RESEARCH ARTICLE

Synthesis and Characterization along with Spectral, Anticancer and Biological Activity Studies of Azo Thiazol Ligand Derived from 2-Amino-5-methylthiazol and its Complex with Ag(I) Ion.

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ABSTRACT

The research included the preparation of new azo compound derived from 2-amino-5-methylthiazol and its Complex with Ag(I) ion. The ligand and its complex were characterized after precipitating and recrystalized by available spectral and analytical techniques such as Fourier-transform infrared spectroscopy (FTIR), UV-visible, atomic absorption, hydrogen-Nuclear magnetic resonance (^{1}HNMR), XRD Analysis and (C.H.N.S) analysis. From the obtained analyzes suggested molar ratios [M:L] was [1:1] for Ag(I)ion . The biological activity of two types of bacteria and two types of fungi was also studied, which proved their great effectiveness against these organisms. In this study, it was studied cytoxicity of ligand and Ag(I) complex on human UBC40 bladder cancer with normal cells by MTT assay. The results obtained indicate of the possibility using the prepared compounds as anti-cancer drugs in the field of pharmacology bladder cancer.

Keywords: Anticancer (bladder cancer), Azo ligand, Biological activity, Metal complex.

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INTRODUCTION

Azo compounds are among the most widely used organic reagents. This is due to its high stability and the speed of its interaction with various metal ions. Many of them are highly sensitive and selective, which made them the focus of the attention of scientists and researchers.[1] The importance of inorganic complexes in life has emerged. The scientific and practical Important natural biologics such as chlorophyll, hemoglobin and vitamin B12 (all of them)with complex structures.[2-4] Azo compounds have many uses in the industrial field.[5-7] They have been used as good dyes depending on effective groups such as azo group, carboxyl, amine, etc.[8,9] Thiazolylazo derivatives are used in the pharmaceutical and medical field.[10] Derivatives of thiazole compounds play a very important in nature and has lots of biological activity as anti-bacterial and anti-microbial,[11,12] anti-viral,[13] anti-candidate,[14] antiproliferative,[15] and anti-inflammatory.[16] In this paper we report on the preparation and identification of new ligand and its silver complex. The biological activity of the prepared compounds against two types of bacteria and two types of fungi that cause many diseases has been studied and proven effective. The anticancer activity against bladder cancer has also been studied by using the lines of bladder cancer cell and Comparison with the healthy cells line by MTT assay.

EXPERIMENTAL

Measurements and Materials

Chemicals from several companies were used with a high degree of purity Fluka, B.D.H and J&K. The ultraviolet-visible (UV-vis.) spectra were measured in a T80-PG-Spectrophotometer, The spectra of infrared were recorded by using Shimadzu device. FT-IR. 8400S Spectrophotometer, The concentration of the silver metal ion under study was determined and estimated by using a flame atomic absorption spectrometer, type Shimadzu (A.A) 680 G Atomic Absorption Spectrophotometer. Magnetic sensitivity was measured using balance magnetic Susceptibillity Model (M.S.B.). Auto, Measuring of proton nuclear magnetic resonance spectrum (^{1}H-NMR) of the compounds prepared using the device 500. MHZ Spectrophotometer (BRUKER) using (DMSO-d6) as solvent and TMS as standard reference Bruker type, The ratios of (C.H.N.S) to ligand and its metal complex were determined using a device: - LECO 923 USA, X-ray diffraction was measured using the Bestic Germany Alminium device,

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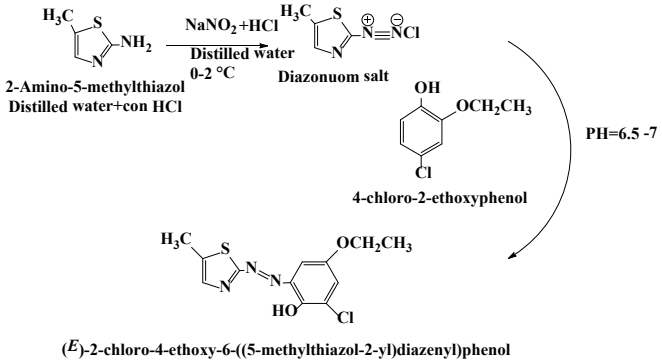
Sterilize the agricultural media and dishes from bacteria using an Autoclave device of the type MAUUOBT)) of English origin,

