Preparation, spectroscopic and anticancer studies of metal antibiotic Chelation Ni(II), Cu(II) and Zn(II) 4,5-dimethyl Thiazolyazo complexes

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Abstract
This work included synthesis of new ligand 2-[2- (4,5-Dimethyl thiazolylazo)- 4,6-dimethyl phenol (DMeTADMeP) by coupling of diazonium salt of 2-amino-4,5-dimethyl thiazole with (2,4-dimethylphenol). Also, preparation of new series of chelate complexes of Ni(II), Cu(II) and Zn(II) metal ions were also prepared by mixing solution of metal salts with solution of ligand at mole ratio[ M:L] [1:2] for Ni(II), Cu(II) and Zn(II) metal ions. The structures of the azo dye ligand and its metal complexes were confirmed by various analytical techniques like by UV-visible, FT-IR, Mass spectroscopic, 1H-NMR, 13C-NMR, (C,H,N,S) element analyses, molar conductance, FESEM and XRD diffraction studies. The antibacterial and antifungal activity of thiazolyl azo dye and its metal complexes were shown activity towards both bacterial Escherichia coli (gram negative), streptococcus (gram positive) and Penicillium as antifungal. The results shows that this kind of compounds plays an important role in the rate of inhibition of the growth of bacterial. The anticancer activity was also conducted of the (DMeTADMeP) and Cu(II)-complex by cells cytotoxicity and screen for in vitro antitumor activity against human Hepatocellular cancer. The results indicated that this type of this compounds play an important role in the rate of inhibition of the growth cells of cancerous, undoubtedly the possibility of using them as anti-cancer drugs in the field of medicine and pharmacy.

1. Introduction
The chemistry of heterocyclic azo compounds represent very interesting field in the study for a long time. In recent years, azo compounds constitute one of the important classes of industrially synthesized organic compounds, potent in drug and cosmetics (1). Thiazolyl azo compounds contain two hybrid atoms, nitrogen and sulfur, this type of compounds is very importance in the field of chemical analysis because its contain more than a group of effective have the ability to form coordinate complexes with different metal ions (2,3). Thiazole compounds are an important class of chemical constituents having various applications in many fields such as leather, polymer, paint and coating industries as dyeing agent (4-6), Also used in chemical operations as spectrophotometry, solid phase extraction, liquid chromatography and electrochemistry (7,8). Previous research has shown that it is an undoubtedly fact that thiazole based-compounds had effective pharmacological importance with antimicrobial (9-11), antitumor (12,13), anti-diabetic (14,15), anticonvulsant (16,17), anti-inflammatory (18), antioxidant (19,20) and more activity properties. Thiazole derivatives are medical important compounds due to the fact that 75% of the available drugs in the market are heterocyclic based drugs and it is considered as one of an important category of the heterocyclic compounds (21-22).
Currently, cancer is one of the critical health problems of the human population and prominent by uncontrolled mechanisms that direct cell proliferation and differentiation \(^{(23)}\). In this study, we report the preparation, identification of new ligand 2-[2- (4,5-Dimethyl thiazolyl)azo]-4,6-dimethyl phenol (DMeTADMeP) and its complexes Ni(II), Cu(II) and Zn(II) ions. The synthesis compounds were studied by various spectral analysis and screened for their biological activities against \textit{Escherichia coli}, \textit{streptococcus}\ as antibacterial and \textit{Penicillium sp}. as antifungal. Moreover, the study of ligand and Cu(II) complex of prescription drug anticancer by using the lines of Hepatocellular carcinoma cells of the type HePG2 and compared with line of the normal cells by MTT assay.

