Synthesis, Spectral Properties And Anticancer Studies of Novel Heterocyclic Azo Dye Ligand Derived From 2-Amino-5-methyl thiazole with Some Transition Metal Complexes

K J AL-adilee1 and H M Hessoon2

1)Department of Chemistry, College of Education, University of AL-Qadisiyah, Dewanyiah, Iraq
2)Department of Chemistry, College of Science, University of AL-Qadisiyah, Dewanyiah, Iraq
E-mail:- Khalid.Jawad@qu.edu.iq , Khalidke_1962@yahoo.com

Abstract
This research included preparation of the thiazolyl azo ligand 2-[2- (5-Methyl thiazolyl)azo]-4-Ethoxy phenol (MeTAEP) and their metal complexes which was prepared by coupling of diazonium salt produced from diazonite d 2-amino-5-methyl thiazole and 4-Ethoxy phenol, New series of chelate complexes of Cu(II), Ag(I) and Au(III) metal ions were also prepared by mixing solution of metal salts with solution of ligand at mole ratio[ M:L] [1:2] for Cu(II) and [1:1] for Ag(I) and Au(III) metal ions. The synthesized compounds were confirmed by elemental analysis, molar conductance measurements, magnetic susceptibility, Mass spectrum,1H- NMR,13C- NMR, FT-IR, UV-Visb., (TGA), FESEM and X-ray diffraction studies. The biological activity of thiazolylazo dye ligand and its complexes were tested against the sensitive organisms Escherichia coli (gram negative), streptococcus (gram positive) as antibacterial and Aspergillus Niger and Penicillium sp. as antifungal, The anticancer activity of the(MeTAEP), Ag(I) and Au(III) complexes were screened for in vitro ; antitumor activity against human breast cancer.

1 Introduction
Azo compounds include about 60-70% of all pigments. The reason for their name is Azo dyes, which is due to the presence of Azo Bridge group (-N = N-), Thiazolylazo compounds contain two hybrid atoms, nitrogen and sulfur atom this type of compounds is of 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 [1,2] . Azo dyes of thiazole and there derivatives compounds are very important class of chemical constituents having different applications in many fields such as paper, polymer, paint and coating industries as a dyeing pigments[3-6]. Thiazolylazo compounds are important in intermediates for the preparation of many applications such as biological activity[7,8], analytical reagents[9,10], clinical fields[11,12], and many drugs including anti the growth of germs[13,14]. In the biological field they were used as anti-cancer[15,16],, antibacterial [17], antioxidant [18], antihypertensive[19], anticoagulant [20], and antifungal [21]. The term cancer is a medical term that refers to a range of diseases characterized by aggressive cells (growth and abnormal cell division) cell growth and spread can not be controlled [22]. The present work describes the prapration and spectral properties of 2-[2- (5-Methyl thiazole)azo]-4-Ethoxy phenol (MeTAEP) containing phenolic-OH function and thiazole moiety. The azo dye ligand (MeTAEP) and their metal complexes were studied by various spectral analysis and screened for their antimicrobial activity against Escherichia coli, streptococcus as antibacterial and Aspergillus Niger and Penicillium sp. as antifungal and anti cancer activity of ligand(MeTAEP), and its metal complexes of Ag(I) and Au(III) complexes on human breast cancer and the normal cells were studied by using MTT assay.

2 Experimental
2.1. Materials and Measurements
Chemicals and solvents in this paper have highest purity provided from many companies BDH, Fluka, sigma and Merck. 13C and 1H- NMR spectra were recorded using DMSO-d6 as a solvent and TMS as an interior reference on a Bruker 300 MHZ spectrophotometer. Mass spectra was obtained by using Mass AB Sciex 3200 QTRAP, IR spectra of the azo ligand and their complexes are recorded as KBr discs using a Shimadzu 8400 S FT-IR spectrophotometer at range (4000-400) cm\(^{-1}\) in wave
number. Elemental analysis (C,H,N,S) was obtained on a Euro EA 1106 elemental analyzer. The electronic spectra of ligand (MeTAEP), Cu(II), Ag(I) and Au(III) complexes measured in absolute ethanol as a solvent (10⁻³ M), (200-1100 nm) in the range using a UV-Vis, T80-PG. double beam spectrophotometer. Atomic absorption measurements were carried out with DMSO solution (10⁻³ M) using a 31 A digital conductivity meter at room temperature. The metal contents of complexes in this study. Magnetic susceptibility measurements of was carried out on Balance Magnetic (MSB-MK1). Apparatus. Molar conductivity measurements were carried out with DMSO solution (10⁻³ M) using a 31 A digital conductivity meter at room temperature. Melting point measurements were carried out with DMSO solution (10⁻³ M) using a 31 A digital conductivity meter at room temperature. Melting point/ SMP, Stuart. TGA analysis were measured by using Perkin Elmer, model (TGA 4000), USA Method. X-ray diffraction are carried out by using a Shimadzu - X-ray diffractometer-(XRD 600). Field-emission Scanning Electron Microscope (FESEM) images of ligand and its metal complexes were taken using TESCAN BRNO-Mira3 LMU made by French-Czech.

