[11, 12]. 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 several variations in the position or shape of the complex bands compared to the absorption of free link bands due to the creation of the mineral complex bands. FT-IR spectra For all complexes, there are new bands observed that were never observed in the ligand spectrum (1-MeCPAI), and this may be attributed to ν (M—O) and ν (M—N) [13]. Thus, FT-IR spectroscopy data suggest that the bonding (1-MeCPAI) is a three - dimensional chelating agent [1-MeCPAI] coordinated with metal ions.

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

Group	(1-MeCPAI)	Ni (II)-Complex	Cu (II)-Complex	Zn (II)-Complex
υ - (OH)	3277 m.	----	----	----
υ - (C-H)	3101 m.	3093 w.	3055 w.	3355w.
υ - (CH3)	2977 w.	2985w.	2977 w.	2977 w.
υ - (C=O)	1717m.	1712 m.	1712m.	1714 m.
υ -(C=N)	1589 s.	1580 s.	1580 s.	1596 s.
υ (COO-) asym.	----	1688 s.	1689 s.	1689 s.
υ (COO-) sym.	----	1319 m.	1311 m.	1288 m.
υ -(N=N)	1427 m.	1427 s.	1419 s.	1419 s.
υ - (C=C)	1488 m.	1500 w.	1550 w.	1550w.

