# Synthesis, Characterization and Evaluation of Biological Activity of new Ligand Derived from 4-Aminoantipyrine, 2-Mercaptobenzoxazole and its Complexes with Some Metal Ions.

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**Abstract**

2-((2-((-4-(benzo[d]oxazol-2-ylamino))-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-ylidene)amino)phenyl (imino)-1,2-diphenylethan-1-ol (LH) was a new Schiff base ligand prepared from the reaction of 2-mercaptobenzoxazole with 4-amino-antipyrene dissolved in absolute ethanol, as a first step to form compound-A, while the second step involved the reaction of benzoin with O-phenylene diamine to produce compound- B, The third step involved the reaction of compounds A and B to produce ligand (LH) as a final product. Five complexes of ligand (LH) were prepared from the reaction with various metal ions Hg (II), Cd (II), Ag (I), Cu (II) and Ni (II). Several different spectroscopy methods were used to prove the structure of ligand (LH) and its complexes along with elemental analysis (CHNS), X-RD, FE-SEM, magnetic measurements, molar conductivity. The molar conductivity values of the prepared complexes exhibited that the complexes have non-electrolytic nature and do not have any molar conductivity. According to the results of the above techniques, the ligand (LH) behaves as a tetradentate (N, N, N, O) and coordinates with the metal ion via the nitrogen atoms of the two azomethine groups, the secondary amine group, and the oxygen atom of the hydroxyl group. Biological activity assays of free ligand (LH) and its metal complexes were performed against Gram-positive and Gram-negative bacteria, Staphylococcus aureus, and Escherichia coli, respectively. Based on the above results, the prepared complexes showed highly effective against bacteria, which were compared with a standard antibacterial. LH and the Ag(I)-complex) have been tested in vitro on breast cancer cells (MCF-7) and healthy human cells for their anticancer and toxicity (HEK-293).

Keywords: Schiff base-benzoxazole, Metal complexes, Antibacterial activity, Anticancer, antioxidant.

## 1. Introduction

As a pyrazolone derivative, 4-aminoantipyrine exhibits a wide range of biological activities, including antimicrobial [1], analgesic [2], and antiviral [3] properties. It is also used in the synthesis of bioactive substances like -lactams [4]. 4-Aminoantipyrine has also been used for safety in resistance to oxidative stress as well as prevention of many diseases that are most cancers, and its entry into many clinical processes [5]. Numerous derivatives of antipyrine were likewise assessed as analgesic [6], anti-inflammatory [7] and anticancer action [8]. Benzoxazole, also known as 1,3 benzoxazol, is an aromatic organic compound of heterocyclic compounds consisting of an oxazole ring that contains one oxygen and one nitrogen atom bonded to the benzene ring [9,10]. It was first presented by researcher Hantzsch in 1887 AD [11]. It has a molecular formula ($C_7H_5NO$) and its molar mass is 119.12 g/mol and its melting point is 27-30°C and its boiling point is 182°C [12]. It is characterized by its white color to a light-yellow color with a pyridine-like smell [13]. It is insoluble in water, but it dissolves in a completely in organic solvents such as ethanol [14]. The benzoxazole compound is an important class of heterocyclic π-electrons [15]. Some reactions of this compound occur in a similar way to the oxazole compound, due to the electronegativity of the nitrogen atom [16]. In pharmaceutical applications, benzoxazole derivatives, which have various biological and pharmacological properties [17] example antituberculotic [18], tuberculosis [19], depression [20], antibacterial [21], antispasmodic [22], andcardiovascular tonic [23], are widely used in drug discovery programs and agricultural chemicals [24]. In general, benzoxazole and its derivatives enter coordination chemistry in the preparation of complexes [25] and in optical applications such as photoluminescence [26] and bleaching and dyeing [27]. It is also used in intermediates to catalyze organic compounds in pharmaceuticals [28] and therapeutic uses [29].

The German chemist Hugo Schiff is the first to prepare Schiff's bases, in 1864, through a condensation reaction between aldehydes or ketones with primary amines [30]. Schiff bases are used in various fields whether it is from a scientific, industrial or agricultural point of view [31], it has

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been found to be of great importance in life processes, such as reactions that involve the transfer of an amine group by a non-enzymatic transamination reaction [32], while others have activity against cancer [33], as well as activity against bacteria and fungi [34]. It is also used as a plant growth regulator, antipyretic [35] and analgesic [36]. Schiff bases are a type of multidentate ligands that are colored complexes with most metal ions, so they constitute selective and sensitive methods for metal determination [37].

This study included the preparation of ligand of Schiff base type and its complexes with metal ions Ni (II), Cu (II), Ag (I), Cd (II) and Hg (II). As well as the biological activity of Ligand and all its complexes were evaluated as anti-bacterial under study, the activity of Ligand and a complex with silver (I) as anti-cancer were also tested.

## 2. Experimental

### Materials and reagent

The chemicals and solvents used in the synthesis have high purity. 2-mercaptobenzoxazole, 4-amainoantipyrin, O-phenylene diamine, Benzoin, silver(I) nitrate, nickel (II) chloride hexahydrate, copper (II) chloride dihydrate, cadmium (II) chloride, mercury (II) chloride, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), Glacial Acetic Acid, DMSO, DMF, EtOH, MeOH, these materials are supplied by companiesو Sigma-Aldrich (Germany), Merck (Germany), Scharlu (España), J&K chemical (China) and others

### Physical Measurements

An internal reference of TMS had been used to obtain the 1HNMR spectra, which were collected using DMSO-d6 as the solvent and a Bruker 500 MHz spectrometer. A dual-beam T80-PG spectrophotometer was used to measure electronic spectra in absolute ethanol as a solvent (UV-Vis). A Shimadzu 8400 S was used to record FT-IR spectra (KBr discs, 400–4000 cm-1). The metallic content of the metal Ni (II), Cu (II), Ag (I) and Cd (II) in the complexes was determined using a Shimadzu AA 6300 atomic absorbance meter. X-ray diffraction (XRD) measurements were carried out using a fluorometer Bestec aluminum X-ray diffraction with irradiance (Cu Kα) (λ = 1.5418° A) within the range (5-80°) 2θ. Field emission scanning electron microscopy (FE-SEM) images were taken on a TESCAN MIRA3, Czech) (Shahid Beheshti University, Iran). Faraday's method, Pascal constants, and magnetic corrections were used to perform magnetic measurements using the magnetic susceptibility model MSB Auto). In ethanol solutions, molar conductance has been measured at room temperature using a 31 A conductivity bridge. The SMP, Stuart tool was utilized to record melting points or decomposition temperatures.

Synthesis of Ligand 2-((2-((-4-(benzo[d]oxazol -2-ylamino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-ylidene) amino) phenyl) imino)-1,2-diphenylethan-1-ol (HL)

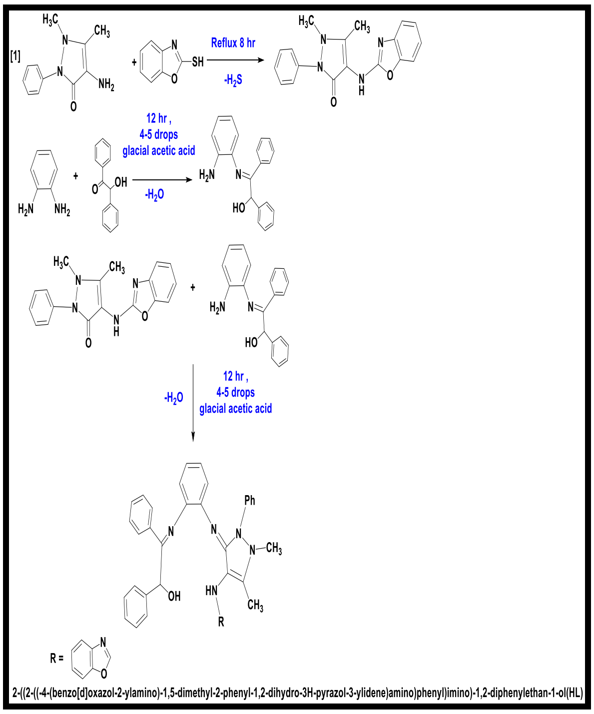
The ligand (LH) was prepared through three main steps:

The first step is the formation compound -A by taking 2.03 g of 4-aminoantipyrine and dissolving it in 25 mL absolute ethanol, then weighing 1.68 g of 2-mercaptobenzoxazole and dissolving in 25 mL absolute ethanol. Then the two solutions are mixed in a round bottom flask, and the mixture refluxed for eight hours, the reaction continues, and the reaction is followed up using lead acetate paper, where the blackening of the paper stops at the end of the reaction. Filtered, dried and recrystallized the product using hot ethanol, then filtered again and dried (yield: 74%, m.p:87-89 oC)

The second step, which is the formation of compound (B), which is by taking a weight of 1.08 gm of O-phenyldiamine and dissolving in 25 ml of absolute ethanol and taking a weight of 2.12 gm of benzoin and dissolving it in 25 ml of absolute ethanol with the addition of 4-5 drops of acetic acid After that, the two solutions are mixed in a round bottom flask, and the reflux process begins for eight hours, after which the solution is filtered and dried, then the product is recrystallized, and then left to dry (yield: 69% , m.p: 102-104 oC)

The third step, compound (A) weighs (1.6 g), and (25 mL) of absolute ethanol, 4-5 drops of glacial acetic acid, and (1.51 g) of compound (B) are mixed together and dissolved in 25 ml of absolute ethanol and the two solutions are mixed in a round bottom flask and the reflux process begins for eight hours, then the solution filtered and dried, then the product is recrystallized, and then left to dry (yield: 81% , m.p: 113-115oC)