2.2. Synthesis of the thiazolyl Azo Dye Ligand (MeTAEP)

The thiazolyl azo Azo Dye ligand was synthesized by following methods proposed by Al-Adilee et al. [23,24] method with some modification. In a two-step, the first step, 2-amino-5-methylthiazol (1.14 g, 0.01 mol) was mixed with 5 ml hydrochloric acid and 30 ml distilled water and diazotized below 5 °C with sodium nitrite NaNO₂ (0.75 gm, 0.01 mol), dissolved in (30) ml distilled water was added drop wise. In the second step the diazonium salt compound was coupled with (1.38 g, 0.01 mol) of 4-ethoxyphenol in alkaline media below 5 °C, The color of the solution was observed in reddish orange, stirred the mixture for one hour at a temperature of 5-0 °C, and filtrate it, wash the precipitate with distilled water several times and re-crystallize using the absolute ethanol solution and then dry the precipitate using oven a 50° C for a few hours. The Scheme 1.below shows the processes of the diazotization coupling:-

2.3. General method of the preparation of metal complexes

The metal complexes were prepared by using Cu(II) and Au(III) chlorides and Ag(I) nitrate where the ligand (MeTAEP) amount of (0.527 g, 0.002 mol) was dissolved in (50) ml of absolute ethanol that was gradually added with stirring a stoichiometric to (0.001 mol) amount of [1:2] [M:L] for Cu(II) chloride salt dissolved in (50) ml of hot buffer solution (ammonium acetate) The mixture was heated to (50-70)°C at 30 min, while the ligand (MeTAEP) amount of (0.263 g, 0.001 mol) added in the same method to a stoichiometric to (0.001 mol) amount of [1:1] [M:L] for Ag(I) nitrate and Au(III) chloride salt. Methanol was solvent for Au(III) chloride salt instead of buffer solution (ammonium
acetate) and the mixture of the this complex was heated to (50 -70)°C at 2 hour . then left over night . The solid complexes were filtered and washed with deionize(DDW) water and little warm ethanol to remove any traces of unreacted materials in the finally obtained complexes were dried under vacuum desiccators over combined CaCl₂.

The analytical data and physical data of ligand (MeTAEP) and there metal complexes are collected in table (1).

**Table (1):** Physical properties and elemental analysis for ligand ((MeTAEP)) and its metal complexes

| Compound | Color          | m.p ⁰C | Yield % | Mf (M.wt)      | Found (Calc.)% |
|----------|----------------|--------|---------|----------------|----------------|
|          |                |        |         |                | C   | H   | N   | S   | M   |
| Ligand   | reddish orange | 117-119| 87      | C₁₂H₁₃N₃SO₂  | (54.74) 54.95 | (4.98) 5.01 | (15.96) 16.37 | (12.18) 12.55 |
| L⁻H(MeTAEP) | Greenis h blue | 247-249| 78      | C₂₄H₂₆N₆S₂O₅Cu | (47.55) 48.01 | (4.32) 4.40 | (13.86) 14.39 | (10.58) 11.05 | (10.48) 10.76 |
| [Cu(L)₂]H₂O | purple         | 140.9-142.5| 67 | C₁₂H₁₆N₃SO₄Ag | (35.48) 35.89 | (3.97) 4.09 | (10.34) 10.83 | (7.89) 8.28 | (26.56) 26.84 |
| [Ag(L)(H₂O)]H₂O | Reddish purple | 214.6-216.4| 76 | C₁₂H₁₄N₃SO₃Cl₂Au | (26.29) 26.67 | (2.57) 2.65 | (7.67) 8.19 | (5.85) 6.14 | (35.93) 36.27 |

**3 Results And Discussion**

3.1. Physical and chemical Characterization of the thiazolyl Azo Dye ligand(MeTAEP) and its metal complexes

the thiazolyl Azo Dye ligand (MeTAEP) was reddish orange crystals but their metal complexes of this ligand vary in colour by depending on metal ions. The metal complexes derived from azo dye ligand(MeTAEP) were stable toward air and insoluble in distilled water and some common organic solvents but soluble in ethanol, dimethylformamide ,methanol, , dimethylsulfoxide, and acetone. However,some physical and analytical data are given in Table.(1).