S = strong , m= medium , w = weak

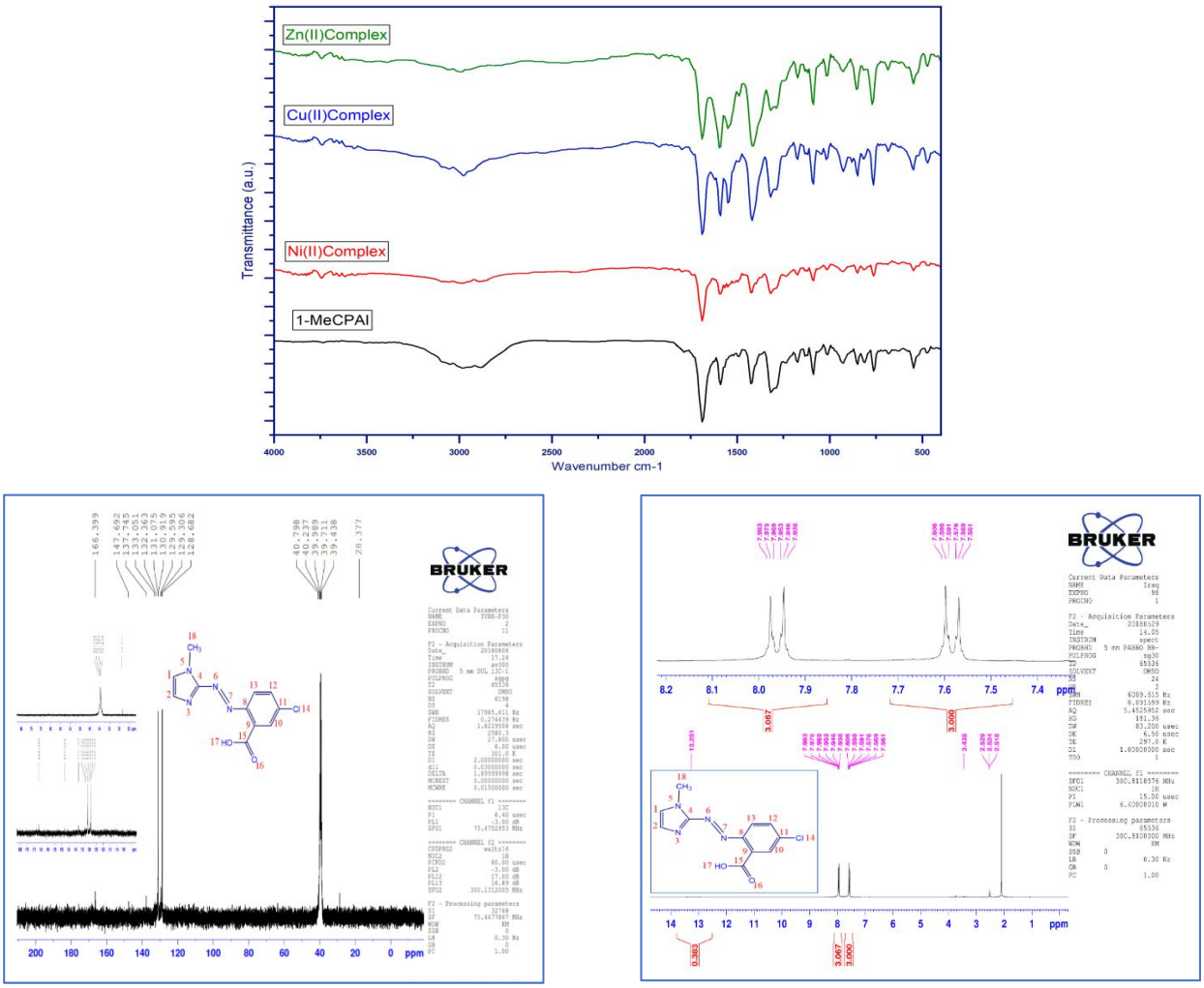


Fig.1. FT-IRspectra of ligand(1-MeCPAI) and metal complexes

^{1}H-NMR (DMSO-d_6) spectrum data of ligand(1-MeCPAI) show : 13.251 (m, 1H ,OH) , 7.606-7.561 (S, 2H, CH imidazol ring), 7.983-7.938 (d, 3H , Ar-H), 3.438 (S, 3H , CH_3), 2.529-2.518 (DMSO-d_6) [14, 15].The ^{13}C-NMR spectrum observed several chemical shift ^{13}C= (28.377, 128.682 , 129.306 , 129.595 , 130.919 , 131.075, 132.363 , 133.051, 137.745,147.692 and 166.399 ppm) to sites with carbon atoms, (6 , 7 , 18 , 17 , 14 , 5 ,10, 11 , 12, 13 , 4 , 15 , 2) respectively. The spectrum also reflected a singlet signal of solvent (DMSO-d_6) at the chemical shift ^{13}C=(39.438-40.798) ppm[16]. ^{13}C - NMR and ^{1}H - NMR spectra of the azo imidazole ligand is illustrated in Figure (2).

(a) **(b)**

Fig.2. (a) ^{1}H-NMR (b) ^{13}C-NMR of ligand(1-MeCPAI)

Mass-spectrum of the ligand (1-MeCPAI) (Figure (3) and Scheme (2)) Molecular peak ion [M^+] assigned at $\frac{m}{z+}$ = 264.1 is corresponding to the original molecular weight of ligand (264.64). There were multiple peaks attributed with molecular ions of Azo Imidazole Ligand at (m/z^+) 237 , 207 ,173 , 156 , 139, 127 ,111, 93, 83, 76,50 and 45 Various fragments of ions were attributable to $[C_{10}H_9ClN_4O]^+$, $[C_9H_7ClN_4]^+$, $[C9H9N4]^+$,$[C_7H_5ClO_2]^+$, $[C_6H_4ClN_2]^+$, $[C_6H_6ClN]^+$, $[C_6H_4Cl]^+$, $[C_6H_7N]^+$, $[C_3H_5lN_3]^+$, $[C_6H_4]^{\cdot+}$, $[C_4H_2]^{\cdot+}$ and $[C_2H_7N]^+$ respectively[17].