2. Experimental

2.1. Materials and Measurements

In this paper all chemicals and solvents have highest purity provided from many companys BDH, Aldrich, sigma and Merck. Mass spectra was taken by using direct probe electron impact agilent 5973 mass spectrometer, \(^1\)H- NMR spectra were recorded usage DMSO-\(d_6\) as a solvent and TMS as an interior reference on a Bruker 500 MHZ spectrophotometer, Infrared spectra from all synthesis compounds are recorded as KBr discs using a Shimadzu 8400S FT-IR spectrophotometer at the wave number (4000-400) cm\(^{-1}\). Elemental analysis (C.H.N.S) were collected on a Euro EA 1106 elemental analyzer. The electronic spectra of the azo ligand (DMeTADMeP) and its metal complexes measured in absolute ethanol as a solvent (10\(^{-5}\)M) in the range of (200-1100) nm using PerkinElmer double beam UV-VIS spectrometer. Magnetic susceptibility measurements for the prepared complexes carried out on a balance magnetic MSB - MK at room temperature using faraday method. X-ray diffraction (XRD) was measured using Siemens model D500. Field-emission Scanning Electron Microscope (FESEM) images of ligand and its metal complexes were taken using TESCAN BRNO-Mira3 LMU made by French-Czech. Molar conductivity measurements were recorded on conductivity with ethanol solution (10\(^{-3}\) M) by using a 31A digital conductivity meter at room temperature.

2.2. Synthesis of the new thiazolyl Azo Dye Ligand

The new thiazolyl azo dye ligand (DMeTADMeP) was synthesized by following methods proposed by researcher Al- Adilee. \textit{etal} \(^{(24-26)}\) with some modification, including two-step, the first step it has been dissolved 2-amino- 4,5- dimethyl thiazol (1.3g,0.01mol) in mixture of 5 ml hydrochloric acid and 40 ml distilled water and diazotized below 5 °C with sodium nitrite NaNO\(_2\) (0.75 gm,0.01 mol), dissolved in (30) ml distilled water was added drop-wise with cooling . In the second step the diazonium salt compound was coupled with (1.5g,0.01mol) of 2,4 -dimethylphenol in alkaline media below 5 °C. The color of the solution was observed in reddish brown. After that stirred the mixture for one hour in an ice-bath and allowed to stand overnight and filtrate it, then wash the precipitate with distilled water several times and purified by recrystallization using the absolute ethanol solution and then dry the precipitate in oven at 50\(^{°}\) C.
for a few hours. The structure was verified by $^1$H-NMR, $^{13}$C-NMR, mass spectrum, Infrared spectra and UV-Vis spectrometry. The Scheme (1) below refers the processes of the diazotization coupling:-

Scheme 1. Synthesis of Azo dye ligand(DMeTADMeP)

2.3. General method of the preparation of metal complexes

The metal complexes were prepared by the addition of suitable metal salts chlorides with the ligand (DMeTADMeP) amount of 0.3 gm (0.002 mole) from ligand, dissolved in 30 mL of absolute ethanol was gradually added with stirring a stoichiometric to (0.001 mole) amount of [1:2] [M:L], for each Ni(II), Cu (II) and Zn(II) chloride salt dissolved in 30 mL hot buffer solution (ammonium acetate) at pH = 7. The mixture of this complex was heated to (50-70) °C at 30 minute, then left all complexes overnight. The precipitates were filtered off, washed with distilled water and little warm ethanol to remove any traces of unreacted materials. After that the obtained complexes were dried under vacuum desiccators over fused CaCl$_2$. The analytical and physical data for synthesized compounds are listed in Table (1).

Table (1):- Analytical and physical data of the ligand (DMeTADMeP) and its complexes.

| Compound | Colour       | m.p °C | Yield (%) | M.f (M.wt)         | Elemental analysis (%): (Found) calc. |
|----------|--------------|--------|-----------|--------------------|---------------------------------------|
| Ligand LH(DMeTADMeP) | reddish brown | 121    | 84        | C$_2$H$_6$N$_2$SO (261.34) | C% 59.75 H% 5.78 N% 16.08 S% 12.26 ........... |
| [Ni(L)$_2$].H$_2$O | green        | 136    | 73        | C$_2$H$_6$N$_2$SO$_2$Ni (597.39) | (60.16) (5.84) (16.54) (12.48) |
| [Cu(L)$_2$].H$_2$O | Bluish green | 191    | 81        | C$_2$H$_6$N$_2$SO$_2$Cu (602.246) | (52.63) (5.15) (14.85) (10.96) (9.82) (10.21) |
| [Zn(L)$_2$].H$_2$O | Greenish purple | 147   | 69        | C$_2$H$_6$N$_2$SO$_2$Zn (604.086) | (51.89) (5.00) (13.91) (10.87) (10.61) (10.82) |