3.2. Metal: Ligand Ratio

In order to know the possible structural formulas of metal complexes, spectroscopic methods are often used, especially because complex solutions are colored. Thus, UV-visible spectra are used for this purpose . There are many methods used to determine the mole ratio. These are the methods of continuous variation method introduced by the (Job) and modified by (Copper and Vosbury) [25] this method carried out at wavelength of maximum absorption (λₘₐₓ) of metal ion and fixed concentration and increasing amount of ligand solutions arrangements (0.25 mL each add up 3 mL). The color of the metal complexes solutions increase the intensity when it reach the required molar ratio . The molar ratio [M:L] [1:2] suggested for the formation of Cu(II) complex and [M:L] [1:1] for the formation of Ag(I) and Au(III) complexes.

3.3. Molar conductivity measurements

The molar conductance of metal complexes were studied at laboratory temperature using DMSO (10⁻⁵) as a solvent. The results were included in table (2).
The values of conductivity indicated that the metal complexes of Ag(I) and Cu(II) ions are non-electrolytes nature but the high value of molar conductivity of the Au(III) complex indicated this complex is 1:1 electrolyte with ionic nature.

3.4. Calculation Stability constants ($\beta$)

Stability constants ($\beta$) values of metal complexes measurement is very important are obtained by measuring the absorbance of the ligand solution and solution of metal ion at fixed wave length ($\lambda_{\max}$) and optimum concentration at pH = 7.0.

The degree of formation of the metal complexes are Calculated by the relationships: 
$$k = \frac{(1-x)}{4} \epsilon L^2 \text{mol}^{-2}$$
and
$$x = \frac{(1-y)}{\epsilon L^2 \text{mol}^{-2}}$$
where $\lambda_{\max}$ and $\lambda_s$ are the absorbance of fully and partially formed metal complex continuously at optimum concentration[26]. The stability constants of metal complexes according to the following sequence :- Cu (II) > Au(III) > Ag(I). The stability constants values are listed in the table(2).

Table (2): Maximum wavelength ($\lambda_{\max}$),Optimal concentration, molar absorptivity ($\epsilon$),stability constants values ($\beta$) and Molar conductivity of metal complexes.

| Metal ion | Optimal Conc.$\times10^{-4}$ M | $\lambda_{\max}$ (nm) | $\epsilon \times 10^3$ L.mol$^{-1}$ cm$^{-1}$ | $\beta$ L$^2$.mol$^{-2}$ | Log $\beta$ | Molar cond. S.cm$^{-1}$ mol$^{-1}$ |
|-----------|-------------------------------|-----------------------|---------------------------------------------|------------------------|------------|----------------------------------|
| Cu(II)    | 1.50                          | 639                   | 7.06                                        | 80 x 10$^3$            | 15.22      | 13.80                            |
| Ag(I)     | 1.00                          | 527                   | 5.3                                         | 11.46 x 10$^4$         | 4.23       | 13.49                            |
| Au(III)   | 1.50                          | 512                   | 11.8                                        | 14.1 x 10$^4$          | 4.59       | 39.76                            |

3.5. $^1$H-NMR Spectra

The $^1$H-NMR spectra of thiazolylazo dye ligand (MeTAEP) and Au(II) complex (figures 1 and 2) were measured in DMSO-d$_6$ as solvent with TMS as an internal reference (300MHz). This compounds have been studied and listed in table (3).

Table (3): $^1$H-NMR spectra of thiazolylazo dye ligand (MeTAEP) and its Au(III) complex

| Ligand L,H$_x$,x,q,m,(H atoms, peak, assign)(s,c,t) | J-J Coupling | $\lambda_{\max}$ Complex J-J Coupling | L,H$_x$,x,q,m,(H atoms, peak, assign) |
|---------------------------------------------------|--------------|--------------------------------------|--------------------------------------|
| 1.3-135(3H,t,16)                                  | 3.02         | 1.28-1.33(3H,t,16)                   | 2.44                                 |
| 2.52 DMSO-d$_6$,2.49 (3H,S,6)                      | 3.92         | 2.52 (DMSO-d$_6$) 2.44 (3H,S,6)      | 4.63                                 |
| 3.96-4.03 (2H,q,15)                               | 1.98         | 4.10,3.87-3.94(4H,S,H,O, q,15)       | 3.00                                 |
| 6.72 (1H,S,10)                                    | 0.72         | 6.58 (1H,S,10)                       | 0.69                                 |
| 7.11-7.14, 7.07-7.10 (2H,d,12,13)                  | 1.92         | 6.72-6.75,6.66-6.69 (2H,d,12,13)     | 1.75                                 |
| 7.81 (1H,S,4)                                     | 1.04         | 6.93 (1H,S,4)                        | 0.86                                 |
| 10.32 (1H,S,14)                                   | 1.02         |                                      |                                      |