### The structural of the ligand (LH) as proven below



*Scheme (1): Synthesis of heterocyclic ligand (L).*

## Synthesis of Metal Complexes

The metal chelates were prepared in a stoichiometric ratio of 1:1 [M: L] by dissolving (0.31 g, 0.5 mmol) of the ligand (LH) in hot ethanol (10 ml) with a 0.5 mmol solution of metal chloride, excluding silver, where silver nitrate was used, dissolved in 10 ml of absolute ethanol. The two solutions were refluxed after mixing for two hours, the formation of precipitates was observed, the products were filtered, the solid complexes were washed with distilled water, then they were washed with 15 ml of hot water, dried and then recrystallized with hot ethanol. The complexes dried. Table (1) gives the analysis and physical properties of the ligand (LH) and its complexe

**Table 1. Physical properties and analytical data for ligand (LH) and its metal complexes.**

| Compounds | color | MP (C°) | Yield % | Mol. Wt | Elemental analysis (%) :)Found) calcd. | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | | C | H | N | M |
| LH | yellow | 113-115 | | $C_{38}H_{32}N_6O_2$<br>604.71 | 75.48 (76.12) | 5.33 (5.79) | 13.90<br>(14.17) | - |
| Ni- complex | Light yellow | 125-127 | | $C_{38}H_{33}ClNiN_6O_3$<br>715.86 | 63.76<br>(64.04) | 4.65<br>(4.75) | 11.74<br>(11.87) | 8.20<br>(8.42) |
| Cu- complex | Dark brown | 131-133 | | $C_{38}H_{33}ClCuN_6O_3$<br>720.72 | 63.33 (63.82) | 4.62 (4.79) | 11.66 (11.85) | 8.82 (8.94) |
| Zn- complex | Light brown | 136-138 | | $C_{38}H_{31}ClZnN_6O_2$<br>704.54 | 64.78<br>(65.06) | 4.44<br>(4.92) | 11.93<br>(12.08) | 9.28<br>(9.63) |
| Ag- complex | Light green | 127-129 | | $C_{38}H_{31}AgN_6O_2$<br>711.57 | 64.14<br>(64.59) | 4.39<br>(4.89) | 11.81<br>(12.05) | 15.16<br>(15.72) |
| Cd- complex | Light brown | 140-142 | | $C_{38}H_{33}ClCdN_6O_3$<br>769.59 | 59.31<br>(59.58) | 4.32<br>(4.76) | 10.92<br>(11.19) | 14.61<br>(14.85) |

LH = ligand

## Cytotoxic studies-MTT assay

The cytotoxicity of Schiff base ligand (LH) and Ag(I)-complex was studied against breast cancer cell line (MCF-7) and normal human cells (HEK-293) by in vitro MTT cytotoxicity assay grown MCF-7cell lines in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Gibco) and 1% penicillin-streptomycin. Incubated at 37 °C in a humidified atmosphere with 5% (v/v) CO, the MTT assay was transformed for use in determining cell viability [39]. For 24 h, cells were seeded at a density of 104 cells/well in 96 dishes). The medium was extracted after 24 h incubation at 37 °C, and cells were treated with concentrations (16, 31, 62, 125, 250 and 500 µg/ml) after a 48-h incubation period at 37 °C, the medium has been withdrawn from the board. Each nicely became then full of 100 µl of MTT reagent (1 mg/ml) in serum-free medium. The medium became extracted after 4 h of incubation, and 200 µl of dimethyl sulfoxide (DMSO) became carried out to each properly. The absorbance of the MTT metabolite dissolved in DMSO became measured the usage of a microplate reader at a wavelength of 570 nm. Triple checks had been achieved at each concentration. % Cellular viability became calculated as (Mean OD of treated cells/Mean OD of untreated cells) ×100[40]. Inhibitory concentration values of 50% (IC50) were measured and IC50 curves were plotted the usage of the x-dose response equation [41].

# 3. Result and Discussion

## Characterization of the ligand (LH) and its metal complexes

The ligand (LH) is an organic compound derived from benzoxazole, and the results of elemental analysis (CHNS) of the ligand and its complexes were consistent with the expected values, and it proved that the ratio of ligand to metal in the prepared complexes is 1:1 (M: L). In normal conditions, all compounds are stable in their solid form, and insoluble in water, while soluble in many organic solvents such as EtOH, MeOH, DMSO, and DMF.

## Metal: Ligand Ratio

The molar ratio method, which was developed by Yu and Jones [42]. By using this method, the amount of ligand in each solution is varied while the amount of metallic remains constant. Then the relationship between the absorbance of each solution versus its molar ratio (VL/VM) at the maximum wavelength, resulting in two straight lines whose intersection point represents the ratio of ligand to metal [43]. Thus, electronic spectra are indicative in the formation of complexes Ni (II), Cu (II), Zn (II), Ag (I) and Cd (II), the molar ratio [M: L] is [1: 1]. These results agree with those expected theoretical results

## Zhu-NMR Spectra

The 1H-NMR spectrum of the ligand (LH) was studied using DMSO-d6 as the solvent and TMS as the internal reference. The spectrum showed two single signals at (S, 3H , δ = 1.05ppm) and (S, 3H , δ =2.72ppm) belonging to the protons of the two methyl groups (C-$CH_3$ and N-$CH_3$), respectively[44] , while the signal at (S, H , δ = 4.86 ppm), which due to proton of methyne group (CH-OH) and signal at (S, H , δ = 6.08ppm) refer to hydroxyl group proton[45].Multiple other signals are observed in (M, 10H , δ= 7.22 – 7.37 ppm) related to the protons of the two phenyl rings of benzoin[46] , Also, several signals appeared at (M ,5H , δ= 7.40 – 7.49 ppm) related to the protons of phenyl ring of pyrazole [47]. The spectrum also gave multiple signals at (M, 4H, δ= 7.52 – 7.80ppm) belonging to the protons of Phenyl ring of O-phenylene di-amine [48]. While multiple signals appeared at (M, 4H, δ= 7.87 – 8.00 ppm) belonging to the phenyl ring protons belonging to Benzoxazole ring [49], the single signals at (S, 1H, δ = 8.16ppm) indicates to the proton of a secondary amine group proton [50].

Fig. (1): $^1$H-NMR Spectrum of the ligand (LH)

Infrared spectra of the ligand (LH) and its metal complexes

Infrared spectra were measured using Shimadzu 8400S device and using potassium bromide (KBr), Infrared spectra were used to identify the functional groups in the ligand and their complexes. The spectrum of the free ligand showed two clear bands at (3409 and 3379 cm 1), which belong to the hydroxyl υ(OH) and secondary amine groups υ(NH), respectively [51]. A band appeared in the spectrum of the ligand at (1681 cm 1), an important band whose appearance indicates the formation of the ligand, which is the azomethine group (C=N) of the Schiff base [52]. Other bands appeared in (3032 cm-1) and (2931 and 2869 cm-1), which belong to the (C-H) aromatic and aliphatic groups, respectively [53], as for the groups (C=C) aromatic, it gave bands at (1450, 1504 cm-1), finally, other bands exhibited at (1596 cm-1) due to the functional groups υ (C = N) of the benzoxazole ring [54].

From observing the spectra of the prepared complexes and comparing with the spectrum of the free ligand, it was found:

The azomethine group υ (C=N) belonging to the Schiff base was shifted towards lower frequencies compared to what it was in the spectrum of the free ligand and appeared at (1635 , 1658 , 1650 , 1668 and 1639 cm-1) in the spectra of the prepared complexes[55], the frequency of the secondary amine group (N-H), which appeared at (3379 cm-1) in the spectrum of the ligand before coordination, was shifted to a lower frequency and appeared at the range (3342-3359 cm-1) in the spectra of the prepared ligand (LH) complexes, the occurrence of this shift is strong evidence of the coordination of the ligand with the metal ions through the nitrogen atoms of the azomethine and the secondary amine groups[56]. The hydroxyl group of the free ligand disappeared in the spectra of the complexes due to the loss of its proton after coordination, which is another evidence of coordination and appearance bands at (424 -486 cm-1), which indicated to υ (M-O). New broad bands also appeared at (3458, 3440 and 3434 cm-1) belonging to the hydroxyl group of the hydrate water in the nickel (II) copper (II) and cadmium (II) complexes, respectively [57], while the bands found at (501-540 cm-1) mainly due to (M-N) group [58].

Table (2): The important Infrared spectral bands of Ligand (LH) and its prepared complexes

| Compounds | υ(O-H) (H2O) | υ(N-H) 2oamine | υ(C-H) aromatic | υ(C-H) aliphatic | υ(C=N Imine | υ(C=N) Benzoxazole ring | υ(C=C) aromatic | υ(M–N)<br>υ(M–O) |
|---|---|---|---|---|---|---|---|---|
| Ligand (LH) | - | 3379 | 3032 | 2931 2869 | 1681 | 1596 | 1504 1450 | — |
| Ni-L complex | 3458 | 3346 | 3039 | 2962 2893 | 1635 | 1596 | 1496 1458 | 501 455 |
| Cu-L complex | 3440 | 3342 | 3070 | 2931 | 1658 | 1626 | 1504 1450 | 509 424 |
| Zn-L complex | - | 3352 | 3055 | 2916 2839 | 1650 | 1596 | 1512 1450 | 516 486 |
| Ag-L complex | - | 3359 | 3062 | 2923 2854 | 1668 | 1596 | 1512 1450 | 540 478 |