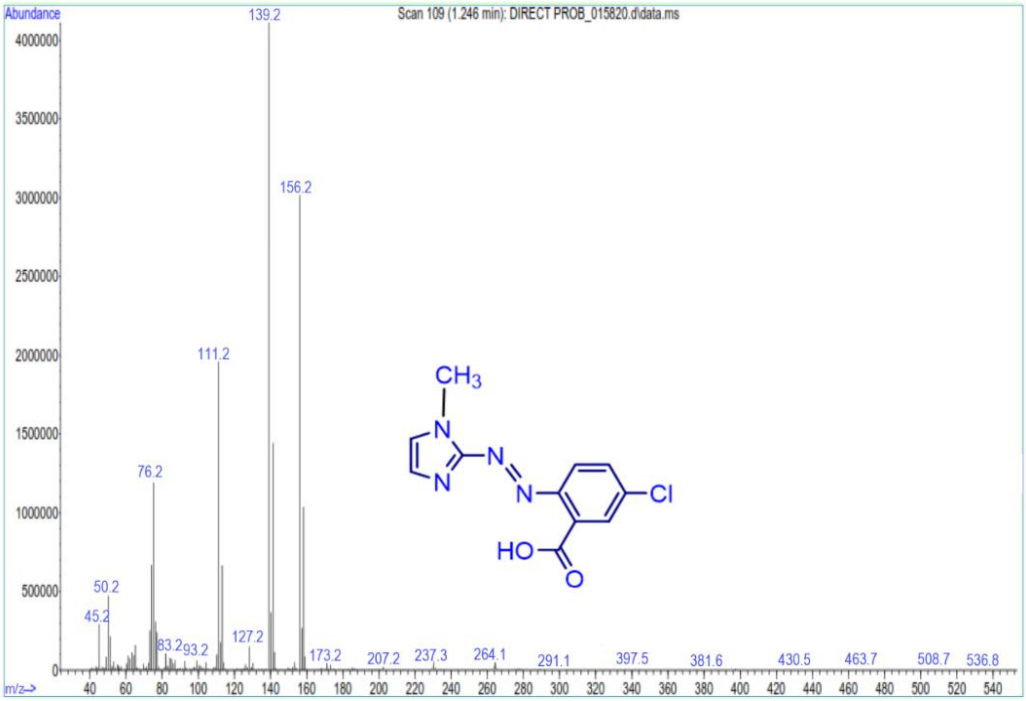
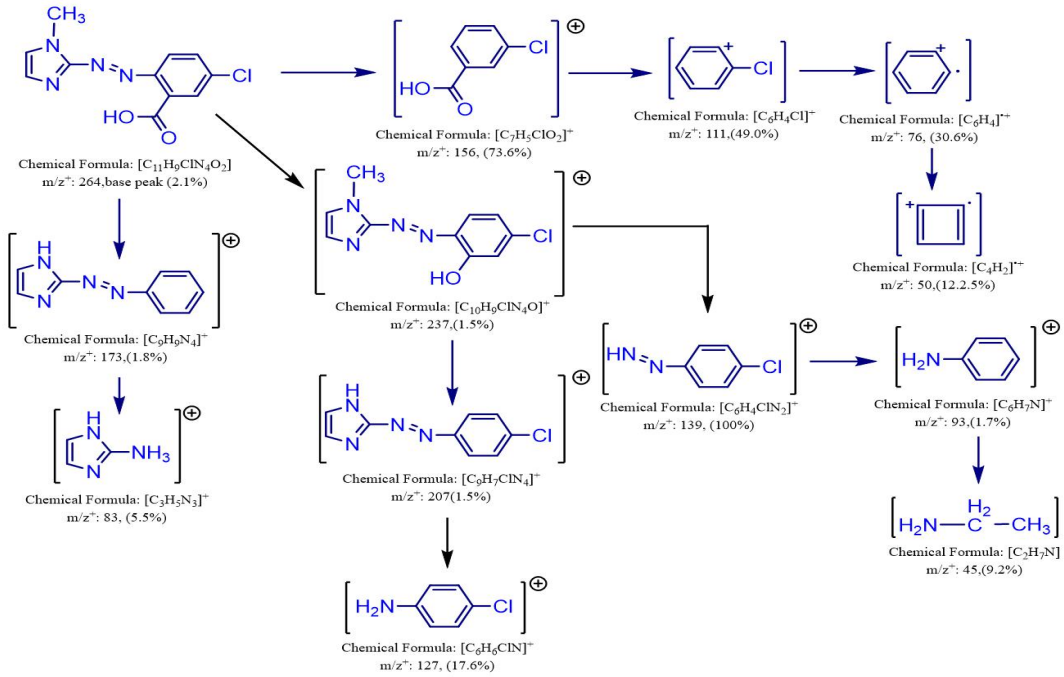


Fig.3.: Mass spectrum of (1-MeCPAI)