Where s= singlet,d= doublet,t= triplet,q= quartet
3.6. $^{13}$CNMR Spectra

The signals were shown at chemical shift $^{13}$C=(12.96, 15.08, 63.98, 101.23, 116.15, 120.05, 124.71, 137.79, 138.84, 142.54, 151.88, 174.92 ppm) to the carbon atoms at the sites, (6,16,15,9,12,10,13,5,4,14,11,2) respectively. The spectrum also showed a singlet signal of solvent (DMSO) at the chemical shift $^{13}$C=(39.99)ppm. The figure(3) shows the $^{13}$CNMR of the thiazolyl azo compound.
3.7. Mass spectrum

The mass spectrum of azo dye ligand (MeTAEP) were showed a molecular ion peak ($M^+$) at $m/z^+ = 264.0$ attributed to the original molecular weight of the ligand (263.32) (figure 4). The result of the expected mass fragment ion have shown in scheme (2) [27].
Scheme (2): Mass spectrum fragmentation of thiazolylazo dye ligand (MeTAEP)
3.8. Infrared spectra of thiazolylazo dye ligand (MeTAEP) and their metal complexes

The IR spectra of the thiazolyl azo ligand (MeTAEP) and their complexes with Cu(II), Ag(I) and Au(III) metal ions are listed in Table (4). The many shifts mentioned in the position or change in the shape of the metal complexes band compared with those absorption band of the free ligand in order to determine the coordination sites that involved in chelation, [28,29]. The Figure (5,6,7,8) the IR in (cm⁻¹) data of thiazolylazo dye the (MeTAEP) ligand and its complexes.

Table (4): The IR (in cm⁻¹) data of thiazolylazo dye the (MeTAEP) ligand and its complexes.

| Group      | Ligand (MeTAEP) | Cu(II)-Complex | Ag(I)-Complex | Au(III)-Complex |
|------------|-----------------|----------------|---------------|-----------------|
| υ-(OH)     | 3464.27 m.br    | *3444.98 m.br  | *3443.05 m.br | *3356.25 m.br   |
| υ (C-H)Ar-ring | 3084.28 W.   | 3091.99 W.    | 3082.35 W.    | 3082.35 W.      |
| υ(-C-H) aliphatic | 2976.26 W. | 2976.26 W. | 2978.19 W. | 2980.12 W.       |
| υ(C=N)     | 1585.54 W.     | 1653.05 W.    | 1624.12 W.    | 1599.04 M       |
| υ(N=N)     | 1494.88 S.     | 1456.3 M.     | 1527.87 S.    | 1508.38 S.      |
| υ (C=C) Ph | 1379.15 W, 677.04 W. | 1344.43 W, 630.74 W. | 1384.94 S, 628.81 W. | 1392.65 M, 692.47 W. |
| υ(C-S) Thia. | 1274.99 S. | 1300.07 S. | 1276.92 S. | 1271.13 W.       |
| υ(C-N) Thia. | 1161.19 S. | 1157.33 M. | 1186.26 S. | 1138.04 W.       |
| υ (M-O)    | 545.87 W.      | 526.58 W.     | 518.87 W.     | 518.87 W.       |
| υ (M-N)    | 439.78 W.      | 422.42 W.     | 420.50 W.     | 420.50 W.       |

S = strong, m= medium, w = weak, br= broad, *(H₂O) outside of sphere coordination

Figure (5): IR Spectrum of thiazolylazo dye ligand (MeTAEP)
3.9. Electronic spectra and Magnetic susceptibility measurements

The electronic absorption spectra of thiazolylazo ligand (MeTAEP) and its metal complexes with Cu(II), Ag(I) and Au(III) ions were recorded with absolute ethanol (10^-4 M) at room temperature.

The electronic spectrum of free ligand show three absorption bands at 477 nm (20964 cm^-1), 393 nm (25445 cm^-1) and 263 nm (38022 cm^-1). The first and second band can be due to n * of the azo group (N=N), and (C=N) group, the third band due to a * transition of (C=C) bond in aromatic and thiazole ring.

The magnetic moment of the Cu(II)-complex has been found to be (1.83 B.M.), which is within the range of values corresponding to octahedral geometry. The electronic spectrum data of Cu(II)-complex exhibited one band at 639 nm (15649 cm^-1). The broadness of the band indicates the three transitions 2B1g (1) 2A1g (2), 2B1g (2) 2B2g (3), and 2B1g (3) 2E2g (1), which are of similar in energy and give only one broad absorption band (2B1g 2E2g). The broadness of the band due to dynamic Jahn-Teller distortion[30,31,32,33].
The magnetic susceptibility for Ag(I)-complex due the electronic configuration $d^{10}$ to be diamagnetic character less of one magnetic value which is characteristic of tetrahedral geometry at 1:1[M:L] and hybridization sp$^3$ symmetry. Ag(I)-complex no d-d transition band of this complex exhibited four band, the first and second band at 527 nm (18975 cm$^{-1}$) and 405 nm (24691 cm$^{-1}$) due to (M L, CT) and the three and four band due to Ligand centered.