Preparation of Thiazolyl azo Ligand[(E)-2-chloro-4-ethoxy-6-((5-methylthiazol-2-yl)diazenyl)phenol]

The ligand was prepared using the method proposed by the researcher Khaled Al-Adly and his group[17,18] (Scheme 1), with some modifications to the method of work. Where 1.14 g (0.01) mol of 2-amino-5-methylthiazole was dissolved by a mixture consisting of 3 mL of (HCl) Con, and 30 mL of (H_2O) distilled of water then the mixture was cooled to a temperature of (2-0)°C. A solution of (0.83 g) 0.012 mol of sodium-nitrit dissolved in 25 ml of distilled of water was added to it in the form of a drop by drop with constant stirring and cooling for 30 minutes, noting that the temperature did not rise. This diazonium chloride solution was then added dropwise, with stirring constant and cooling, to a 1.72 g (0.01) mol solution of 4-chloro-2-ethoxyphenol dissolved in a mixture of 65 mL ethanol with 13 mL 7% sodium hydroxide solution cooled to 0°C. After completing the addition process, the mixture was left for one hours. The precipitate was filtered and was washed with cold distilled water. It was recrystallized using absolute ethanol. The formation of a reddish orange precipitate was observed. The precipitate was placed in a thermal oven at a temperature of 60°C for 24 hours.

Preparation of Ag(I) Complex

Solid metal complex was prepared based on optimum conditions of concentration and molar- ratio, where the molar- ratio was [1:1], [M:L], And that is by adding the (0.297g,0.001mol) for ligand that was prepared and it dissolved in 25 mL in absolut ethanol solvent and that was added step by step with astoiciometric amount of 0.001 mol [1:1) [M:L]. for Ag (I) salt dissolved in (60) mL of buffer solution at pH =7. The mixture solution was heated at 50°C for 1 hour, then left it to overnight. The Purple precipitate was filtered and washed with absolute ethanol and dried in desicator. The analytical and physical data of the azo ligand and silver complex are showed in the Table 1.



Scheme 1: Synthesis of azo thiazole ligand

RESULT AND DISCUSSION

1HNMR Spectrum

The spectra of the prepared ligand showed in Figure 1 and used solvent (DMSO-d6) in (TMS) were measured as internal reference (500MHz). The results obtained are shown in the Table 2.[19-22]

Magnetic Susceptibility and the Electronic Spectra Measurement

The spectra of prepard ligand and silver ions were measured in absolut ethanol have (0.0001 M) at lab temperature. The results obtained are listed in the Table 3 and Figures 2.

The value of the effective magnetic moment of the silver complex gave diamagnetic properties,indicate tetrahedral geometry.

Infrared–spectra of Azo Ligand and its Silver Complex

Table 4 shows the stretching vibration of the bands for functional group which appeared to azo ligand and its Ag(I)