Scheme(2) :- Mass spectrum fragmentation of (1-MeCPAI)

The Ligand UV-Vis-spectrum (1-MeCPAI) displays three UV-visible bands at 417 nm in the first field (23980 cm^{-1}) attributed to a n → π* transition of the azo (- N = N-) group, This band revealed a coordinated red change between the azo group's metal ion and azo atom.. The second band located at 296 nm (33783 cm^{-1}) corresponds to the π →π* transition of the(C=N) group of imidazole. While the third band observed at 246 nm (40650 cm^{-1}) is assigned to the π→π* transition of the (C-C) of imidazole and phenyl rings. The electronic spectrum of Nickle (II) complex exhibits three absorption bands at about 957nm (10449 cm^{-1}), 420nm (23809 cm^{-1}) and 228 nm (43859 cm^{-1}) which may be attributed to $^3A_2g \rightarrow {}^3T_2g(F)$, $^3A_2g \rightarrow {}^3T_1g_{(F)}$, and $^3A_2g \rightarrow {}^3T_1g_{(p)}$,the magnetic moment of Nickle (II) complex was found at 2.79 B.M which was very close to the octahedral environment. The Copper (II) complex displays a broad asymmetric band around at 480 nm (20833 cm^{-1}). The broadness band indicates

the three transitions transitions $^2B_1g \rightarrow {}^2A_1g$ ($dx^2-y^2 \rightarrow dz^2$) ($\nu_1$), $^2B_1g \rightarrow {}^2B_2g$ ($dx^2-y^2 \rightarrow dyz$) (ν_2), and $^2B_1g \rightarrow {}^2Eg(\nu_3)$, (charge transfer), which are of similar energy and gives rise to only one broad absorption band ($^2B_1g \rightarrow {}^2Eg$), the magnetic moment of Copper (II) complex was found at 1.73 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 Zinc (II) complex was studied did not any d-d transitions, the magnetic susceptibility appeared the complex has diamagnetic moments because of the complex is diamagnetic having a d^{10} system the absorption band at 459nm (21786 cm^{-1}).The electronic transitions and suggested geometry Table(4)[18-20] .

Table (4): Maximum wavelength, electronic spectra, magnetic moments, geometry ,Conductivity and hybridization

Compounds	λ_{max} (nm.)	Abs. Bands (cm^{-1})	Transitions	μ_{eff} (B.M)	Geometry	Hybridization	Λ_m $S.mol^{-1}.cm^2$
1-MeCPAI	246	40650	$\pi \rightarrow \pi^*$	------	------	------	------
	296	33783	$\pi \rightarrow \pi^*$				
	417	23980	$n \rightarrow \pi^*$				
Nickel (II) Complex	228	43859	$^3A_2g \rightarrow {}^3T_1g_{(P)}$	2.79	Octahedral regular	Sp^3d^2 (high spin)	8.94
	420	23809	$^3A_2g \rightarrow {}^3T_1g_{(F)}$				
	957	10449	$^3A_2g \rightarrow {}^3T_2g_{(F)}$				
Copper (II) Complex	480	20833	$^2B_1g \rightarrow {}^2Eg$	1.73	distorted (Z-in or Z-out)	Sp^3d^2	13.17
Zinc (II) Complex	459	21786	$d\pi (Zn)^{+2} \rightarrow \pi^*$ (L)	Dia	Octahedral regular	Sp^3d^2	10.37

XRD Analysis

The phase composition and crystal size of prepared ligand and its complexes can be examined using the X-ray diffraction analysis. Figure (5). Shows the diffraction patterns of ligand (1-MeCPAI) and its complexes. All XRD patterns of prepared materials estimated the crystalline structure due to the presence of sharp diffraction peaks whereas amorphous and poor-ly-ordered phases, generate broad diffraction peaks were absence. According to Scherrer's equation (1), the average crystal size (D) of nanocomposite materials was calculated and placed in a Table (5).