Au(III)-complex has less of one magnetic value indicate square planer geometry[23]. The electronic spectrum data exhibited one band at 512 nm (19193 cm$^{-1}$) due to $^1A_{1g} \rightarrow ^1B_{1g}$ transition. The electronic spectral results of the azo ligand (MeTAEP) and there metal complexes are listed in table(5) and figures (9).

### Table (5): Electronic spectra (nm and cm$^{-1}$), magnetic moments, proposed geometry and hybridization of prepared metal Complexes

| Compounds               | $\lambda_{\text{max}}$ (nm) | Absorption bands (cm$^{-1}$) | Transitions              | $M_{\text{eff}}$ (B.M) | Geometry                  | Hybridization       |
|-------------------------|------------------------------|------------------------------|--------------------------|------------------------|---------------------------|---------------------|
| L=(MeTAEP)              | 477                          | 20964                        | $n\rightarrow\pi^*$     | **           | **                        | **                  |
|                         | 393                          | 25445                        | $n\rightarrow\pi^*$     | **           | **                        | **                  |
|                         | 263                          | 38022                        | $\pi\rightarrow\pi^*$   | **           | **                        | **                  |
| [Cu(L)$_2$].H$_2$O      | 639                          | 15649                        | $^2B_{1g} \rightarrow ^2Eg$ | 1.83       | Octahedral distorted (Z-in or Z-out) | Sp$^2$d$^2$ |
| [Ag(L)(H$_2$O)]        | 527                          | 18975                        | $M \rightarrow L, CT$   | Dia         | Tetrahedral               | Sp$^3$              |
|                         | 405                          | 24691                        | $M \rightarrow L, CT$   | Dia         | Tetrhaedral               | Sp$^3$              |
|                         | 257                          | 38910                        | Ligand centered         | Dia         | Tetrahedral               | Sp$^3$              |
|                         | 247                          | 40485                        | Ligand centered         | Dia         | Tetrhaedral               | Sp$^3$              |
| [Au(L)Cl].Cl.H$_2$O    | 521                          | 19193                        | $^1A_{1g} \rightarrow ^1B_{1g}$ | Dia        | Square planer              | dsp$^2$             |

### Figure(9): Uv-visible spectra of thiazolylazo dye ligand (MeTAEP) and its metal complexes with Cu(II), Ag(I) and Au(III) ions

#### 3.10. Thermal Analysis

The thermal degradation of the thizolylazo ligand and its metal complexes were investigated by thermogravimetric (TG) analysis under nitrogen atmosphere (20 mL/min). TGA curve of samples were done in range of (40-900) $^\circ$C at heating rate (20$^\circ$C/min). The thermal stability data are presented...
in table (6) and a representative TGA,DTG, diagram is given in Figure (10). The data from the thermogravimetric analysis clearly indicated that the decomposition of the complexes proceeds in one, two or three steps[34].

**Table (6): Thermal analyses data for (TGA,DTG,) of thiazolylazo dye ligand (MeTAEP) and metal complex**

| Compounds          | TG range(°C) | DTG max (°C) | % Estimated(Calculated) | Total mass loss (Mass loss - (C5H11O) - (C5H2NS)) | Assignment                  |
|--------------------|--------------|--------------|------------------------|--------------------------------------------------|-----------------------------|
| Ligand = (MeTAEP)  | 105.28-281.8 | 241.15       | 33.485(33.093)          | 75.021(74.162)                                   | - (C3H11O)                  |
|                    | 281.8-904.3  |              | 41.536(41.069)          |                                                  | - (C3H2NS)                  |
|                    |              |              |                        |                                                  | C2N2O                       |
| [Cu(L)2].H2O       | 119.99-258.41| 251.97       | 12.643(13.028)          | 60.413(60.267)                                   | - (H2O+CH3+OC2H5)           |
|                    | 258.4-903.8  |              | 47.77(47.239)           |                                                  | - (C18H10N2S)               |
|                    |              |              |                        |                                                  | Cu C8H8N2SO4                 |
| [Ag(L)(H2O)].H2O   | 90.91-203.73 | 107.81,161.13| 2.569(4.43)             | 41.04(39.922)                                   | - (H2O) outer -sphere       |
|                    | 203.73-282.55| 246.34       | 12.458(11.093)          |                                                  | coordination (-OC6H4)       |
|                    | 282.55-902.4 |              | 25.986(24.399)          |                                                  | (-H2O) inner-sphere         |
|                    |              |              |                        |                                                  | coordination+C8H8N2          |
|                    |              |              |                        |                                                  | AgC8H8N2SO4                 |
| [Au(L)Cl].Cl.H2O   | 100.34-175.26| 116.1        | 2.92(3.28)              | 56.45(56.32)                                    | - (H2O)                     |
|                    | 175.26-904.2 | 234.41,371.74| 53.53(53.04)            |                                                  | - (C16H16SN3OCl2)           |
|                    |              |              |                        |                                                  | AuC5H5O                     |