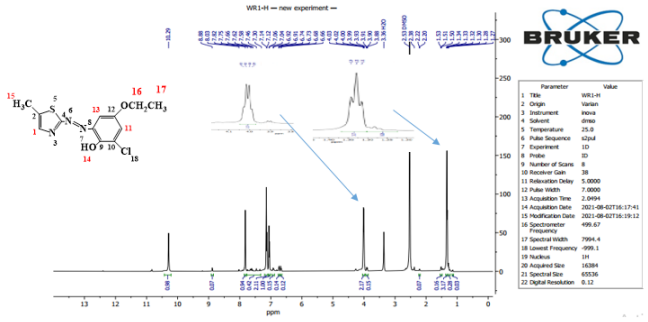


Figure 1: ^{1}H-NMR -spectrum of prepard ligand

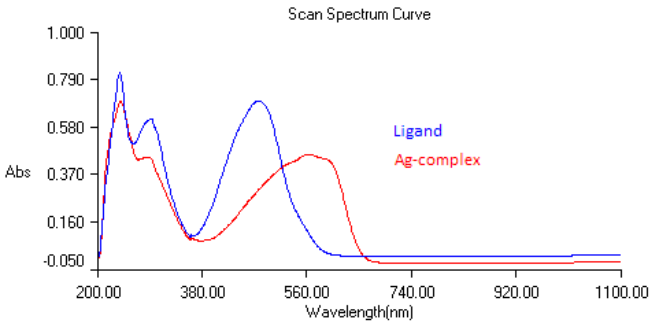


Figure 2: UV-vis ligand spectra and Ag(I)complex

Table 1: Analytical and physical properties for azo ligand and Ag(I)complex

Compounds	*Color*	*Melting point C°/*	*Yield percentage*	*M.f (molecular weight)*	*Found (Calc.)%*				
					C	*H*	*N*	*S*	*M*
Ligand	reddish orange	133.3–134.7	85	C12H12ClN3O2S	(48.41) 47.44	(4.06) 3.97	(14.11) 13.99	(10.77) 10.21	—
Ag(I)complex	purple	217.3–218.4	79	C12H13AgClN3O3S	(34.10) 33.98	(3.10) 2.98	(9.94) 9.23	(7.59) 7.22	(25.52) 24.11

complex in the the range of measurement {400–4000} cm^{-1}. These bands suffered a noticeable change in the intensity, shape and location in spectra of the silver complex, which indicates the involvement of the electron pair belonging to the Nitrogen atom of the azo bridge group in the process of coordination with the silver ions under study to form the complexes.[23-25]

X-Ray-Diffraction Anlysis (XRD)

The prepard azo ligand and silver complex were analyzed in their solid state within -angular range- 2Θ-(5°-80°) to know some properties of structural, crystal structures and crystal sizes. The (Debye Scherer -equation) was used in order to calculate the crystal size of prepard compound, as a follows[26-28]

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

where D = average of crystal size ; k = shape factor whose value is usually about 0.9; λ represents the X ray- wavelength; β ;-the line broadening in half of maximum intensity at the radians; θ ;- Bragg angle

The patterns of XRD and the crystallographic data for azo ligand and their complex illustrated in Figure 3 and Table 5.

Study of the Activity of Antibacteria and Antifungi

The Activity of the prepared compounds on two types from bacteria and two types from fungi was studied. The prepared compounds proved their good efficacy against bacteria and fungi. The following Table 6 shows the results obtained.[29,30]

Table 2: ^{1}H-NMR spectra of azo compound

Ligand L_1H,δ, ppm, (H atoms, peak, assignment)
1.32-134(3H,t,17)
2.53 DMSO-d6,2.38 (3H,S,15)
3.99-4.03 (2H,q,16)
6.92 (1H,S,13)
7.14 (1H,S,11)
7.82 (1H,S,1)
10.29 (1H,S,14)

Where q ;- quartet ,s;- singlet, t;- triplet

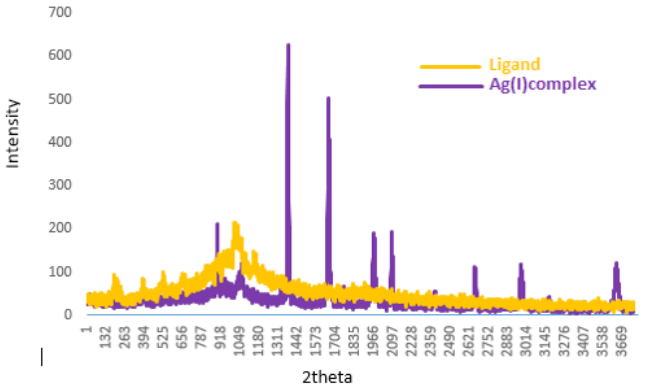


Figure 3: X-RD data for prepared compoud

Table 3: Electronic spectra -$cm^{-1}$and nm., Hybridization and geometry proposed of Ag(I)Complex

Compoundes	*λ_{max} nm*	*Absorption bands (cm^{-1})*	*Transetion*	*Geometry of prepard compound*	*Hybridization*
Azo ligand	474	21097	$n \rightarrow \pi^*$		
	292	34246	$\pi \rightarrow \pi^*$		
	238	42016	$\pi \rightarrow \pi^*$		
[Ag(L)(H_2O)]	560	17857	M→L,CT	tetrahedral	Sp3
	282	35460	Ligand centered		
	240	41666	Ligand centered		