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

…….. (1)

And d spacing (d) can calculated from Bragg's law:

$$d = \frac{n\lambda}{2\sin\theta}$$

…….. (2)

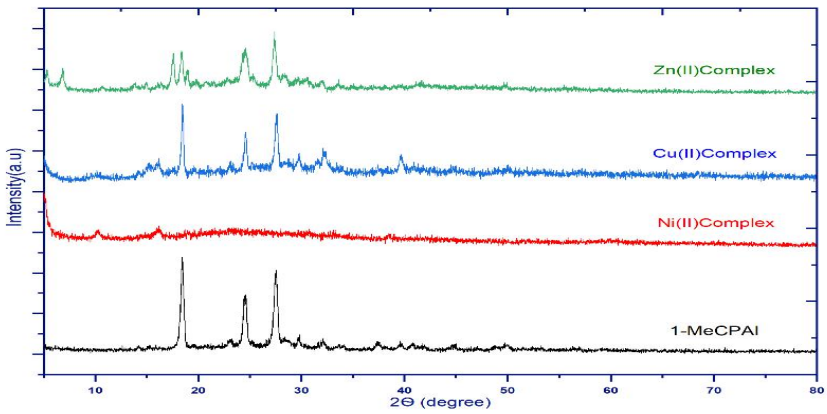
And Dislocation density (δ) also calculated from the following relationship:

$\delta = 1/D^2$ …….. (3)

Where *k* is the shape factor which usually takes a value of about (0.94), λ is the incident x-ray wavelength (0.15040 nm for CuK), β is full width at half maximum (FWHM). θ : is diffraction angle at maximum intensity peak and n is Bragg's constant (1, 2, 3, ..) [21, 22].

Table (5): Ligand (1-MeCPAI) crystal data and metal complexes

Compound	No.	2 Θ observed	d – spacing (A°)	(I/I◦) %	F W H M	Crystallite Size. (nm)	Lattice Strain	$\delta_{DX10^{15}}$ (lin m^{-2})
1-MeCPAI	1	18.5005	4.79201	100	0.33750	24.91	0.0090	1.61
	2	27.5455	3.23558	85	0.40430	21.14	0.0072	2.23
	3	24.5626	3.62134	58	0.40140	21.16	0.0080	2.23



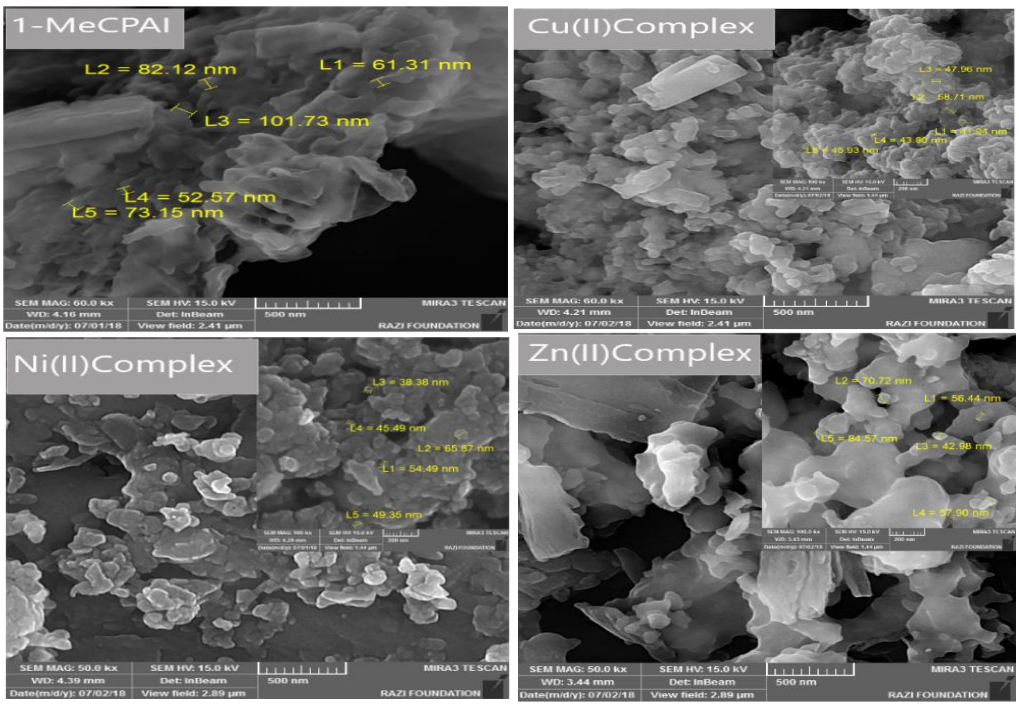
Ni(II)- complex	1	5.1958	16.99452	100	0.69000	12.04	0.0664	6.89
	2	4.6046	19.17509	34	0.40000	20.77	0.0434	2.31
	3	16.1070	5.49831	24	0.77000	10.89	0.0237	8.43
Cu(II)-complex	1	18.4548	4.80377	100	0.22970	36.61	0.0062	0.74
	2	27.5600	3.23391	79	0.30160	28.33	0.0054	1.24
	3	24.5556	3.62235	56	0.26540	32.01	0.0053	0.97
Zn(II)-complex	1	27.4509	3.24652	100	0.38300	22.31	0.0068	2.00
	2	18.4113	4.81502	93	0.33400	25.18	0.0090	1.57
	3	24.5037	3.62991	68	0.35000	24.27	0.0070	1.69