**Figure (10): TGA-DTG- Curves of thiazolylazo dye ligand (MeTAEP) and its metal complexes**
According to the results obtained from the different techniques, the proposed structural formula of prepared metal complexes are shown in the following figures (11, 12, 13).

**Figure (11):** The proposed chemical structure formula of the Cu(II)- complex

**Figure (12):** The proposed chemical structure formula of the Ag(I)- complex

**Figure (13):** The proposed chemical structure formula of the Au(III)- complex

### 3.11. X-ray diffraction analysis

Thiazolylazo dye ligand (MeTAEP) and its complexes were analyzed in their solid state using x-ray diffraction within the angular range of $2 (5^\circ - 80^\circ)$. To determine some of the structural properties such as crystalline structure, crystalline size microstrains and dislocation density to determine their purity and defects in crystalline structure when converting the ligand under study into
metallic complexes. The Debye-Scherer equation was used to calculate the crystalline size of the ligand and its complexes, as follows [35]

$$ D_p = \frac{0.94 \lambda}{\beta \cos \theta} $$

Where $ D_p $ = Average Crystallite size; $\beta$ = is the line broadening at half the maximum intensity in radians; $\theta$ = Bragg angle; $\lambda$ = X-ray wavelength.

The following equation was also used for the calculation of the Microstrains [36]

$$ S = \cos \theta/4\pi $$

Where $ S $= Microstrains $\beta$ = is the line broadening at half the maximum intensity in radians; $\theta$ = Bragg angle

while The following equation was used to calculate the Dislocation density [37]

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

$\delta$ = Dislocation density

In addition, we found that ligand and their metal complexes under study have a crystalline size less than 100 nm, which is within the nanoscale. At the same time, these results reinforce our previous measurements of (Field-emission Scanning Electron Microscope) FESEM analysis. The following table (7) shows the diffraction angles, $d_{observed}$, relative strength, crystal size, crystalline stress, FWHM peak width, Microstrains, and Dislocation density of ligand and its metal complexes under study.

**Table (7):** The diffraction angles, $d_{observed}$, relative strength, crystal size, crystalline stress, FWHM peak width, Microstrains, and Dislocation density of thiazolylazo dye ligand (MeTAEP) and its metal complexes

| Compound | Number | $d_{observed}$ | $(I/I_0)$ | FWHM | Crystallite Size (nm) | Lattice Strain | $\delta_{oh}$ $10^6$ (lin m$^{-1}$) | $Sx_{10^6}$ (lin$^2$ m$^{-4}$) |
|----------|--------|----------------|-----------|-------|----------------------|---------------|---------------------------------|-------------------------------|
| Ligand   | 1      | 26.2799        | 3.38846   | 100   | 0.23339              | 36.44         | 0.00444                        | 0.75                          | 5.694                        |
|          | 2      | 12.6727        | 6.97958   | 27    | 0.272               | 30.71         | 0.0107                         | 1.06                          | 6.758                        |
|          | 3      | 22.5794        | 3.93472   | 14    | 0.5067              | 16.71         | 0.011                          | 3.58                          | 12.422                       |
| Cu(II)-complex | 1 | 7.7517 | 11.39585 | 100   | 0.2393              | 34.76         | 0.0154                         | 0.827                         | 5.968                        |
|          | 2      | 22.2464        | 3.99286   | 36    | 0.2007              | 42.15         | 0.0045                         | 0.562                         | 4.923                        |
|          | 3      | 12.4626        | 7.09676   | 31    | 0.2464              | 33.89         | 0.0098                         | 0.87                          | 6.123                        |
| Ag(I)-complex | 1 | 38.0852 | 2.36092 | 100   | 0.2317              | 37.9          | 0.0029                         | 0.695                         | 5.72                         |
|          | 2      | 44.2543        | 2.04506   | 29    | 0.3023              | 29.639        | 0.0032                         | 1.138                         | 7.000                        |
|          | 3      | 7.0075         | 12.60434  | 26    | 0.2843              | 29.25         | 0.0203                         | 1.168                         | 7.094                        |
| Au(III)-complex | 1 | 38.167 | 2.35605 | 100   | 0.3164              | 27.76         | 0.0044                         | 1.3                           | 7.475                        |
|          | 2      | 44.3594        | 2.04046   | 29    | 0.4567              | 19.63         | 0.0049                         | 2.595                         | 10.573                       |
|          | 3      | 64.5579        | 1.4424    | 20    | 0.3700              | 26.53         | 0.0026                         | 1.42                          | 7.820                        |

and the following figure (14) represents the X-ray diffraction spectra of the thiazolyl azo ligand and its prepared metal complexes:-
Figure (14): The X-Ray diffraction spectra of thiazolylazo dye ligand (MeTAEP) and its complexes