Table 4: Stretching vibration of function groups for ligand and its Ag(I) complex

Compounds	*v(OH)*	*V(CH3)*	*v(C=N)*	*v (N=N)*	*v(C=C)ph*	*v(C-S)Thia*	*v(C-N)Thia*	*v(M–N)*	*v(M–O)*
Ligand	3345.12m.br	3032.22w	1579.45w	1467.21s	1402.56 w 665.12 w	1278.76 s	1169.32 s	---------	-------
[Ag(L)H_2O]	*3454.44m.br	2899.34m	1588.78m	1477.34 s	1432.22m 644.13 w	1265.32 s	1156.43 s	532w	425w

* = the band belong to coordinate water molcule
br : broad,, w: weak,, m : medium,, S:-strong

Table 5: X-ray diffraction data for azo ligand and Ag(I)-Complex diffraction data

Compound	*No.*	*°2Θ observed*	*d observed (A°)*	*I/I₀) %)*	*FWHM*	*Crystallite Size. (nm)*	*Lattice Strain*
Ligand	1	25.7098	3.46229	100	1.34000	6.35	0.0256
	2	24.3922	3.64625	47	0.2234	38.01	0.0045
	3	23.0350	3.85792	38	0.4544	18.64	0.0097
Ag(I) complex	1	32.6563	2.73993	100	0.17380	31.589	0.0041
	2	38.1990	2.35415	72	0.22570	38.92	0.0028
	3	46.8485	1.93768	33	0.14760	61.29	0.0015

Table 6: Activity of antibacteria and antifungi (zone for inhibition in mm) of the azo and silver complex.

	Anti-bacterial Activity		*Anti-fungal Activity*	
Prepared compound	*Streptococcus*	*Escherichia-coli*	*Penicillium*	*Aspergillus niger*
Ligand	+++	+++	+++	+++
Ag(I) complex	++	++	++	++

(+++):-highly effective (inhibition zone > 12 millimeter;;(++);- moderate effective - (inhibition zone = 9--12 millimeter

Table 7: Effect azo ligand on bladder cancer cells and normal cells

	Ligand			
	Cancerous lines cell of bladder cancer UBC40		*Normal lines cells WRI-68*	
Con. [µg.mL^{-1}]	*(Mean)*	*(Standrd. error of mean)*	*(Mean)*	*(Standrd. error of mean)*
6.25	97.33	0.434	94.33	0.321
12.5	95.21	0.534	93.650	0.502
25	91.65	0.511	88.34	0.302
50	90.05	1.28	85.78	0.281
100	85.15	2.12	84.11	1.112
200	70.97	1.48	83.32	3.243
400	55.99	2.81	81.65	3.021

Table 8: Effect of silver complex on bladder cancer cells and normal cells

	Silver complex			
	Cancerous lines cell of bladder cancer UBC40		*Normal lines cells WRI-68*	
Con. [µg.mL^{-1}]	*(Mean)*	*(Standrd. error of Mean)*	*(Mean)*	*(Standrd. error of Mean)*
6.25	90.78	0.455	95.54	0.443
12.5	88.76	0.456	94.67	0.456
25	83.33	0.499	87.42	0.288
50	80.23	1.39	86.99	0.276
100	70.39	2.01	85.21	1.011
200	61.44	1.22	81.24	3.222
400	60.34	2.55	80.99	3.001

In vitro Cytotoxicity Examination

The effect prepared compound on the growth of cell lines was studied by MTT test, where the bladder cancer viability cells was 55.99% at 400 µg/mL and viability for normal cells was 81.65 %at the same concentration when using the ligand, and the bladder cancer viability cells was 61.44% at 200 µg/mL and viability for normal cells was 81.24 %at the same concentration when using the Ag(I)complex,[31,32] Table 7 and Table 8 and Figures 4 and 5 shows the effect of ligand and Ag(I).complex on bladder cancer UBC40 cell and it compared with cell of normal lines at the same amount by (MTT) assay under 37°C.