3. Results and Discussion

3.1. Physical and chemical properties of the azo dye ligand (DMeTADMeP) and its metal complexes
The heterocyclic azo dye ligand (DMeTADMeP) was reddish brown crystals but the metal complexes of this ligand vary in colour by depending on the metal ions. All synthesis compounds were soluble in common organic solvents such as methanol, acetone, ethanol and chloroform but completely soluble in dimethylsulfoxide and dimethylformamide. Additionally, the metal complexes derived from azo dye ligand (DMeTADMeP) were stable toward air and sparingly soluble in distilled water indicated the stability of the complexes. Table (1) represent some physical and analytical data.

### 3.2. Metal :Ligand Ratio
The possible structural formula for the formation metal complexes was studied by molar ratio method at the wave lengths of maximum absorption . The results are summarized in Table (2) by used UV-visible spectra from this purpose. There are various methods used to determine the mole ratio such as continuous variation method introduced by the (Job) and modified by (Copper and Vosbury) . The color solutions of metal complexes increase the intensity as an approach point of intersection ratio [M:L] and the continued stability of colour at passing this point which indicates that the metal complexes formed in constant of solution. The molar ratio [M:L] in all metal complexes was found to form [1:2], these results are in agreement with the values reported for some thiazolylazo complexes.

### 3.3. Conductivity Measurements
The molar conductance of prepared complexes were studied at room temperature in ethanol as a solvent , these values indicating that there is no conductivity existence and appear no chloride ions are present outside the coordination spheres . The results are seen in Table (2).

### 3.4. Calculation stability constants (β)
Stability constants (β) of complexes are obtained by measuring the absorbance of the ligand solution and metal ion solution at fixed wavelength (λ\text{max} ) ,and optimum concentration at pH = 7. The degree of formation of the complexes are calculated from the relationship, β = (1-α)/4α \text{C}^2 and α = Am-As/Am, where Am and As are the absorbance of fully and partially formed metal complex respectively at optimum concentration . The stability follows the sequence: Ni(II) > Cu(II) > Zn(II). Also, this agreement with Irving-Williams series of stability constant . The stability constants values for the prepared metal complexes are given in Table (2).

### Table (2): Maximum Wavelength (λ\text{max}),Optimal Concentration, Stability Constants Values (β and Logβ ) and Molar Conductivity of Complexes.

| Metal ion | Optimal Conc. X10^{-4} | λ\text{max} (nm) | β L^2.M^{-2} | Log β | MolarCond. S.cm^{-1}.mol^{-1}.1 |
|-----------|------------------------|-----------------|-------------|-------|---------------------------------|
| Ni(II)    | 1.5                    | 622nm           | 43.1 x 10^4 | 9.63  | 12.06                           |
| Cu(II)    | 1.75                   | 648nm           | 21.1 x 10^4 | 9.32  | 10.29                           |
| Zn(II)    | 2.0                    | 617nm           | 11.87 x 10^4| 9.07  | 9.76                            |

### 3.5. 1H-NMR Spectra
The 1H-NMR Spectra was carried out in DMSO-d6 as solvent by using TMS as an internal reference. The 1H-NMR Spectra of azo dye ligand (Figure 1) shows a single peak at δ =11.01ppm (s, 1H) due to the
presence of the OH group in 16 position. The singlet peaks of phenyl protons in positions (11-H and 13-H) are observed at \( \delta = 6.97 \text{ppm} \) (s, 2H) and \( \delta = 7.19 \text{ppm} \) (s, 2H) respectively. While the singlet peaks of the protons methyl groups are observed at \( \delta = 2.34, 2.25, 2.41 \) and \( 2.20 \text{ppm} \) (s, 12H) respectively in 6, 7, 17 and 18 positions. The singlet at \( \delta = 2.29 \text{ppm} \) (s, solvent proton) \(^{(26)}\).