Fig.5. XRD patterns of (1-MeCPAI) with some complexes.

FESEM characterizing of Ligands and complexes

The ligand (1-MeCPAI) and the complexes were shown in the Figure (6). in different morphologies. [23, 24]. Differing the surface morphology of metal complexes by modifying the ions, FESEM micrographs of metal complexes have shown

Fig.6.FESEM images of (1-MeCPAI) with some complexes.



Biological Activities

Azo imidazole compounds are highly effective in inhibiting many different types of bacteria and fungi. The reason is that the ability of their solutions to dissolve the outer cell wall leads to the depletion and killing of the cell's fluids. The hybrid atom is the nitrogen in which it binds to certain elements in the body of the cell such as copper ions, cobalt, iron, zinc, monovalent manganese, and monovalent potassium that are needed by the bacterial cell, leading to the formation of complexes with these elements Leads to cell death due to loss of these components. The study studied the effectiveness of ligands under study and its metallurgical complex with ions. The study included the use of two strains of *Staphylococcus* and *pneumonia Klebsilla* and it also evaluation of the antifungal activity by *Alternaria alternata* [25, 26]. These types were used to determine the inhibitory influence of these species on their development. The data of antimicrobial activates of the newly synthesized compounds are given in Table (6) and summarized in the Figure (7). Our experiment revealed that, the ligand and some transition metal complexes show good antimicrobial activity against tested bacterial and fungal. Therefore, the imidazole group compounds may have an advantage in that they are used in the treatment of more severe clinical diseases than bacteriostatic agents.

Table (6):- biological activity data of synthesis compounds

No.	Compound	*Staphylococcus*	*pneumonia Klebsilla*	*Alternaria alternata*
		Diameter of inhibition zone (mm)	Diameter of inhibition zone (mm)	Diameter of inhibition zone (mm
1	1-MeCPAI	+++	+++	+
2	Ni (II) Complex	+	+	-
3	Cu (II) Complex	+++	+++	+
4	Zn (II) Complex	+	+	-

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

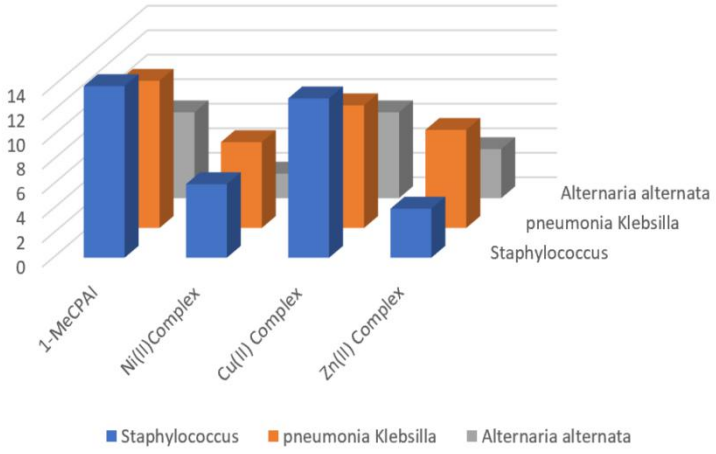


Fig. (7): Biological ligand statistic representation (1-MeCPAI) with prepared complexes.

