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## Synthesis, Spectroscopic Characterization, Thermal Stability and antimicrobial activity of Schiff base, $\beta$ -lactam and Zn (II), Cu (II) complexes derived from Sulfamerazine.

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### ABSTRACT

Schiff base derived from *o*-vanillin and Sulfamerazine,  $\beta$ -lactam and Zn(II), Cu(I) complexes have been synthesized and characterized by IR, <sup>1</sup>HNMR, MASS spectrometry, molar conductance and thermal analysis. The schiff base acts as a monobasic bidentate ligand in complex formation thermal analysis indicate the presence of lattice water molecules in complexes. The molar conductance measurements indicate the non electrolyte behaviour of the complexes in DMF solution. The antimicrobial activities of compounds were tested against four bacterial clinical isolates (human pathogenic) strains as 1 gram +Ve bacteria (*Staphylococcus aureus*), 3 gram -Ve bacteria (*Escherichia coli*, *Pseudomonas aeruginosa* and *Proteus spp.*) to develop novel class of anti microbial agents with varied mode of action. The results of bioassay showed that the newly synthesized  $\beta$ -lactam emerged as laed with MIC (mg/ml) values with mentioned gram +Ve, While the complexes-schiff base showed highly antimicrobial activity toward mentioned gram -Ve bacterai. These results compared with standard drugs (Cephalexin;30 $\mu$ g/disc, Ciprofloxacin;5 $\mu$ g/disc, Oxacillin;1 $\mu$ g/disc, Cloxacillin;1 $\mu$ g/disc, Aztreonam;30 $\mu$ g/disc, Ampicillin;10 $\mu$ g/disc, Clarithromycin;15 $\mu$ g/disc, Novobiocin;30 $\mu$ g/disc)

**Keywords:** Sulfamerazine, antimicrobial activity,  $\beta$ -lactam, Thermal stability.

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## INTRODUCTION

Schiff bases have received much attention due to their characteristic properties and applications such as structural variety [1], varied coordinating ability [2,3], thermal stability [4,5], catalysis properties [6,7], biological activities [8-10] etc. Schiff bases derived from sulfa drugs have gained considerable importance due to their pronounced biological activities. Schiff bases derived from sulfa drugs are good ligands due to the presence of different active sites such as heterocyclic moieties NH, OH and SO<sub>2</sub> groups, this led to the formation of stable complexes with d-block transition metals with a wide range of applications.  $\beta$ -lactams are synthetic antibiotics active against Gram-positive and Gram-negative bacteria by inhibiting the synthesis of the peptidoglycan layer from the cell wall [11]. The highly strained  $\beta$ -lactam nucleus is stabilized by means of the fusion of a variety of either 5-membered or 6-membered heterocyclic moieties to give rise to a wide spectrum of newer antibiotics [12].

## EXPERIMENTAL

### Materials

Analar grade Cu(CH<sub>3</sub>COO)<sub>2</sub>·H<sub>2</sub>O and Zn(CH<sub>3</sub>COO)<sub>2</sub>·2H<sub>2</sub>O as well as o-vanillin were obtained from Fluka. Sulfamerazine and chloroacetyl chloride were obtained from Sigma and used as received. Muller-Hinton agar, Nutrient agar, MCKONKY agar and Nutrient Broth were obtained from HiMEDIA. Standard drugs were obtained from Bioanalyse.

### Instrumentation and spectral measurements

Melting points were recorded by using a Fisher-Johns melting point apparatus. IR spectra were recorded by using Shimadzu FTIR-8300 spectrophotometer in the region 4000-400 cm<sup>-1</sup> in KBr pellet. The mass spectra were scanned by EI technique at 70 eV with an Agilent Technologies 5975C spectrometer. <sup>1</sup>H NMR spectra were scanned on a Bruker 400 MHz spectrometer with TMS as the internal standard and DMSO-d<sub>6</sub> as solvent. The molar conductance of complexes was measured using a conductometer coming model 441 at room temperature using DMF as a solvent. The thermal analysis (TG and DTG) were carried out in a dynamic argon atmosphere (20 ml/min) with a heating rate of 10 °C/min using a Rheometric Scientific and TGAQ50 (USA). Sterilizer Aesthica UV-Ray (USA). Incubator (HI 900 D) at 37 °C. Autoclave (HIRAYAMA) at 120 °C.