3.6. \(^{13}\)CNR Spectra

The \(^{13}\)C NMR spectrum observed several chemical shift \(^{13}\)C = (10.37, 14.68, 15.33, 19.84, 129.45, 125.46, 95.45, 128.77, 136.96, 137.25, 150.54, 155.97 and 161.69 ppm) to the carbon atoms at the sites, (6, 7, 18, 17, 14, 5, 10, 11, 12, 13, 4, 15, 2) respectively. The spectrum also showed a singlet signal of solvent (DMSO) at the chemical shift \(^{13}\)C = (39.89) ppm. \(^{13}\)CNR spectra of the thiazolyl azo ligand is illustrated in (Figure 2).
3.7. Mass spectral studies

The mass spectrum of the ligand (DMeTADMeP) (Scheme 2, Figure 3) assigned to molecular peak ion \([M^+]\) at \(m/z^+ = 261.1\) is corresponding to the original molecular weight of ligand (261.34). The mass spectrum of azo dye ligand showed several peaks attributed to the molecular ions at \((m/z^+)\) 232 , 216 , 204 , 190 , 77 and 53 were due to various fragments ions \([\text{C11H10N3OS}]^+, [\text{C11H10N3S}]^+, [\text{C10H10N3S}]^+, [\text{C9H8N3S}]^2+, [\text{C6H5}]^2+\) and \([\text{C4H5}]^+\) respectively. On the other hand, the mass spectrum of Cu(II) complex (Scheme 3, Figure 4) observed \([\text{M}^+]\) at \(m/z^+ = 602.246\) is due to the molecular weight supporting the suggested structure form the prepared complex \((7,25)\). The molecular ion peaks of Cu(II) - complex display different \(m/z^+\) values with different intensities. The mass spectrum contain molecular ion peaks at \((m/z^+)\) 602, 322, 268, 226, 166, 140, 127 and 67 were due to various fragments ions \([\text{C26H28N6S2O2Cu}]^+, [\text{C13H13N3SOCu}]^+, [\text{C11H13N2SCu}]^+, [\text{C9H8NSCu}]^+, [\text{C9H12NS}]^+, [\text{C8H12S}]^+ [\text{C7H12S}]^+\) and \([\text{C5H7}]^+\) respectively. This data is in good agreement with the corresponding molecular formulae.

Figure 3: Mass spectrum of thiazolylazo dye ligand (DMeTADMeP).

Figure 4: Mass spectrum of Cu(II) complex \([\text{Cu(L)}_2].\text{H}_2\text{O}\)
Scheme (2): Mass spectrum fragmentation of thiazolylazo dye ligand (DMeTADMeP).

Scheme (3): Mass spectrum fragmentation of Cu(L)₂.H₂O.
3.8. Infrared spectra studies

IR spectra have proven to be the most suitable technique to give enough information to elucidate the nature of bonding of the ligand and complexes. The spectra of all metal complexes Ni (II), Cu(II) and Zn (II) complex showed medium broad bands around (3329, 3360, 3566) cm\(^{-1}\) suggesting the existence of water molecules in the structure of metal complex\(^{(25)}\). The many shifts in the position or change in the shape of the complexes bands compared with those absorption bands of free ligand due to the formation of the metal complexes bands. The IR spectra of all complexes, there are new bands observed which are never been observed in the azo dye ligand spectrum, this may attributed to \(\nu(M\rightarrow O)\) and \(\nu(M\rightarrow N)\). Thus, the IR spectral data lead to suggest that the ligand (DMeTADMeP) behaves as a tridentate chelating agent coordinating with metal ions\(^{(33,34)}\) and their probable assignments are given in Table (3). Figures 5 represent the spectra in (cm\(^{-1}\)) data of ligand, Ni(II), Cu(II) and Zn(II) complexes.