Cytotoxic activity

One important topic of the current study is assessing anticancer activities of the (1-MeCPAI) ,Nickel(II)complex and Copper(II) complex against prostate cancer cell lines PC3 and normal cells (WRL-68)[27, 28]. Further, potentially bioactive molecules having cytotoxic activity are required for the treatment of cancer. These compounds were examined for their anti-cancer properties against prostate cancer cell lines (PC3) using five different concentrations, and the viability of the cells cultured by the MTT protocol was used. The ligand (1-MeCPAI) was observed that has high ratio of the inhibition of prostate carcinoma cell (PC3) is (47.57%) at 400 µg/mL, Few effects at the same concentration were observed in normal cell (WRL-68). On the other hand, the Copper (II) complex was showed that the best rate of inhibition of (PC3) (48.69%) at 400 µg/mL concentration and the nature cellular of (WRL-68) was little effect with the same concentration .While, Nickel (II)Complex was showed that the best rate of inhibition of (PC3) (55.36%) at 400 µg/mL concentration and the nature cellular of (WRL-68) was little effect with the same concentration. In addition, it is well known that the growth inhibitory activities and antitumor activity were both expressed by IC50 parameter (half-maximum inhibitory concentration), this concentration kills approximately half of the cells. The IC50 values in Figure (8). of the ligand (1-MeCPAI), Ni (II) and Cu (II) complexes were found to be 94.48 ,217.8 and 145.2 µg/mL respectively against PC3 cells. On the other hand, The IC50 values of the ligand (1-MeCPAI), Nickel (II) and Copper(II) complexes were found to be 213.6 , 206.2 and 204.5 µg/mL respectively against nature cells line .The results are summarized in Table (7) and (8) show the effect of these compounds on prostate cancer cell lines PC3 and compared with the nature cell line (WRL-68) of the same concentration using a 24-hour 3 - (4, 5-dimethyl thiazol-2-yl) - 2,5diphenyl tetrazolium bromide (M T T) assay at 37 ° C.

.Table (7): Effect (1-MeCPAI) on PC_3-cells and compared to the normal line of cells of the same concentration

Con.(µg.mL^{-1})	1-MeCPAI			
	Cancerous line cells of prostate PC3		Normal line cells of prostate WRL	
	Cell Viability%	Std. Error of Mean	Cell Viability%	Std. Error of Mean
400	47.57	1.738	75.89	1.189
200	61.23	3.478	84.22	0.342
100	80.48	1.307	92.75	1.099
50	87.04	0.703	96.03	0.278
25	96.95	0.193	96.88	0.176
12.5	97.57	0.812	93.90	0.991
6.20	96.76	0.898	95.64	0.429

Table (8): Effect Copper(II) complex on PC3 cells and compared with the normal cell line of the same concentration

Con.(µg.mL^{-1})	Copper (II) complex		Nickel (II)Complex	
	Cancerous line cells of prostate PC3	Normal line cells of prostate WRL	Cancerous line cells of prostate PC3	Normal line cells of prostate WRL

	Cell Viability%	Std. Error of Mean	Cell Viability %	Std. Error of Mean	Cell Viability %	Std. Error of Mean	Cell Viability %	Std. Error of Mean
400	48.69	1.504	73.03	0.133	55.36	1.416	85.03	1.756
200	57.95	3.747	84.45	0.102	73.23	0.510	92.71	0.703
100	75.58	1.116	94.21	1.142	88.19	0.267	98.15	3.195
50	88.62	1.808	95.18	0.301	95.56	0.301	99.23	2.606
25	97.69	0.372	94.83	0.315	95.29	0.154	96.22	1.138
12.5	96.37	0.444	95.60	0.522	96.33	0.234	96.84	1.023
6.20	96.26	0.682	95.72	0.353	96.22	0.102	95.52	0.992