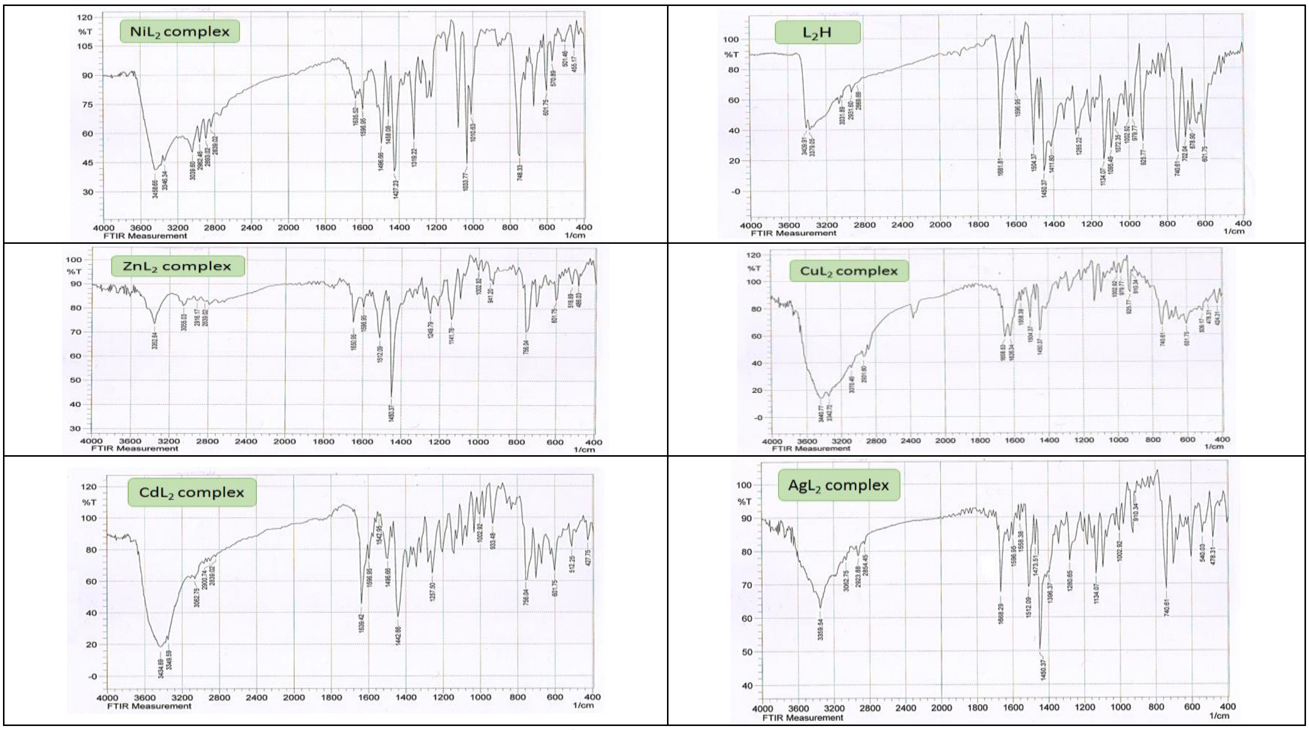


Fig. (2): The FTIR spectra of the ligand (LH) and its metallic complexes

## Electronic spectra and magnetic susceptibility measurements

Metal complexes are considered colored compounds, and these colors vary from one complex to another according to the metal present in the complex, and from one ligand to another. In addition, the change of colors is an important guide for the occurrence of the coordination. The difference in colors results in the absorption bands differing in intensity and position, which is another indication of consistency.

Electronic (d-d) transitions in complexes appear in the visible region at the range (380-800 nm), but the electronic absorption of the ligand occurs in the UV region at (200-380 nm), as well as new peaks appear indicating the charge transfer between the metal and the ligand that it appears at the end of the ultraviolet region and the beginning of the visible region, transitions of relatively high intensity compared to the (d–d) transitions.When observing the electronic spectrum of the ligand (LH) in Figure (3), the spectrum of the ligand showed three absorption peaks at 206 nm (48544 cm-1), 252 nm (39683 cm-1) and 305nm (32787 cm-1), the first and second peak belongs to the (π-π*) transitions, and the last peaks belong to the(n-π*) transitions belonging to the azomethine group (C=N) [59].

## Nickel (II) - Complex

The spectrum of the nickel complex showed three absorption peaks centered at 219 nm (45662 cm-1), 289 nm (34602 cm-1) and 339 nm (29499 cm-1), all of which are due to the spectrum of the ligand field, while the absorption peaks at 426 nm (23474) cm-1), 514 nm (19455 cm-1) and 628 nm (15924 cm-1), which are attributed to the transitions 3A2g (F) → 3T1g (p), 3A2g (F) → 3T1g (F) and 3A2g (F) →3T2g(F), refers to the regular octahedral geometry (hybridization -sp3d2). The magnetic moment is given a value of (3.16 BM) [60].

## Copper (II) - Complex

The spectrum of the Cu (II) complex shows peaks at 218,266 and 360 nm. (45872,37594 and 27778 cm-1) belonging to the spectrum of the ligand field and a broad peak at (619 nm) (16155 cm-1), which is due to the transition (2B1g →2Eg), the appearance of this peak, which includes a shoulder, indicates a deformation in the complex due to the Jan-Teller effect, and these transitions indicate that the above complex is octahedral deformed, The magnetic moment is given a value of (1.74 BM), these indicate The value indicates the presence of one non-pair electron[61].

## Zinc (II), silver(I) and Cadmium (II) Complexes

Zinc (II), Silver (I) and cadmium (II) electronic spectra show no (d-d) transitions because electrons are saturated in (d) orbitals, where the above complexes showed a number of peaks within the range 221-336 nm (45249-29762 cm-1) which belong to the Ligand field , while the peaks at 376, 377, 394nm (26596, 26525, 25381 cm-1), all refer to The charge transitions of the type (metal - ligand), (M → L) in silver (I), Zinc (II)and cadmium (II), respectively. Previous studies have shown that these complexes have regular tetrahedral geometry [62].

Table (3): - Electronic spectra (nm, cm-1), magnetic moments, geometry and hybridization

| Compounds | λ (nm) | υ- (Cm-1) | Transitions | μeff (B.M) | Geometry |
|---|---|---|---|---|---|
| Ligand($L_2H$) | 206 | 48544 | π-π* | – | – |
| | 252 | 39683 | π-π* | | |
| | 305 | 32787 | n-π* | | |
| Ni- complex | 219 | 45662 | Ligand Field | )Para.(<br>3.16 | Octahedral<br>sp$^3$d$^2$<br>Regular |
| | 289 | 34602 | Ligand Field | | |
| | 339 | 29499 | Ligand Field | | |
| | 426 | 23474 | $^3A_2g$ (F) →$^3T_1g$ (P) | | |
| | 514 | 19455 | $^3A_2g$ (F) →$^3T_1g$(F) | | |
| | 628 | 15924 | $^3A_2g$ (F) →$^3T_2g$(F) | | |
| Cu- complex | 218 | 45872 | Ligand Field | )Para.(<br>1.74 | Octahedral<br>sp$^3$d$^2$<br>distorted |
| | 266 | 37594 | Ligand Field | | |
| | 360 | 27778 | Ligand Field | | |
| | 619 | 16155 | $^2B_1g$ → $^2Eg$ | | |
| Zn- complex | 224 | 44643 | Ligand Field | (Dia.) | Tetrahedral<br>sp$^3$<br>Regular |
| | 281 | 35587 | Ligand Field | | |
| | 324 | 30864 | Ligand Field | | |
| | 376 | 26596 | Charge transfer (MLCT) | | |
| Ag- complex | 225 | 44444 | Ligand Field | (Dia.) | Tetrahedral<br>sp$^3$<br>Regular |
| | 283 | 35336 | Ligand Field | | |
| | 336 | 29762 | Ligand Field | | |
| | 377 | 26525 | Charge transfer (MLCT) | | |
| Cd- complex | 221 | 45249 | Ligand Field | (Dia.) | Tetrahedral<br>sp$^3$<br>Regular |
| | 281 | 35587 | Ligand Field | | |
| | 324 | 30864 | Ligand Field | | |
| | 394 | 25381 | Charge transfer (MLCT) | | |

B.M= Bohr magneton

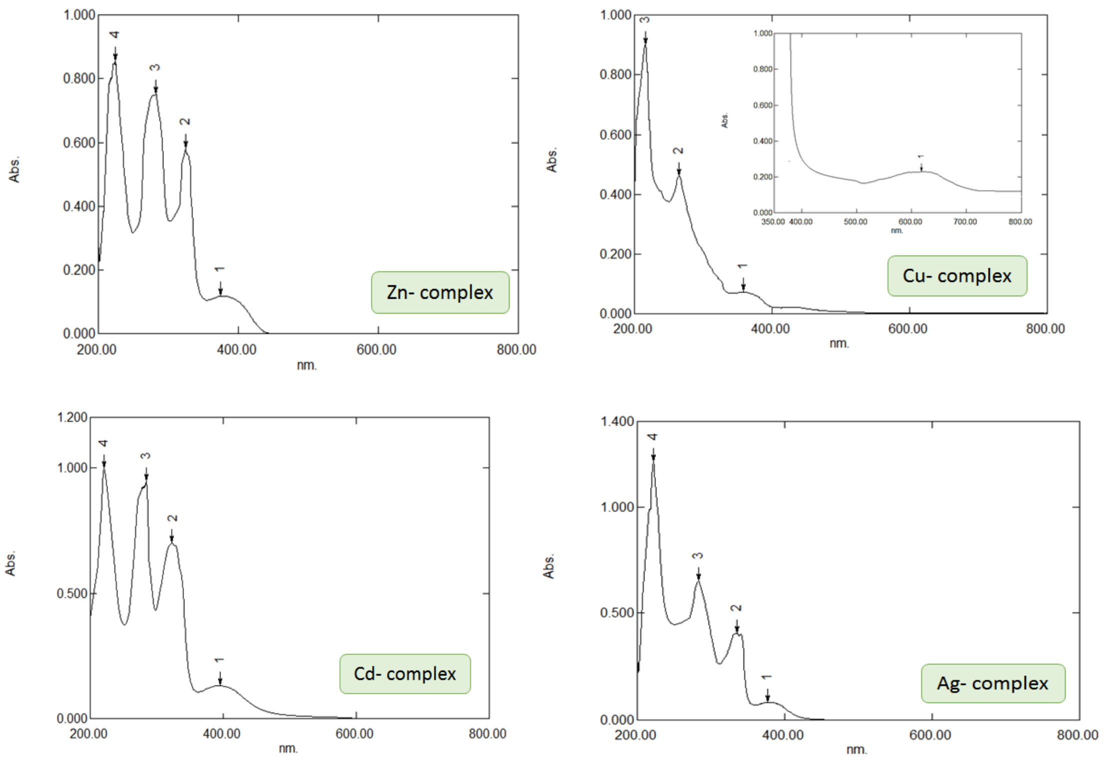


*Fig. (3): Electronic spectra of the ligand (LH) and its metallic complexes*

Depending on the techniques used and the results it reached, we can suggest the structural formulas of the prepared complexes as in Figure (4).