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

The properties of the surface of the ligand and its metal complexes were studied in terms of size, shape of particles and aggregates, with a cross section of 1.44 m, and a magnification force of Mag = 100.00. KX. FESEM micrographs of the metal complexes are revealed that the surface morphology of metal complexes is changed by changing the metal ions [38,39].

The image of the FESEM analysis of ligand shows that it has a homogeneous crystalline surface with an average size of 56.01 nm while the FESEM analysis of the Ag(I) complex has been shown to be in the form of heterogeneous crystals with a lower percentage of aggregates with an average size of 83.51 nm either the FESEM analysis of the Au(III) complex showed spherical circumference particles with an average size of 24.098 nm.

The FESEM images shown in Fig. 15 below show that ligand and its complexes have a grain size less than 100 nm, it is within the nanoscale range. Where the surface area increases effectively and thus enters the (Quantitative effect) to create new energy levels that make the movement of the electron more free. Characteristics of the ligand and its complexes enabled us to study in medicine, and the susceptibility of these compounds to the inhibition of many types of cancers such as breast cancer.

Fig. (15): FESEM images of thiazolylazo dye ligand (MeTAEP) and its complexes
4 Pharmacology Results

4.1. Antimicrobial and antifungal activity

Antimicrobial activity was carried out by the cup-plate method [40]. The effectiveness of the ligand and their complexes were studied by using of two types of Bacteria, *Streptococcus* (Gram positive Bacteria) and *Escherichia coli* (Gram Negative Bacteria) and two types of Fungi *Aspergillus Niger* and *Penicillium sp.* were used to determine their inhibitory effect on the growth of these organisms. The results of the antimicrobial activity were statistically presented as shown in table(8) and figure(18):

Table (8):- Antimicrobial activity screening data (zone of inhibition in mm) of the thiazolylazo dye ligand (MeTAEP) and its metal complexes.

| Comp. No.                  | Anti-bacterial Activity | Anti-fungal Activity |
|----------------------------|-------------------------|----------------------|
|                            | Streptococcus          | E.coli               | Penicillium | Aspergillus niger |
| Ligand = (MeTAEP)          | ++                      | ++                   | +++       | +++              |
| [Cu(L)2].H2O               | ++                      | -                    | +++       | +++              |
| [Ag(L)(H2O)]               | +                       | +++                  | +++       | +                |
| [Au(L)Cl].Cl.H2O           | -                       | -                    | +++       | +++              |

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

Figure (16): Statistical representation for biological activity of thiazolylazo ligand (MeTAEP) and its metal complexes.

4.2. In vitro cytotoxicity assay

Chemotherapy is the major approach for both metastasized and localized cancer[41] The test (MTT) was used to examine the viability of cells. The ligand was observed that the best rate of inhibition of breast cancer (MCF-7) was found (35.11%) at 400 g / ml while the rate of normal cell (WRL-68) - inhibition was observed with the same concentration of 23.63%.

On the other hand, Ag(I)-complex was showed the best rate of inhibition of (MCF-7) 25.97% at 200 g / ml concentration and the normal cellular cell inhibition ratio of (WRL-68) 13.85% with the same concentration , while the Au(III)-complex was observed that the best rate of inhibition of breast cancer (MCF-7) (27.47%), at the concentration of 200 g / ml and The results showed that the normal cell inhibition rate (WRL-68) with the same concentration above was 13.19%. The following tables
9, 10 and 11 and figures 17, 18 and 19 are illustrated effect the ligand and its metal complex on MCF-7 cells and compared with the normal cell line of the same concentration using a 24-hour MTT test at 37 °C.