Table (3):- The IR frequencies (in cm\(^{-1}\)) data of ligand and its complexes.

| Group | Ligand (DMeTADMeP) | Ni(II) Complex | Cu(II) Complex | Zn(II) complex |
|-------|--------------------|---------------|----------------|---------------|
| \(\nu\) OH | 3466 W.br | 3329. m.br* | 3360 m.br* | 3566.50 m.br* |
| \(\nu\) (CH\(_3\))\(_2\) | 2918 W. | 2922 W. | 2922 W. | 2922 m.br |
| \(\nu\) (C-O) | 1278 S. | 1292 S. | 1286 S. | 1263 S. |
| \(\nu\) (C=N) | 1637 S. | 1641 m. | 1651 S. | 1676 S. |
| \(\nu\) (N=N) | 1496 S. | 1413 m. | 1448 m. | 1456 S. |
| \(\nu\) (C=C)Ph | 1566 S. | 1512 S. | 1597 m. | 1577 m. |
| \(\nu\) (C=S) Thia | 1215 S. | 1232 S. | 1219 m. | 1236 S. |
| \(\nu\) (C-N)Thia | 1120 W. | 1170. S. | 1012 m. | 1161 m. |
| \(\nu\) (M-O) | ............ | 667 W. | 601 W. | 642 W. |
| \(\nu\) (M-N) | ............ | 556 W. | 540 W. | 536 W. |

S=strong, m=medium, w=weak, br=broud, *= (H\(_2\)O) outside of sphere coordination.

Figure 5 : IR Spectrum of ligand and Cu and Zn(II)-complex
3.9. Electronic spectra studies

The electronic spectra of the azo dye ligand (DMeTADMeP) and complexes were studied in absolute ethanol (10^{-3} M) as a solvent at room temperature and used to confirm the geometry of the complexes. The characteristics of these bands are summarized in Table (4). The free ligand shows three bands in UV-visible region. The first band at 412 nm (24271.84 cm\(^{-1}\)) attributed to a n→π* transition of the azo (-N=N-) group, this band showed a red shift coordinated between the metal ion and the nitrogen atom of azo group \(^{29}\). The second band located at 325 nm (30769.23 cm\(^{-1}\)) corresponds to the π →π* transition of the (C=N) group of thiazole. While the third band observed at 270 nm (37037.04 cm\(^{-1}\)) is assigned to the π→π* transition of the (C-C) of thiazole and phenyl rings \(^{31}\). The electronic spectrum of Ni(II) complex exhibits three absorption bands in absolute ethanol (10^{-3} M) at about 622 nm (1677 cm\(^{-1}\)), 313 nm (31948.8 cm\(^{-1}\)) and 266 nm (37593.98 cm\(^{-1}\)) which may be attributed to \(^3\)A\(_2\)\(_g\)→\(^3\)T\(_2\)\(_g\)(F) (\(v_1\)), \(^3\)A\(_2\)\(_g\)→\(^3\)T\(_1\)\(_g\)(P) (\(v_2\)), and \(^3\)A\(_2\)\(_g\)→\(^3\)T\(_1\)\(_g\)(F) (\(v_3\)), transitions respectively in an octahedral structure \(^{35-37}\). The Cu (II) complex displays a broad asymmetric band around at 641 nm (15600.62 cm\(^{-1}\)). The broadness of this band indicates the three transitions \(^2\)B\(_1\)\(_g\)→\(^2\)A\(_1\)\(_g\) (dx\(^2\)-y\(^2\) → dz\(^2\)) (\(v_1\)), \(^2\)B\(_1\)\(_g\)→\(^2\)B\(_2\)\(_g\) (dx\(^2\)-y\(^2\) → dyz) (\(v_2\)), and \(^2\)B\(_1\)\(_g\)→\(^2\)E\(_g\)(v\(_3\)), (charge transfer), which are of similar energy and gives rise to only one broad absorption band (\(^2\)B\(_1\)\(_g\)→\(^2\)E\(_g\)) and the structure of this complex distorted octahedral geometry (Z-in or Z-out) according to Jahn–Teller effect \(^{30,38,39}\). The electronic spectra of Zn (II) complex was studied in absolute ethanol (10^{-3} M) did not any d-d transitions because of the complex is diamagnetic having a d\(^{10}\) system the absorption band at 613 nm (16313.21 cm\(^{-1}\)) . The band may be assigned to a charge transfer [d\(\pi\)(M\(^{2+}\)→π*(L))] transition where π*(L) was believed to be primarily dominated by the LUMO of the azoimine chromosphere \(^{40,41}\). UV-Visible spectra of the ligand and its metal complexes are shown in Figure 6.