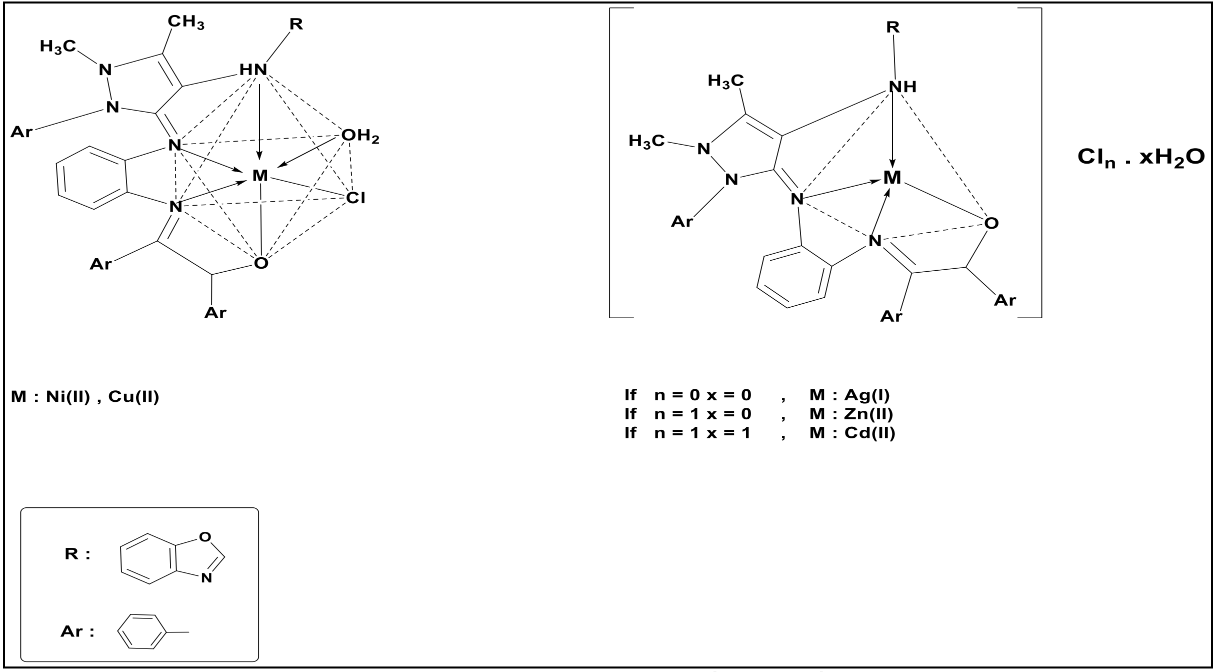


*Fig. (4): The suggested structure formula of (LH).*

## X-ray diffraction study (XRD)

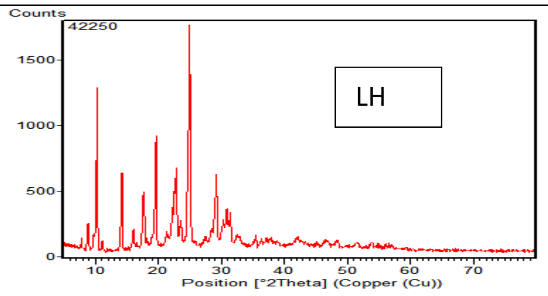
The crystal structures of ligand (LH) and its metal complexes in their solid state were studied using X-ray diffraction within (angular range) 5°-80° 2ϴ in order to know some structural properties such as crystal structure and crystal size, also calculating the macrostrains and dislocation density to estimate the extent of its purity and the defects in the crystal structure when converting ligand to metal complexes. There are some diffraction peaks that show due to micro-strains such as the lack of lattice deformation, as well as crystal faulting as a result of the occurrence of distortions of the crystal, as well as the domain size of the crystal and the distribution of domain size [63,64]. The X-ray diffraction spectrum showed the presence of sharp peaks for ligands and metal complexes, which indicates that they form a crystal lattice and others contain broad peaks indicative of non-crystalline structures amorphous structure The sharpness of the peaks depends on the crystal arrangement and the properties of the crystal lattice as well as on the crystal levels. e-Scherer was used to calculate the crystal size of ligand (LH) and its metal complexes. The following equation was used to calculate the decay density

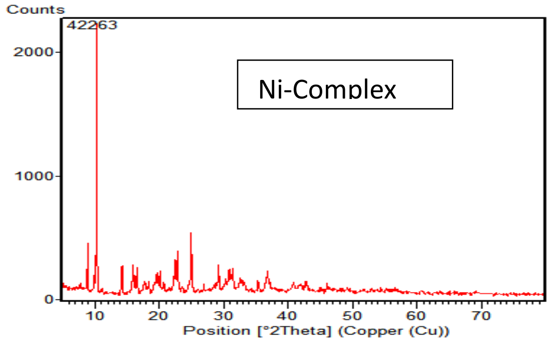
$$\delta=1/D2$$

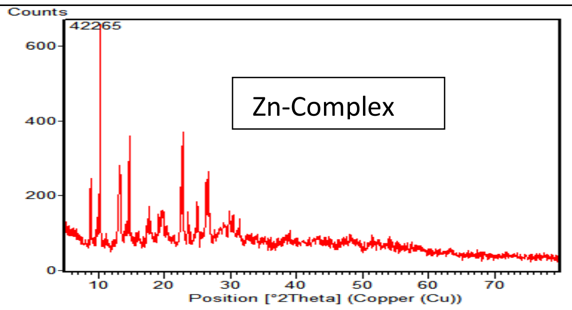
by analyzing the X-ray spectra, showing the clear difference in the data that it was previously mentioned from crystalline size, density of dissolutions, as well as the spacing between crystalline levels of ligand and its prepared metal complexes, and this confirms the occurrence of the coordination process between ligands and metal ions [65,66].

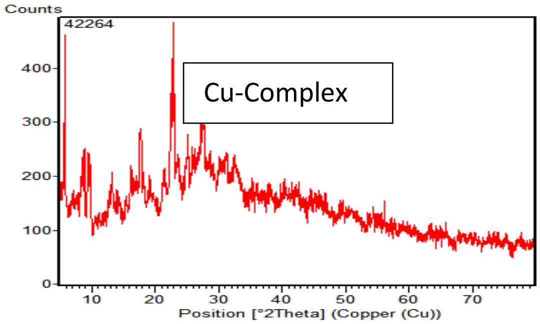
Table 4. Crystallographic data for (L) and chelate complexes

| Compound | No. | °2ϴ observed | d observed (A°) | I/I°) %( | Width | FWHM | Crystallite Size. (nm) | Lattice Strain |
|---|---|---|---|---|---|---|---|---|
| LH<br>$C_{13}H_{15}N_3OS$ | 1 | 10.286 | 8.593 | 77.6 | 0.0933 | 0.187 | 44.57 | 0.0091 |
| | 2 | 14.204 | 6.230 | 36.5 | 0.1005 | 0.201 | 41.62 | 0.007 |
| | 3 | 19.636 | 4.517 | 51 | 0.1028 | 0.206 | 40.9 | 0.0052 |
| | 4 | 22.842 | 3.890 | 34.6 | 0.2730 | 0.546 | 15.51 | 0.0118 |
| | 5 | 24.952 | 3.565 | 100 | 0.1390 | 0.278 | 30.580 | 0.0055 |
| [Ni(L) Cl.$H_2O$] | 1 | 8.926 | 9.899 | 17.7 | 0.0462 | 0.092 | 90.51 | 0.0051 |
| | 2 | 10.324 | 8.561 | 100 | 0.0488 | 0.098 | 85.05 | 0.0047 |
| | 3 | 24.967 | 3.563 | 21.3 | 0.0742 | 0.148 | 57.45 | 0.0029 |
| [Cu (L) Cl.$H_2O$] | 1 | 5.781 | 15.274 | 100 | 0.0532 | 0.106 | 78.42 | 0.0092 |
| | 2 | 9.576 | 9.228 | 43.7 | 0.3749 | 0.750 | 11.11 | 0.0391 |
| | 3 | 17.698 | 5.007 | 42.2 | 0.1803 | 0.361 | 23.27 | 0.0101 |
| | 4 | 22.799 | 3.897 | 83.1 | 0.1507 | 0.301 | 28.13 | 0.0065 |
| | 5 | 27.662 | 3.222 | 41.3 | 0.2245 | 0.449 | 19.04 | 0.008 |
| [Zn(L)] Cl | 1 | 8.890 | 9.939 | 26.4 | 0.0565 | 0.113 | 73.64 | 0.0071 |
| | 2 | 10.295 | 8.585 | 100 | 0.0489 | 0.098 | 85.05 | 0.0047 |
| | 3 | 14.730 | 6.009 | 39.6 | 0.0574 | 0.115 | 72.789 | 0.0039 |
| | 4 | 22.806 | 3.896 | 43.8 | 0.0859 | 0.172 | 49.239 | 0.0037 |
| [Ag ($L_2$)] | 1 | 5.592 | 15.790 | 100 | 0.1323 | 0.265 | 31.36 | 0.0237 |
| | 2 | 16.836 | 5.261 | 7.2 | 0.1316 | 0.263 | 31.91 | 0.0078 |
| | 3 | 22.594 | 3.932 | 8 | 0.1867 | 0.373 | 22.7 | 0.0081 |
| [Cd($L_2$)] Cl.$H_2O$ | 1 | 6.781 | 13.025 | 30.8 | 0.0939 | 0.118 | 44.23 | 0.0138 |
| | 2 | 10.375 | 8.519 | 100 | 0.0512 | 0.102 | 81.72 | 0.0049 |
| | 3 | 19.708 | 4.501 | 31.8 | 0.1664 | 0.333 | 25.3 | 0.0084 |
| | 4 | 22.858 | 3.887 | 33.1 | 0.0573 | 0.115 | 73.65 | 0.0025 |
| | 5 | 25.026 | 3.555 | 33.8 | 0.0725 | 0.145 | 58.64 | 0.0029 |