Table (9): Effect the ligand on MCF-7 cells and compared with the normal cell line of the same concentration using a 24-hour MTT test at 37 °C

| Con. (µg.mL⁻¹) | Ligand (MeTAEP) | Cancerous line cells of breast cancer MCF-7 | Normal line cells WRI-68 |
|---------------|----------------|---------------------------------------------|--------------------------|
|               | Mean           | Std. Error of Mean                          | Mean                     | Std. Error of Mean |
| 6.25          | 96.64          | 0.306                                       | 95.72                    | 0.3535            |
| 12.5          | 96.30          | 0.637                                       | 95.60                    | 0.5221            |
| 25            | 97.84          | 0.402                                       | 94.83                    | 0.3158            |
| 50            | 94.06          | 1.24                                        | 95.18                    | 0.3014            |
| 100           | 89.16          | 2.076                                       | 94.21                    | 1.142             |
| 200           | 76.93          | 1.353                                       | 87.79                    | 3.354             |
| 400           | 64.89          | 2.953                                       | 76.37                    | 3.218             |

Table (10): Effect the Ag(I)-complex on MCF-7 cells and compared with the normal cell line of the same concentration using a 24-hour MTT test at 37 °C

| Con. (µg.mL⁻¹) | Ag(I)-complex | Cancerous line cells of breast cancer MCF-7 | Normal line cells WRI-68 |
|---------------|---------------|---------------------------------------------|--------------------------|
|               | Mean          | Std. Error of Mean                          | Mean                     | Std. Error of Mean |
| 6.25          | 96.10         | 0.8839                                      | 96.03                    | 0.2783            |
| 12.5          | 97.11         | 0.6103                                      | 94.21                    | 1.220             |
| 25            | 96.3          | 0.5044                                      | 95.95                    | 0.4821            |
| 50            | 96.33         | 0.2346                                      | 95.49                    | 0.3063            |
| 100           | 92.28         | 0.5760                                      | 93.83                    | 1.725             |
| 200           | 74.03         | 0.7227                                      | 86.15                    | 1.843             |
| 400           | 73.15         | 0.6581                                      | 79.71                    | 1.103             |
Table (11): Effect the Au(III)-complex on MCF-7 cells and compared with the normal cell line of the same concentration using a 24-hour MTT test at 37 °C

| Con. (μg.mL⁻¹) | Au(III)-complex | Cancerous line cells of breast cancer MCF-7 | Normal line cells WRI-68 |
|---------------|----------------|---------------------------------------------|--------------------------|
|               | Mean | Std. Error of Mean | Mean | Std. Error of Mean |
| 6.25          | 95.68 | 0.1683 | 95.68 | 0.2346 |
| 12.5          | 95.99 | 0.5445 | 96.64 | 0.4065 |
| 25            | 95.99 | 0.5681 | 95.37 | 0.5219 |
| 50            | 94.91 | 0.6124 | 95.83 | 0.1769 |
| 100           | 87.46 | 1.168 | 94.29 | 1.057 |
| 200           | 72.53 | 0.3279 | 86.81 | 0.8739 |
| 400           | 66.40 | 0.6955 | 78.86 | 0.702 |

Figure (17): Comparison of viability and inhibition for carcinoma and normal cells at 400 g/ml concentration for ligand.
It is important to note that we have reached through the tests conducted between ligand and its complexes and the cells of the cancer lines of the breast MCF-7 and normal cells is the -called Inhibition Concentration Fifty (IC50)[42]. This concentration kills approximately half of the cells. In the case of ligand was showed selective cytotoxicity against cancer cell line with IC50 = 198.7 g / ml, while it was 253.5 g / ml for normal human cells, the Ag(I)-complex was observed against cancer cell line with IC50 = 124.9 g / ml while it was 188.2 for normal human cells, either Au(III) complex was showed against cancer cell line with IC50 = 136.1 g / ml, while it was 204.5 g / ml for normal human cells, The following Figures from(20) to(22) are shown IC50 (g/ml) values of the carcinoma cell lines and normal cell Line for ligand and its complex.
5 Conclusions

In this article, Thiazolylazo ligand 2-[2-(5-Methyl thiazole)azo]-4-Ethoxy phenol (MeTAEP) and their complexes of Cu(II), Ag(I) and Au(III) metal ions were synthesized and spectral identification. The antibacterial and antifungal activity of the Azo ligand(MeTAEP) and prepared complexes were studied. The ligand and their metal complexes are found to have biological activities toward antifungal and antibacterial.

The structure of Thiazolylazo ligand(MeTAEP) and their metal complexes were confirmed by elemental analysis and spectroscopic techniques. The geometry proposed for the Cu(II) complex is
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The biological activity conducted cells viability and cytotoxicity assays on thiazolylazo ligand (MeTAEP) and its Ag(I) and Au(III) complexes by using the lines of cancerous of breast cancer (MCF-7) and compared with line of the ordinary cells it can be concluded that azo ligand and their complexes possess good cytotoxic property and selectivity against human breast cancer (MCF-7) cells and the possibility of using them as a drugs with some modifications to treat some cancerous diseases that affect humans.

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