Table (4) :- Electronic spectra (in nm and cm\(^{-1}\)) , magnetic moments, proposed geometry and hybridization of complexes.

| Compounds                | \(\lambda_{\text{max}}\) (nm) | Absorption Bands(cm\(^{-1}\)) | Transitions | Meff (B.M) | Geometry          | Hybridization               |
|--------------------------|-------------------------------|-------------------------------|-------------|-------------|-------------------|-----------------------------|
| DMeTADMeP (LH)           | 412                           | 24271.84                      | n→π*        | ............ | ........            | ........                     |
|                          | 325                           | 30769.23                      | π→π*        | ............ | ........            | ........                     |
|                          | 270                           | 37037.04                      | π→π*        | ............ | ........            | ........                     |
| [Ni (L\(_2\)]           | 622                           | 16077.17                      | \(^3\)A\(_2\)\(_g\)→\(^3\)T\(_2\)\(_g\)(F) | 2.81        | Octahedral (Regular) | sp\(^3\)d\(^2\) (high spin) |
|                          | 313                           | 31948.88                      | \(^3\)A\(_2\)\(_g\)→\(^3\)T\(_1\)\(_g\)(P) |             |                   |                             |
|                          | 266                           | 37593.98                      | \(^3\)A\(_2\)\(_g\)→\(^3\)T\(_1\)\(_g\)(F) |             |                   |                             |
| [Cu (L\(_2\)]           | 641                           | 15600.62                      | \(^2\)B\(_1\)\(_g\)→\(^2\)E\(_g\) | Dia         | Octahedral (Distorted) (Z-in or Z-out) | sp\(^3\)d\(^2\) |
|                          | 613                           | 16313.21                      | d\(\pi\)(Zn\(^{2+}\)→π*(L\(_{\text{H}}\)) | Dia         | Octahedral (Regular) | sp\(^3\)d\(^2\) |


3.10. X-ray diffraction analysis

The (XRD) of ligand (DMeTADMeP) and complexes were scanned in the range 2θ = (5°-80°) value. The (XRD) patterns of ligand and complexes are illustrated in Figure 7 and the crystallographic data for ligand and complexes are summarized in Table (5). The ligand has sharp diffraction peaks indicating crystalline nature while for Ni (II) and Cu(II) complexes we re observed broad peak due to the amorphous nature. To determine some of the structural properties such as crystalline structure, and dislocation density to record their purity and defects in crystalline structure when converting the ligand to metal complex. The crystallite size is evaluated by the Scherrer equation (42,43):

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

Dislocation density can be calculate by using the equation (44):

$$\delta=\frac{1}{D^2}$$

Where $D_p$ = average crystallite size, $\beta$ = line broadening at half the maximum intensity in radians, k is Blanks constant (0.891), $\theta$ = Bragg angle, $\lambda$ = X-ray wavelength, S= Macrostrains, $\delta$= Dislocation density.

The result illustrated that some complexes have a crystallite size less than 100 nm, which is within the nanoscale. Furthermore, these results support our previous measurements of FESEM (Field-emission Scanning Electron Microscope) analysis.

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

| Compound | No | $2\theta_{observed}$ (°) | $d_{observed}$ (Å) | I/Io (%) | FWHM | Crystallite size (nm) | Lattice strain | $\delta_p X10^{10}$ (lin m$^{-2}$) |
|----------|----|---------------------------|---------------------|----------|------|----------------------|---------------|------------------|
| DMeTADMeP| 1  | 11.174                    | 7.91228             | 100      | 0.184| 45.33                | 0.0002        | 0.486            |
|          | 2  | 26.163                    | 3.40327             | 34.97    | 0.17 | 50.13                | 0.0032        | 0.397            |
|          | 3  | 11.62                     | 7.60720             | 22.13    | 0.16 | 52.15                | 0.0069        | 0.367            |
|          | 4  | 9.86                      | 8.96193             | 15.57    | 0.26 | 32.05                | 0.0132        | 0.973            |
|          | 5  | 13.47                     | 6.56662             | 8.49     | 0.24 | 34.83                | 0.0089        | 0.824            |
| [Ni (L)₂] | 1  | 8.36                      | 10.56379            | 100      | 0.40 | 20.81                | 0.0239        | 2.309            |
|          | 2  | 16.17                     | 5.47727             | 52.34    | 0.31 | 27.05                | 0.0095        | 1.366            |
Table 1: X-Ray diffraction spectra of thiazolylazo dye ligand (DMeTADMeP) with some complexes.