IC_{50} inhibition, concentration, fifty of the ligand and its complex were examined. when using the ligand, the (IC_{50}) of cancrous cells was 129.2 µg/mL, while it is 268.7 µg/mL for normal cells. While when using the complex the (IC_{50}) of cancrous cells was 73.8 µg/mL, while it 213.6 µg/mL for normal cells. The results obtained show the possibility to use the compounds with some modifications as the treatment of

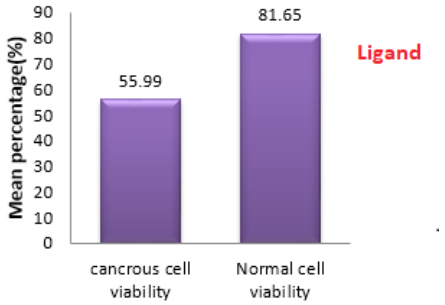


Figure 4: Viability of cancrous cell and viability normal cells at 400 µg/mL for ligand

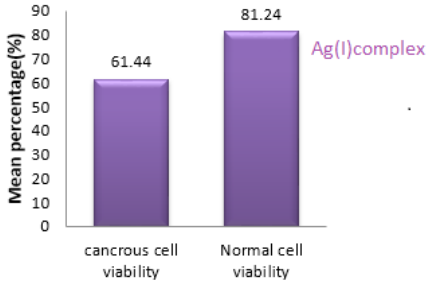


Figure 5: Viability of cancrous cell and viability normal cells at 200 µg/mL for Ag(I)complex

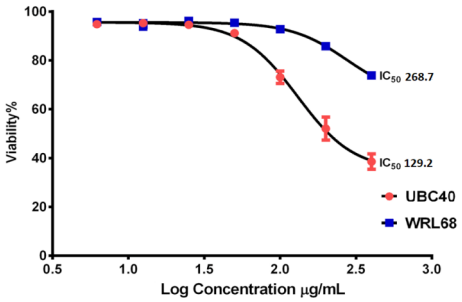


Figure 6: IC_{50} µg/mL value for the ligand

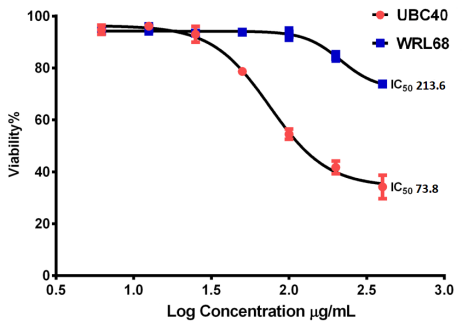


Figure 7: IC_{50} µg/ml value for the complex

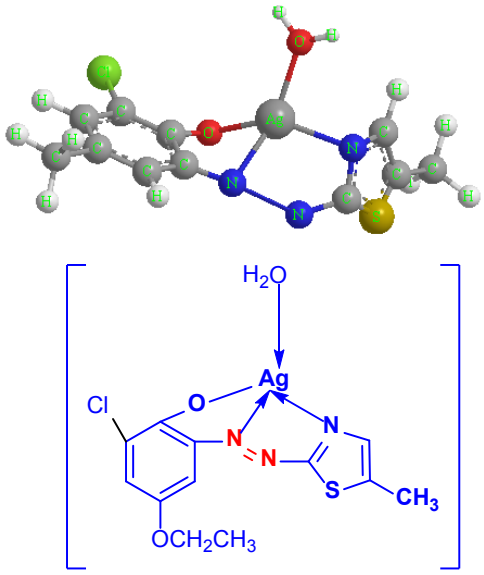


Figure 8: The geometrical structure of the silver complex

some human cancers. Figures 6 and 7 show values IC_{50} µg/mL for ligand and complex.

According to the spectroscopic and analytical results, the proposed the geometrical structure of the silver complex is tetrahedral as shown in the Figure 8.

CONCLUSION

In the current work, the ligand and the silver complex were prepared, which were identified by the available spectroscopic methods. The biological activity of the prepared compounds was studied as anti-bacterial and anti-fungal, and it had good efficacy. In addition, the toxic activity was studied as an anti-bladder cancer, as it had good activity. It can be used as anti-bladder cancer drug with some modifications.

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