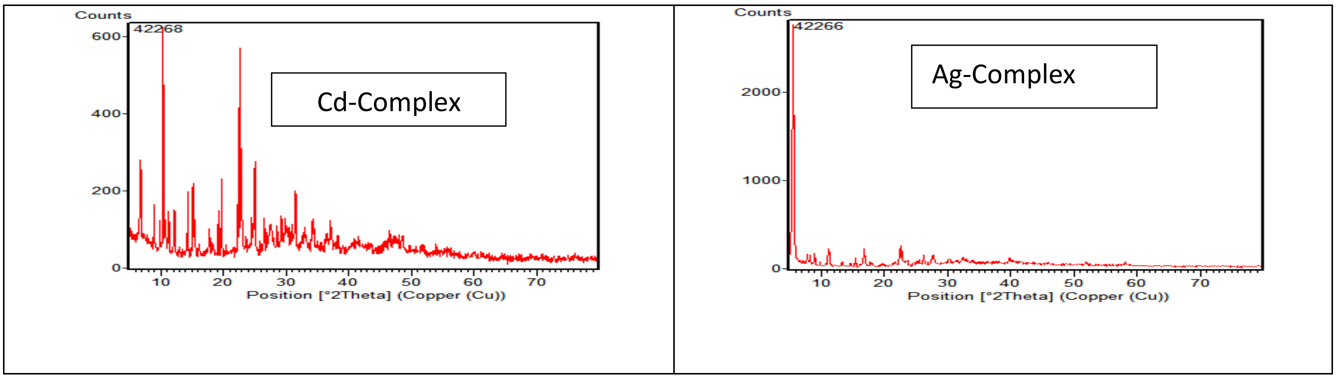


*Fig. (5): XRD patterns of Ligand (LH) and its complexes*

## Scanning Electron Microscope

The scanning electron microscope technique was used to study the surface properties of ligand (LH) particles and its prepared metal complexes with some metal ions, namely nickel (II), copper (II), silver (I), Zinc (II) and cadmium (II), respectively in terms of the shape and size of the particles and clusters and their distribution with a cross-sectional distance of 200nm and magnification strength Mag=135kx. Through FESEM analysis images of ligand (LH) it appeared in the form of homogeneous crystals with an average particle size of 34.64 nm, while the image of FESEM analysis of nickel (II) complex appeared in the form of adjacent column crystals with an average size of minutes 155.80 nm, the image of FESEM analysis of copper (II) complex It appeared in the form of heterogeneous crystals and the average size of the minutes was 84.14 nm. Also, the FESEM analysis image of the complex of silver (I) appeared in the form of heterogeneous crystal clusters and the average particle size was 56.45 nm, also, the image of the FESEM analysis of the complex of zinc (II) appeared in the form of large heterogeneous crystal particles with an average size of 148.34 nanometers. As for the FESEM analysis image of the cadmium (II) complex, it appeared in the form of heterogeneous crystals, with an average particle size of 105 nm [67]. Through this analysis the results obtained in the figure (6) below showed that the benzoxazole ligand and some of its prepared complexes are with a grain size of less than 100 nanometers, that is, it is within the nanoscale, while others are outside the nanoscale. These results enabled us to study the ligand and silver (I) compound in the field of medicine and their ability to eliminate many types of cancers, including breast cancer, and the possibility of using it as a drug, and this is what will be clarified in this study [68].

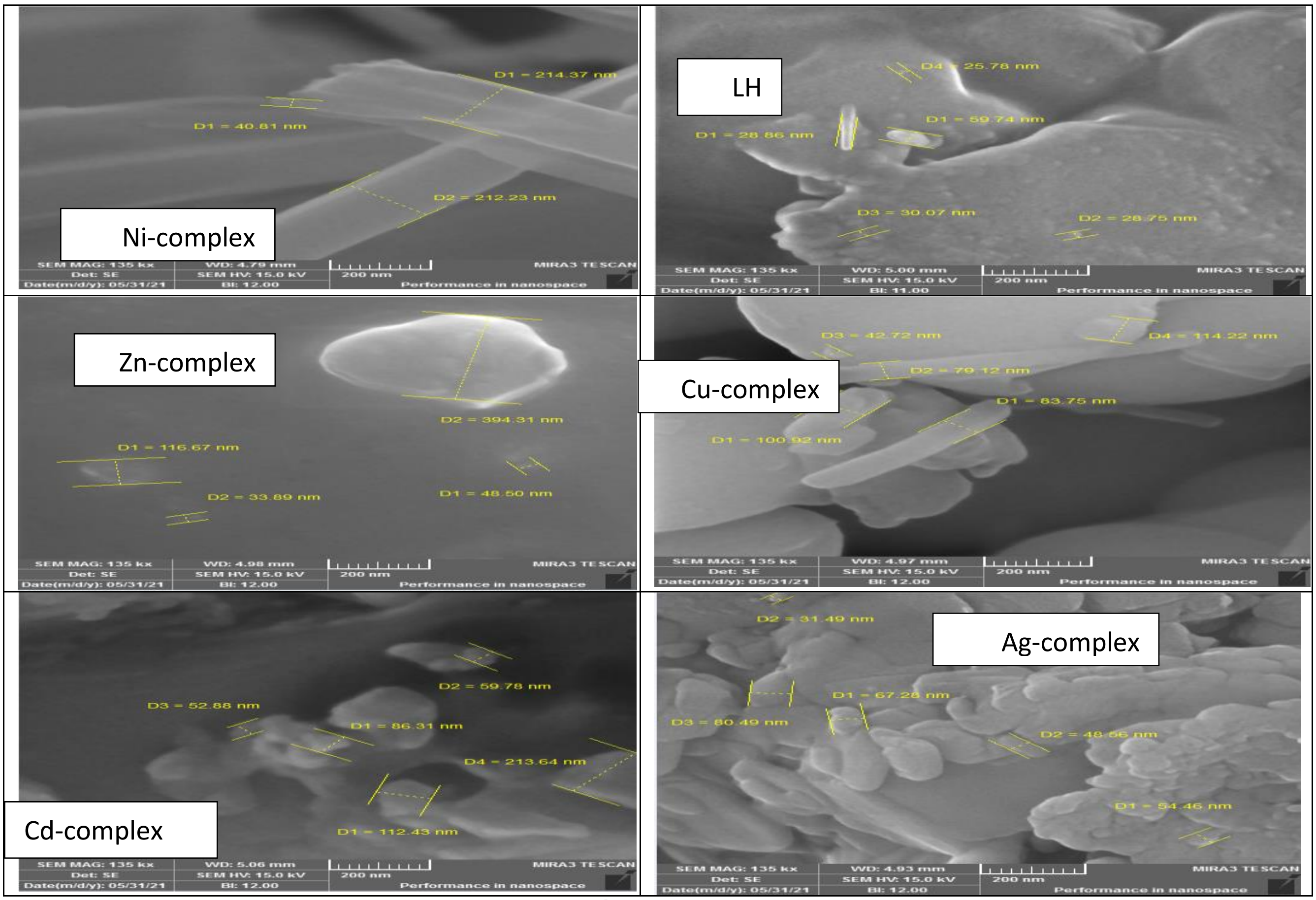


*Fig. (6): FE-SEM images of ligand (L) and it chelate complexes.*

## Molar Conductivity Measurement

Molar conductivity measurements were used to determine whether the prepared compounds had ionic or non-ionic properties, where it was observed that the molar conductivity is directly proportional to the charged species in the solution, many organic solvents such as methyl cyanide, nitromethane, dimethyl sulfur dioxide (DMSO), dimethylformamide (DMF), ethanol and others are often used because they have a high dielectric constant and low viscosity [69]. The molar conductivity of the ligand (LH) complexes was measured using absolute ethanol as a solvent at a concentration of (1*10-3M) at laboratory temperature. It was noticed that the zinc (II) and cadmium (II) complexes had conductivity values (36.7 and 36.5 ohm-1cm2mole-1), respectively. These values indicate that these complexes have an ionic character in a ratio of (1:1), while the nickel (II) and copper (II) and silver (I) showed a relatively low conductivity (11.8, 9.7 and 8.3 ohm-1cm2mole-1), these values indicated that they are complexes with non-ionic properties [70].

# 4. Pharmacology Results

## Anticancer effect

The efficacy of ligand (LH) and its complex with Ag(I) as anticancer agents against breast cancer cell line (MCF-7) and normal human cell line (HEK-293) was tested by MTT assay after 24 h incubation at 37°C and at concentrations (16, 31, 62, 125, 250 and 500 mcg/mL). The ligand inhibits the growth of cancer cells at a concentration of 500 µg/ml with a cytotoxic substance. Effectiveness (93.24%). On the other hand, Ag(I)-Complex inhibited the growth of cancer cells to (93.25%) at a concentration of 500 µg/ml, while the ligand had no significant effect on healthy cells (HEK-293) at this concentration. It can also be concluded that all examined compounds were inhibited the maximum whilst concentrations (500 µg/ml) have been incubated for (24)h, whilst the lowest concentrations (16 µg/ml) had been inhibited. Selectivity Index (SI), which indicates the cytotoxicity of the compound. Selectivity in opposition to most cancers cells even as preserving normal cellular fitness, turned into measured using a ratio of IC50 values for each cell line. For human cells, the Ag(I) complicated confirmed selective cytotoxicity towards cancer cellular traces with IC50 = 165 µg/ml, however it became very healthful on ordinary cell lines with IC50 = 261 µg/ml. Table 6 and 7 show survival rate values have been acquired after 24h of treatment with different concentrations of the chosen compounds in breast cancer and HEK-293 cells, as well as IC50. Figures (8-11) show the survival rates of breast most cancers cells and HEK-293 cells observed with the chosen compounds [71].