|   | 2θ (°) | Intensity (°) | Percentage | 2θ (°) | Intensity (°) | Percentage |
|---|-------|--------------|------------|-------|--------------|------------|
| 1 | 8.93  | 9.89671      | 100        | 2     | 20.98        | 4.23000    |
| 2 | 19.71 | 4.49972      | 39.01      | 4     | 26.22        | 3.39547    |
| 3 | 27.84 | 3.20151      | 30.44      | 5     | 12.33        | 7.17094    |

3.11. FESEM (Field-emission Scanning Electron Microscope) analysis.

This technique was used to study the surface properties such as the surface morphology, shape of particles and aggregates with a cross section of 1.44 m, and a magnification force of Mag = 100.00 KX from ligand and complexes. Furthermore, FESEM micrographs of the metal complexes showed that the surface morphology of metal complexes are changed by changing the metal ions (45,42). The FESEM of ligand seemed as a needle crystals shaped morphology with average size 177.15 nm. The analysis of FESEM for Ni(II) complex seemed as contiguous columns crystal type with average size 66.54 nm while the analysis of FESEM for Cu(II) seemed as peripheral spherical shape with average particle size 110.30 nm, either the FESEM image of Zn(II) complex seemed heterogeneous surfaced with average particle size 84.31 nm. Characteristics of the ligand and complexes it enabled us to study in the field of pharmacy and medicine, and the susceptibility of these compounds to eliminate and reduce of many types of cancers such as Hepatocellular cancer. The ligand and complexes showed different morphologies as illustrated in Figure 8.
Figure 8: FESEM images of (a) ligand (b) Ni (II-) complex (c) Cu (II)- complex (d) Zn (II)- complex.

4. Antimicrobial activity

4.1. Antibacterial and antifungal activity

The antibacterial activity study was performed by used cup-plate method (46,47), stock solutions of all synthesized compounds were prepared in DMSO and used in experiments. The antibacterial activity of the ligand (DMeTADMeP) and complexes were screened against two types of bacteria, *Streptococcus* (Gram-negative Bacteria) and *Escherichia coli* (Gram Negative Bacteria) and it is also evaluation of the antifungal activity by screen against the Penicillium sp. 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 Figure 9. Our experiment revealed that, the ligand and some transition metal complexes show good antimicrobial activity against tested bacterial and fungal. Therefore, the thiazole group compounds may have an advantage in that they are used in the treatment of more severe clinical diseases than bacteriostatic agents.
Table (6):- Antimicrobial activity data of synthesis compounds.

| Comp .No | Anti- bacterial Activity | Anti- fungal Activity |
|----------|--------------------------|-----------------------|
|          | Streptococcus            | E.coli                | Penicillium sp.      |
| LH (C_13H_15N_3OS) | + +                     | +                     | +                     |
| [ Ni(L)_2].H_2O    | +                       | -                     | -                     |
| [ Cu(L)_2].H_2O    | + +                     | -                     | + + +                 |
| [Zn (L)_2].H_2O    | + +                     | -                     | -                     |

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

Figure 9 : Statistical representation for biological activity of ligand (DMcTADMeP ) with prepared complexes.