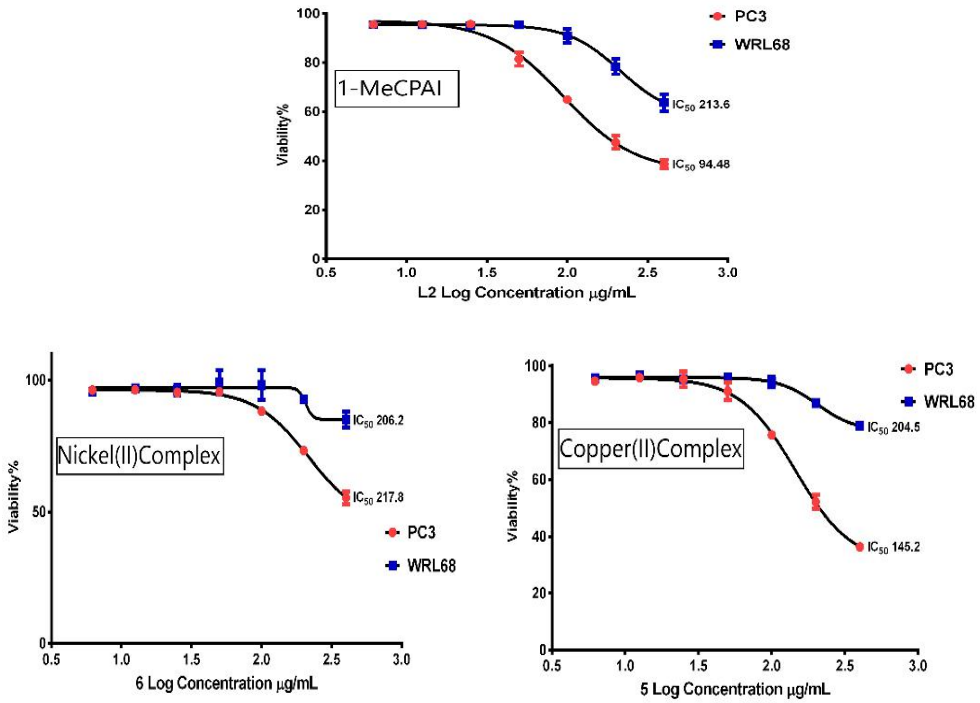
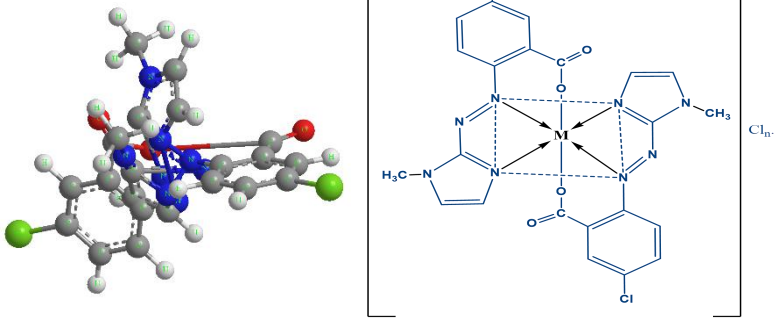


Fig.8: IC50(µg/mL) values of the carcinoma cell line and normal cell line 1-MeCPAI, Ni (I I) Complex and Cu (II) complex

CONCLUSION

In this study, The new azo dye ligand derived from 2-amino-4-chloro benzoic acid, synthesis and spectral characterization have been published with 1-methyl imidazole (1-MeCPAI) and its metal complexes with Ni(II),Cu(II) and Zn(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 Figure (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 1-MeCPAI) and Copper(II) complex yielded better results in comparison with Nickel(II) complex. Particularly, (1-MeCPAI) and copper(II) complex showed a selective cytotoxicity, harming the cancerous cell lines while not impairing the normal cells, which is considered as the key to the future of cytotoxic therap. The results indicated that this type of this compounds play an important role in the rate of inhibition of the growth cells of cancerous,it might be considered a promising agent for further structural modifications and pharmacological evaluation. undoubtedly the possibility of using them as anti-cancer drugs in the field of medicine and pharmacy.



M= Cu (II), Ni (II) and Zn(II); n=0

Fig.9.Proposed structural formula of metal complexes

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