Table 5. Evaluation of cytotoxicity of (L), MCF-7 cancer cell line after incubation (24 hr) at (37oC) and HEK-293 cell line.

| Concentration (µg/mL) | LH | | | | | |
|---|---|---|---|---|---|---|
| | Cancer line cells MCF-7 | | | Normal line cells HEK-293 | | |
| | Cell Viability | | % Cell Inhibition | Cell Viability | | % Cell Inhibition |
| | Mean | SD | | Mean | SD | |
| 16 | 92.12547 | 4.49845 | 7.87453 | 95.78005 | 11.59981 | 4.21995 |
| 31 | 85.41347 | 4.22192 | 14.58653 | 91.30435 | 13.31594 | 8.69565 |
| 62 | 79.82014 | 3.11996 | 20.17986 | 87.0844 | 8.28857 | 12.9156 |
| 125 | 74.62163 | 8.03131 | 25.37837 | 82.99233 | 6.10766 | 17.00767 |
| 250 | 18.97346 | 2.19184 | 81.02654 | 79.92327 | 2.39782 | 20.07673 |
| 500 | 6.75586 | 2.50077 | 93.24414 | 72.63427 | 7.22478 | 27.36573 |
| IC50 | 127 | | | 438.99 | | |

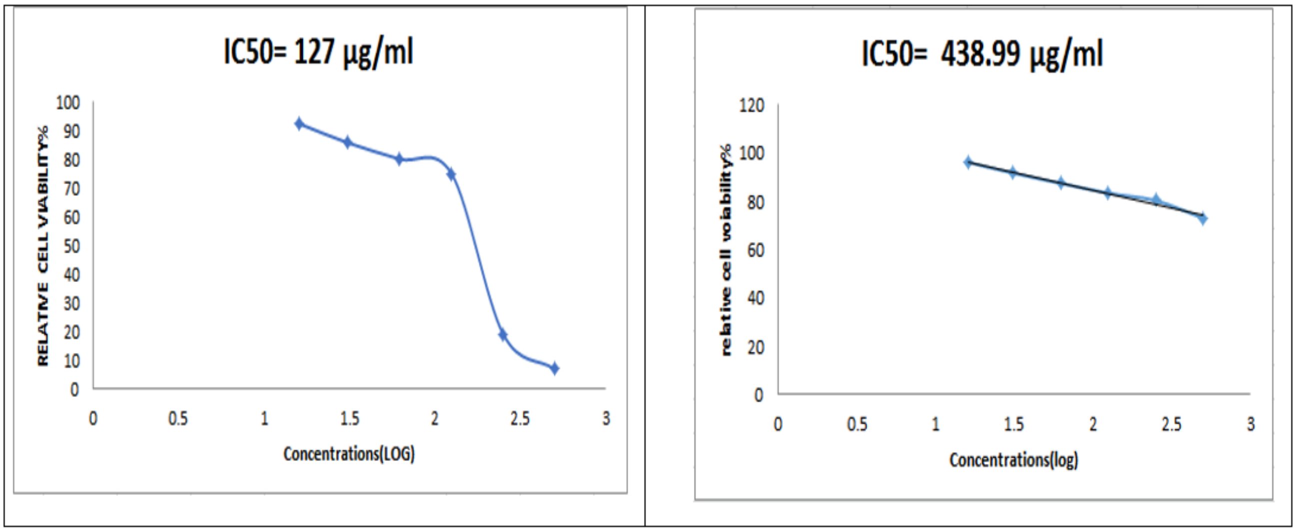


*Fig. (7): IC50 for ligand (LH) in MCF-7 cancer cell line and the normal cells HEK-293.*

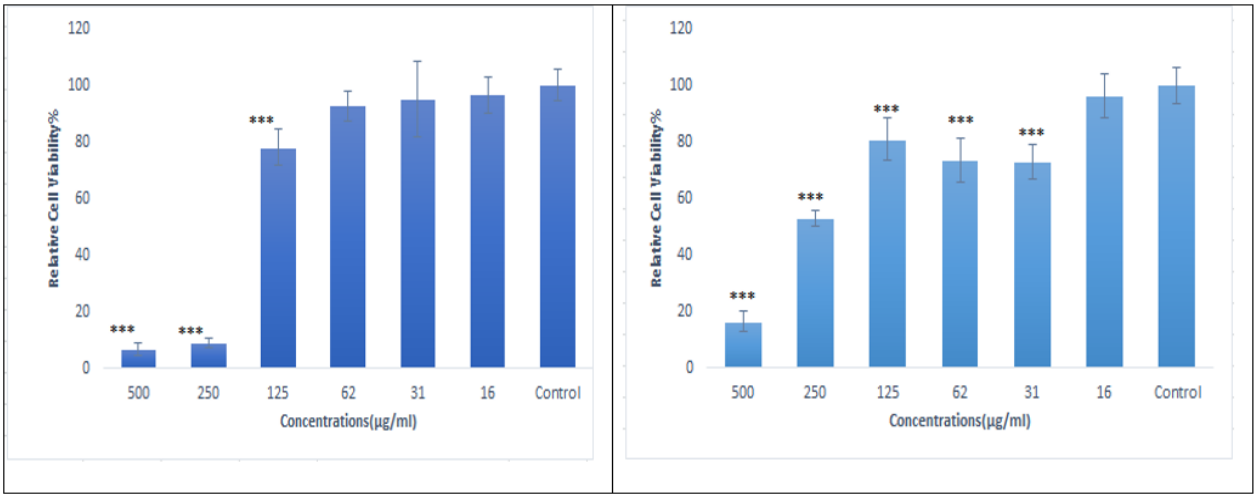


*Fig. (8): % Cell viability for ligand (L) MCF-7 cancer cell line and the normal cells HEK-293.*

*Table 6. Evaluation of cytotoxicity of Ag(I)-Complex in MCF-7 cancer cell line after incubation (24 hr) at (37oC) and HEK-293 cell line.*

| Concentration (μg/mL) | Ag(I)-Complex | | | | | |
|---|---|---|---|---|---|---|
| | Cancer line cells MCF-7 | | | Normal line cells HEK-293 | | |
| | Cell Viability | | % Cell Inhibition | Cell Viability | | % Cell Inhibition |
| | Mean | SD | | Mean | SD | |
| 16 | 96.60013 | 6.525412 | 3.39987 | 96.0084 | 7.838134 | 3.99160 |
| 31 | 94.80149 | 13.35768 | 5.19851 | 72.68908 | 6.040784 | 27.31092 |
| 62 | 92.60803 | 5.452864 | 7.39197 | 73.21429 | 7.692347 | 26.78571 |
| 125 | 78.04343 | 6.220471 | 21.95657 | 80.67227 | 7.520891 | 19.32773 |
| 250 | 8.86159 | 1.476512 | 91.13841 | 52.83613 | 2.940426 | 47.16387 |
| 500 | 6.749287 | 2.141133 | 93.25072 | 16.28151 | 3.640581 | 83.71849 |
| IC50 | 165 | | | 261 | | |

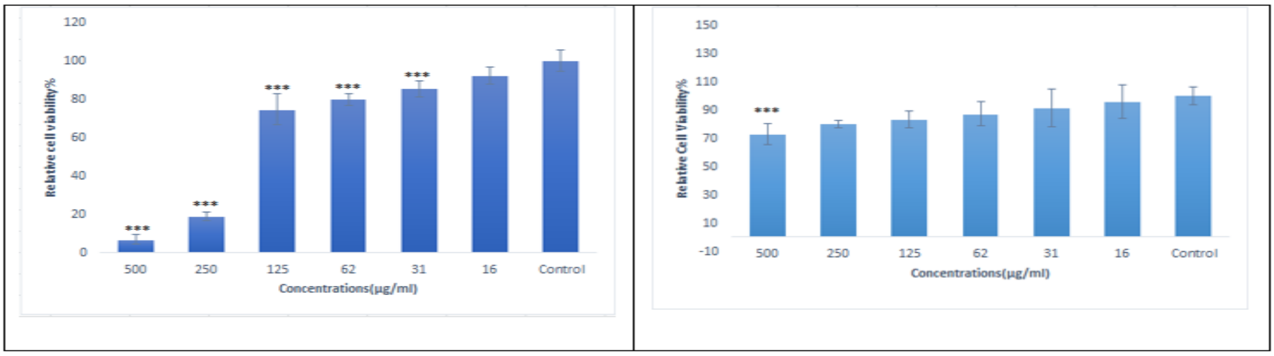


*Fig. (9): % Cell viability of Ag(I)-Complex MCF-7 cancer cell line and the normal cells HEK-293.*

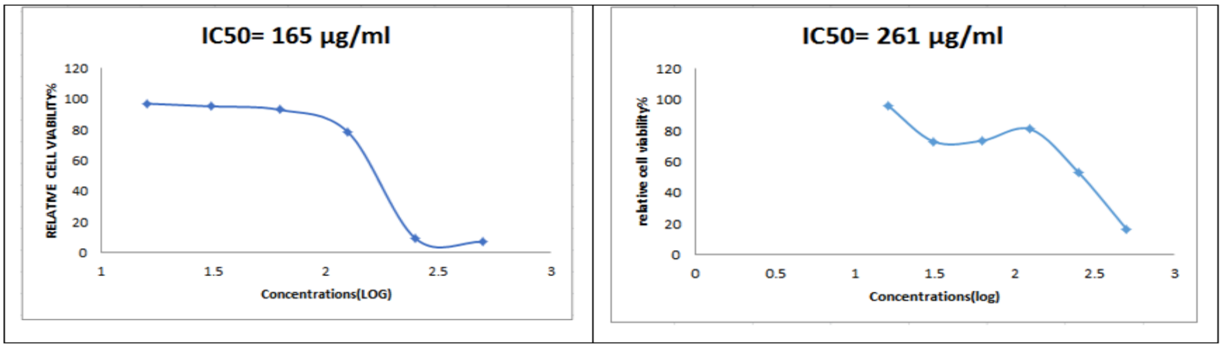


*Fig. (10): IC50 for Ag(I)-Complex in MCF-7 cancer cell line and the normal cells HEK-293.*