4.2. Cytotoxic activity

Chemotherapy is noticed as a primary methodology for both limited and metastasized cancer treating(48,49). One important topic of the current study is assessing anticancer activities of ligand and Cu complex against human Hepatocellular carcinoma cell lines HepG2 and natural cell. Further, potentially bioactive molecules having cytotoxic activity are required for the treatment of cancer. The thiazolylazo ligand and Cu (II) complex were screened for anticancer property against HepG2 cell lines using five different concentrations, and the viability of cultured cells are used by MTT protocol. The ligand was observed that has high ratio of the inhibition of Hepatocellular carcinoma cell (HepG2) is (41.66%) at 25 μg/mL and the normal cell (WRL-68) was observed few effect with the same concentration. On the other hand, Cu (II)-complex was showed that the best rate of inhibition of (HepG2) 37.95% at 25 μg/mL concentration and the nature cellular of (WRL-68) was little effect with the same concentration. The percentage of cell viability from synthesis compounds is depicted in Figures 10 and 11. Furthermore, it is concluded that, a measurable changes of inhibitory activity from HepG2 cells treated with ligand and Cu
complex compounds and compared with the untreated cells are given in Tables (7) and (8).

Table (7) - Effect the ligand on HePG2 cells and compared with the normal cell line of the same concentration using 24h MTT test at 37 °C

| Concentration (μg.mL⁻¹) | ligand (DMATADMeP) | | | | | | Cancer line cells of HepG2 | Normal line cells WRI-68 |
|------------------------|--------------------|---|---|---|---|---|---|---|---|
|                        | Mean               | SD | Mean | SD | Mean | SD |
| 25                     | 59.803             | 2.4728 | 84.27656959 | 2.999709961 |
| 50                     | 49.215             | 3.3065 | 760639903 | 96233392.2 |
| 100                    | 17.497             | 1.3504 | 9,.9269327 | 2.98077847 |
| 200                    | 4.0377             | 0.3962 | 313396.937 | .69967.03 |
| 400                    | 1.3908             | 0.795 | .69967.03 | .6932.269 |

Table (8) - Effect the ligand on HepG2 cells and compared with the normal cell line of the same concentration using 24h MTT test at 37 °C.

| Concentration (μg.mL⁻¹) | Cu(II)-complex | | | | | | Cancer line cells of HepG2 | Normal line cells WRI-68 |
|------------------------|----------------|---|---|---|---|---|---|---|---|
|                        | Mean           | SD | Mean | SD | Mean | SD |
| 25                     | 46.747         | 1.3248 | 3.33203793 | 9.207027 |
| 50                     | 35.307         | 1.8853 | 39.0993933 | 39.3.3963.9 |
| 100                    | 7.6267         | 1.175 | 976.0.93.9 | 62339936.9 |
| 200                    | 1.6151         | 0.3962 | 339062360 | .0.02799.9 |
| 400                    | 1.7048         | 0.7796 | 6080.,69397 | 61.299.3970 |

Figure 10: Comparison of viability and inhibition for carcinoma and normal cells for the ligand
In addition, it is well known that the growth inhibitory activities and antitumor activity were both expressed by IC\textsubscript{50} parameter\(^{(50)}\). This concentration kills approximately half of the cells. The IC\textsubscript{50} values of the thiazolylazo ligand and Cu (II) complex were found to be 38.60 and 25.14 µg/mL respectively against HepG2 cells. On the other hand, the ligand was showed selective cytotoxicity against normal cell line with IC\textsubscript{50} = 88.27 µg/mL, while the Cu(II)-complex was observed against nature cell line with IC\textsubscript{50} = 82.07 µg/mL. The screening results are shown IC\textsubscript{50} (µg/mL) values of the carcinoma cell lines and natural cell line for ligand and Cu(II) complex in Figures 12 and 13. The results showed the possibility of using that the type of compounds as antitumor drugs in the field of medicine.
5. Conclusion

In this paper, we reported the synthesis and spectral characterization of new azo dye ligand derived from 2-amino-4,5-dimethyl thiazole with 2,4-dimethylphenol (DMeTADMeP) 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 FTIR, $^1$H-NMR, $^{13}$C-NMR, mass and UV-Visb spectral studies. On the basis of their analytical and spectral data, the geometry structure proposed for all metal complexes is octahedral. 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 activities toward antibacterial and antifungal. The biological activity studied in cells viability and cytotoxicity assays on ligand and Cu (II)-complex by using the lines of cancerous liver cells, of the type (HePG2) and compared with line of the nature cells. Therefore, through tests conducted to identify the possibility of using the thiazolylazo ligand and Cu(II)-complex as anticancer drugs development in future.

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