## 5. Conclusions

Five complexes were prepared with metal ions Ni (II), Cu (II), Ag (I), Hg (II) and Cd (II) derived from Schiff base-type ligand. Various analytical methods were used to determine the structures of the ligand (LH) and its metal complexes, The most important of these techniques are elemental analysis, molar conductivity, atomic absorption, magnetic susceptibility measurements, melting points, X-ray diffraction, and scanning electron microscope, in addition to spectroscopic methods (1H-NMR, IR, and UV-Vi's spectra) and molar ratio measurements, which proved that the ratio of metal to the ligand (1:1). As a result of the molar conductivity measurements, it was found that the chloride ion is present outside the coordination sphere in zinc (II) and cadmium (II) complexes only which gives the electrolyte nature of 1:1, but not in the rest of the other metal complexes. Infrared spectra proved that the tetradentate ligand is coordinated to the metal ions through the nitrogen atoms of the azomethine groups, the nitrogen of the secondary amine group and the oxygen atom of the hydroxyl group as the donor atoms.In the cytotoxicity assays performed on ligand (LH) and Ag(I) complex the use of breast cancer cellular line MCF-7 , and as compared with HEK-293 , the anticancer performance assayed the viability of the cells, it is able to be concluded that the ligand and Ag(I) complex have good cytotoxic property and selectivity towards breast cancer cell line (MCF-7) cells.

## Reference

[1] AL-Rawi, Muna S., Huda A. Hassan, and Dheefaf F. Hassan. "Synthesis, anti-bacterial and anti-cancer activities of Some Antipyrine Diazenyl Benzaldehyde Derivatives and Antipyrine-Based Heterocycles." *Iraqi National Journal of Chemistry* 17.2 (2017).

[2] Alsahib, S. A., and S. R. Baqer. "Synthesis, identification of some new derivatives of 4-amino antipyrine bearing six and seven membered rings." *Journal of Physics: Conference Series*. Vol. 1853. No. 1. IOP Publishing, 2021.

[3] Mnguni, Malitsatsi J., and Andreas Lemmerer. "A structural study of 4-aminoantipyrine and six of its Schiff base derivatives." *Acta Crystallographica Section C: Structural Chemistry* 71.2 (2015): 103-109.

[4] Al-Labban, Hutham Mahmood Yousif, Hawraa Mohammed Sadiq, and Ahmed Abduljabbar Jaloob Aljanaby. "Synthesis, Characterization and study biological activity of some Schiff bases derivatives from 4-amino antipyrine as a starting material." *Journal of Physics: Conference Series*. Vol. 1294. No. 5. IOP Publishing, 2019.

[5] Shoaib, Mohammad, et al. "Synthesis of 4-aminoantipyrine derived Schiff bases and their evaluation for antibacterial, cytotoxic and free radical scavenging activity." ||| *Bangladesh Journal of Pharmacology*||| 10.2 (2015): 332-36.

[6] Abdul khaderJailani, N. M., A. Xavier, and A. Ramu. "Synthesis and Characterisation of Transition Metal Complexes Derived from 4-Aminoantipyrine and Thiosemicarbazide Based Schiff Base." *Moroccan Journal of Chemistry* 6.4 (2018): 6-2.

[7] Yasar, Qazi, and Zahid Zaheer. "4-aminoantipyrine analogs as anti-inflammatory and antioxidant agents: synthesis, biological evaluation and molecular docking studies." *International Journal of Pharmaceutical Investigation* 11.1 (2021): 14-22.

[8] Kurdekar, Gurunath S., et al. "4-Aminoantipyrine-based Schiff-base transition metal complexes as potent anticonvulsant agents." *Medicinal Chemistry Research* 21.9 (2012): 2273-2279.

[9] Alheety, Nuaman. "Synthesis, Characterization and Antimicrobial Activity Study of Some New Substituted Benzoxazole Derivatives." *Baghdad Science Journal* 16.3 (2019): 616-616.

[10] Nayak, D. N., and N. Panda. "Synthesis of 2-aryl benzoxazoles from aldoximes." *MOJ Biorg Org Chem* 1.5 (2017): 176-182.

[11] Kamal, U. D. D. I. N., NAIM MOHD Javed, and K. U. M. A. R. Arun. "Biological potential of benzoxazole derivatives: an updated review." *Asian J Pharm Clin Res* 13.8 (2020): 1-14.

[12] Kumar, S. U. N. I. L., et al. "Synthesis and evaluation of some benzothiazole derivatives as antidiabetic agents." *Int. J. Pharm. Pharm. Sci* 9.2 (2017): 60

[13] Ramineni, Srinivasulu, Ravi Kumar Kannasani, and VV Satyanarayana Peruri. "Efficient one-pot synthesis of benzoxazole derivatives catalyzed by Zinc triflate." *Green Chemistry Letters and Reviews* 7.1 (2014): 85-89.

[14] Kakkar, Saloni, et al. "Benzoxazole derivatives: design, synthesis and biological evaluation." *Chemistry Central Journal* 12.1 (2018): 1-16.

[15] Porcelli, L., et al. "Synthesis, characterization and biological evaluation of ureidofibrate-like derivatives endowed with peroxisome proliferator-activated receptor activity." *Journal of medicinal chemistry* 55.1 (2012): 37-54.

[16] Chikhale, Rupesh V., et al. "Facile and efficient synthesis of benzoxazole derivatives using novel catalytic activity of PEG-SO3H." *Arabian Journal of Chemistry* 10.5 (2017): 715-725.

[17] Laeeq, S. H. A. I. N. D. A., ANUP K. Sirbaiya, and HEFAZAT H. Siddiqui. "Benzoxazole: progress report on chemistry, synthesis and biological activities." *Indo American journal of Pharmaceutical Research* 3.12 (2013): 8550-8562.

[18] Jamel, Hayder Obaid, et al. "Synthesis and Characterization of the Ligand (S, S'-Bis (Benzo [d] Oxazol-2-yl) 2, 2'-((Methylenebis (4, 1 Phenylene)) Bis (Azanediyl)) Diethanethioate (BMPAE) and it's Complexes with Some Transition Metals Ions." *Journal of Global Technology* 8.9 (2017): 76-85.

[19] Kumar, K. Ravi, P. V. V. Satyanarayana, and B. Srinivasa Reddy. "NaHSO 4-SiO 2-Promoted Solvent-Free Synthesis of Benzoxazoles, Benzimidazoles, and Benzothiazole Derivatives." *Journal of Chemistry* (2013).

[20] Susanne, Flavien, et al. "Match-making reactors to chemistry: a continuous manufacturing-enabled sequence to a key benzoxazole pharmaceutical intermediate." *Organic process research & development* 21.11 (2017): 1779-1793.

[21] Mohammed, Omar A., and Omar S. Dahham. "Synthesis, Characterization, and Study of Antibacterial Activity of Some New Formazan Dyes Derivatives, Derived from 2-Mercapto Benzoxazole."

*IOP Conference Series: Materials Science and Engineering*. Vol. 454. No. 1. IOP Publishing, 2018.

[22] Zhong, Wenying, et al. "Benzoxazole Derivative K313 Induces Cell Cycle Arrest, Apoptosis and Autophagy Blockage and Suppresses mTOR/p70S6K Pathway in Nalm-6 and Daudi Cells." *Molecules* 25.4 (2020): 971.

[23] Zheng, Xunan, Wei Liu, and Dawei Zhang. "Recent advances in the synthesis of oxazole-based molecules via van Leusen oxazole synthesis." *Molecules* 25.7 (2020): 1594.

[24] Ghoshal, Tanay, and Tarun M. Patel. "Anticancer activity of benzoxazole derivative (2015 onwards): a review." *Future Journal of Pharmaceutical Sciences* 6.1 (2020): 1-24.

[25] Shallal, Muhammed Abdel Hasan. "Synthesis, Coordination, Chemical Analysis, Biological Activity of Ligands (Imidazole, Oxazole, thaizole) Derivatives with Zn." Vol. 2. (2017).

[26] Ohshima, Asuka, et al. "The effect of meta-substitution on the photochemical properties of benzoxazole derivatives." *Bulletin of the Chemical Society of Japan* 80.3 (2007): 561-566.

[27] H Sultan, Asmaa. "Synthesis and Identification of some Benzoxazole Derivatives via Mannich Reaction." *Rafidain Journal of Science* 25.6 (2014): 49-54.

[28] Chang, Weichieh, Yukai Sun, and Yungtzung Huang. "One-pot green synthesis of benzoxazole derivatives through molecular sieve-catalyzed oxidative cyclization reaction." *Heteroatom Chemistry* 28.2 (2017): e21360.

[29] Mohammed, J. M., and Samaa A. Raouf. "Synthesis and Structural Studies on some Transition Metal Complexes of Bis-(benzthiazole-2-thio) Ethane, Pyridine and Bis-(benzoxazole-2-thio) Ethane Ligands." *International Journal of Enhanced Research in Science, Technology & Engineering ISSN* (2017): 2319-7463.

[30] Shah, Sayed Suliman, et al. "Synthesis and antioxidant activities of Schiff bases and their complexes: An updated review." *Bio Interface Res Applied Chem. vol* 10 (2020): 6936-6963.

[31] Hadi, Muna Abass, and Ibtihal Kadhim Kareem. "Synthesis, Characterization and Spectral Studies of a new Azo-Schiff base Ligand Derived from 3, 4-diamino benzophenone and its Complexes with Selected Metal Ions." *Research Journal in Advanced Sciences* 1.1 (2020).

[32] Kajal, Anu, et al. "Schiff bases: a versatile pharmacophore." *Journal of Catalysts* 2013 (2013).

[33] Kareem, Ibtihal Kadhim, Fawzi Yahya Waddai, and Ghusoon Jawad Abbas. "Synthesis, Characterization and biological activity of Some Transition Metal Complexes with New Schiff Base Ligand Type (NNO) Derivative from Benzoin." *Journal of Pharmaceutical Sciences and Research* 11.1 (2019): 119-124.

[34] Ibatte, S. N. "Synthesis and Characterization of N2O2 type Metal Complexes derived from 4-Aminoantipyrine, 4-Nitrobenzaldyhyde and Acetylacetone." *Asian Journal of Research in Chemistry* 10.2 (2017): 166-173.

[35] Al Zoubi, Wail, et al. "A new azo-Schiff base: Synthesis, characterization, biological activity and theoretical studies of its complexes." *Applied Organometallic Chemistry* 32.1 (2018): e3895.

[36] Alhaidry, Wesam Abed AL Hassan, and Hayder Obaid Jamel. "Synthesis and characterization of new benzothiazole-derived ligand and its complexes with some transitional metal ions with evaluation of their biological activities." *Journal of Pharmaceutical Sciences and Research* 10.12 (2018): 3241

[37] Kareem, Ibtihal Kadhim, and Muna Abass Hadi. "Synthesis and characterization of some transition metal complexes with new azo-schiff base ligand 3, 4-bis (((1E, 2E)-2-((2-((4-((Z)-(3-Hydroxyphenyl) Diazenyl) Naphthalen-1-yl) amino) ethyl) imino)-1, 2-Diphenylethylidene) Amino) Phenyl)(phenyl) Methanone." *Egyptian Journal of Chemistry* 63.1 (2020): 301-313.

[38] Speidel, K-H., O. Kenn, and F. Nowacki. "Magnetic moments and nuclear structure." *Progress in Particle and Nuclear Physics* 49.1 (2002): 91-154.

[39] Loffredo, Jeremy, et al. "Integrated multi-omic analyses reveals clinical relevance of endometrial cancer cell line models." *Gynecologic Oncology* 162 (2021): S11.

[40] Kumar, P., A. Nagarajan, and P. D. Uchil. "Analysis of cell viability by the MTT assay. Cold Spring Harb. Protoc. (6)." (2018). [41].

[41] Gasparini, Luciana S., et al. "In vitro cell viability by CellProfiler® software as equivalent to MTT assay." *Pharmacognosy magazine* 13. Suppl 2 (2017): S365.

[42] Al-Adilee, Khalid J., Hassan A. Habeeb Al-Shamsi, and Mohammed N. Dawood. "Synthesis, Spectral Characterization and photo Thermal Decomposition Studies of New Hetrocyclic Azo Dye Compound Derived from Imidazole with Some Transition Metal Complexes." *Research Journal of Pharmaceutical, Biological and Chemical Sciences* 7 (2016): 2882-2905.

[43] Al-Adilee, Khalid Jawad, and Azal Shakir Waheeb. "Preparation, spectroscopic and anticancer studies of metal antibiotic Chelation Ni (II), Cu (II) and Zn (II) 4, 5-dimethyl Thiazolylazo complexes." *Journal of Physics: Conference Series*. Vol. 1664. No. 1. IOP Publishing, 2020.

[44] Pluth, Michael D., and Zachary J. Tonzetich. "Hydrosulfide complexes of the transition elements: diverse roles in bioinorganic, cluster, coordination, and organometallic chemistry." *Chemical Society Reviews* 49.12 (2020): 4070-4134.

[45] Kumar, Jat Rakesh, Jat Jawahar L, and D. P. Pathak. "Synthesis of benzimidazole derivatives: as anti-hypertensive agents." *E-Journal of chemistry* 3.4 (2006): 278-285.

[46] Uyar, Zafer, et al. "Synthesis of well-defined bisbenzoin end-functionalized poly (ε-caprolactone) macrophotoinitiator by combination of ROP and click chemistry and its use in the synthesis of star copolymers by photoinduced free radical promoted cationic polymerization." *Designed Monomers and Polymers* 20.1 (2017): 42-53.

[47] Jiang, Jie, et al. "Synthesis and characterization

of the ligand based on benzoxazole and its transition metal complexes: DNA-binding and antitumor activity." *Journal of Inorganic Biochemistry* 104.5 (2010): 583-591.

48] Issa, Y. M., H. B. Hassib, and H. E. Abdelaal. "1H NMR, 13C NMR and mass spectral studies of some Schiff bases derived from 3-amino-1, 2, 4-triazole." *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 74.4 (2009): 902-910

[49] Kim, Ji-Heung, et al. "Synthesis and characterization of novel aromatic condensation polymers containing rigid benzoxazole pendent groups." *Journal of applied polymer science* 92.1 (2004): 178-185.

[50] Presterl, Elisabeth, et al. "Bacteriology: Selected bacteria and diseases." *Basic Microbiology and Infection Control for Midwives*. Springer, Cham, 2019. 67-84.

[51] Şahin, Ömer, et al. "New platinum (II) and palladium (II) complexes of coumarin-thiazole Schiff base with a fluorescent chemosensor properties: Synthesis, spectroscopic characterization, X-ray structure determination, in vitro anticancer activity on various human carcinoma cell lines and computational studies." Journal of Photochemistry and Photobiology B: Biology 178 (2018): 428-439.

[52] Heravi, Majid M., and Vahideh Zadsirjan. "Prescribed drugs containing nitrogen heterocycles: an overview." RSC advances 10.72 (2020): 44247-44311.

[53] Aleabi, Suad H., Mahmood Radhi Jobayr, and Shatha H. Mahdi1and Ebtisam MT Salman. "Properties evaluation of a composite of unsaturated polyester resin reinforced with mullite." *Solid State Technology* 63.1 (2020).

[54] Samota, Mahesh Kumar, and Gita Seth. "Synthesis, characterization, and antimicrobial activity of palladium (II) and platinum (II) complexes with 2-substituted benzoxazole ligands." Heteroatom Chemistry: An International Journal of Main Group Elements 21.1 (2010): 44-50.

[55] Sarkar, Shuranjan, et al. "Structure and Heme-Independent Peroxidase Activity of a Fully-Coordinated Mononuclear Mn (II) Complex with a Schiff-Base Tripodal Ligand Containing Three Imidazole Groups." *Bulletin of the Korean Chemical Society* 31.11 (2010): 3173-3179.

[56] Sathiyaraj, Subbaiyan, Ganesan Ayyannan, and Chinnasamy Jayabalakrishnan. "Synthesis, spectral, DNA binding and cleavage properties of ruthenium (II) Schiff base complexes containing PPh3/AsPh3 as co-ligands." *Journal of the Serbian Chemical Society* 79.2 (2014): 151-165.

[57] Sattar, Rabia, et al. "Synthetic transformations and biological screening of benzoxazole derivatives: A review." *Journal of Heterocyclic Chemistry* 57.5 (2020): 2079-2107

[58] Seijas, J. A., and M. P. Vázquez-Tato. "Carballido, -Reboredo, M. R; Crecente-Campo, J." *Synlett* 313 (2007).

[59] Singh, Laliteshwar P., et al. "Synthesis and antimicrobial activity of some 2-phenyl-benzoxazole derivatives." *Der Pharma Chemica* 2.4 (2010): 206-212.

[60] Slabbert, C., and M. Rademeyer. "One-dimensional halide-bridged polymers of metal cations with mono-heterocyclic donor ligands or cations: A review correlating chemical composition, connectivity and chain conformation." *Coordination Chemistry Reviews* 288 (2015): 18-49.

[61] Bhunia, Falguni, Nimai Chandra Saha, and Anilava Kaviraj. "Effects of Aniline–an aromatic amine to some freshwater organisms." *Ecotoxicology* 12.5 (2003): 397-404.

[62] So, Ying-Hung, et al. "Synthesis and photophysical properties of some benzoxazole and benzothiazole compounds." *Macromolecules* 29.8 (1996): 2783-2795.

[63] Gaber, Mohamed, et al. "Chromone Schiff base complexes: synthesis, structural elucidation, molecular modeling, antitumor, antimicrobial, and DNA studies of Co (II), Ni (II), and Cu (II) complexes." *Journal of the Iranian Chemical Society* 16.1 (2019): 169-182.

[64] Xu, Ran, et al. "Computer-aided blended extractant design and screening for co-extracting phenolic, polycyclic aromatic hydrocarbons and nitrogen heterocyclic compounds pollutants from coal chemical wastewater." *Journal of Cleaner Production* 277 (2020): 122334

[65] Moharram, A. M., et al. "Biodiversity of microbiota in cephalosporin-manufacturing environments at T3A factory, Assiut, Egypt." *Journal of Basic & Applied Mycology (Egypt)* 5 (2014): 1-13.

[66] El-Sonbati, A. Z., et al. "Geometrical structures, thermal stability and antimicrobial activity of Schiff base supramolecular and its metal complexes." *Journal of Molecular Liquids* 215 (2016): 423-442.

[67] Lee, Taekhee, et al. "Asbestos fiber length and width comparison between manual and semi-automated measurements." *Journal of Occupational and Environmental Hygiene* (2022): 1-11.

[68] Javed, Irfan, Seung Wook Baek, and Khalid Waheed. "Effects of dense concentrations of aluminum nanoparticles on the evaporation behavior of kerosene droplet at elevated temperatures: The phenomenon of microexplosion." *Experimental thermal and fluid science* 56 (2014): 33-44.

[69] Kolthoff, I. M_. "Acid-base equilibriums in dipolar aprotic solvents." *Analytical Chemistry* 46.13 (1974): 1992-2003.

[70] Nicholls, David. Complexes and first-row transition elements. Macmillan International Higher Education, 2017.

[71] Kyhoiesh, Hussein Ali Kadhim, and Khalid J. Al-Adilee. "Synthesis, spectral characterization, antimicrobial evaluation studies and cytotoxic activity of some transition metal complexes with tridentate (N, N, O) donor azo dye ligand." *Results in Chemistry* 3 (2021): 100245.