### Synthesis

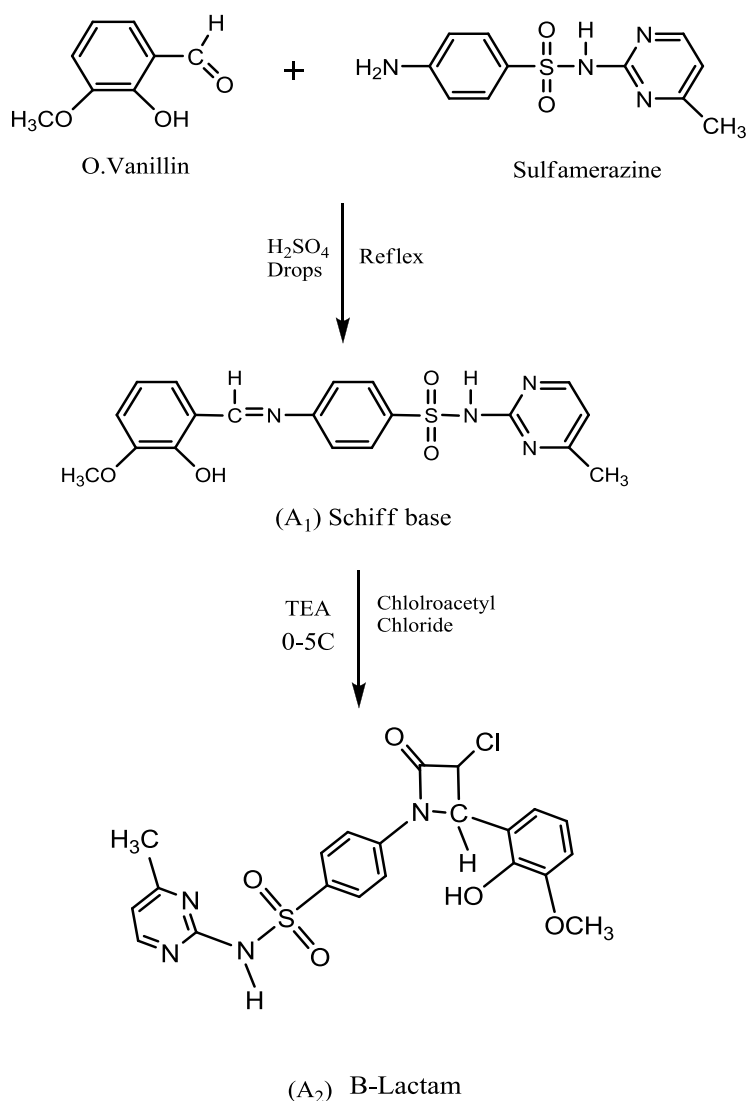
**Synthesis of Schiff base ligand (A<sub>1</sub>): 4-(2-hydroxy-3-methoxybenzylideneamino)-N-(4-methylpyrimidin-2-yl) benzenesulfonamide.**

2 mmole (0.538 g) of sulfamerazine (S.M) dissolved in 10 ml ethyl acetate was mixed with 2 mmole (0.304 g) of o-vanillin dissolved in 10 ml absolute ethanol. 2 drops of H<sub>2</sub>SO<sub>4</sub> was

added as a catalyst. The resulting mixture was refluxed for 2hrs. The orange solid precipitate which formed during the reaction filtered hot and washed with cold ethanol. The orange crystal product is produced in 75% yield and melting at 202-203°C.

**Synthesis of  $\beta$ -lactam (A<sub>2</sub>) :4-(3-chloro-2-(2-hydroxy-3-methoxyphenyl)-4-oxoazetidin-1-yl)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide.**

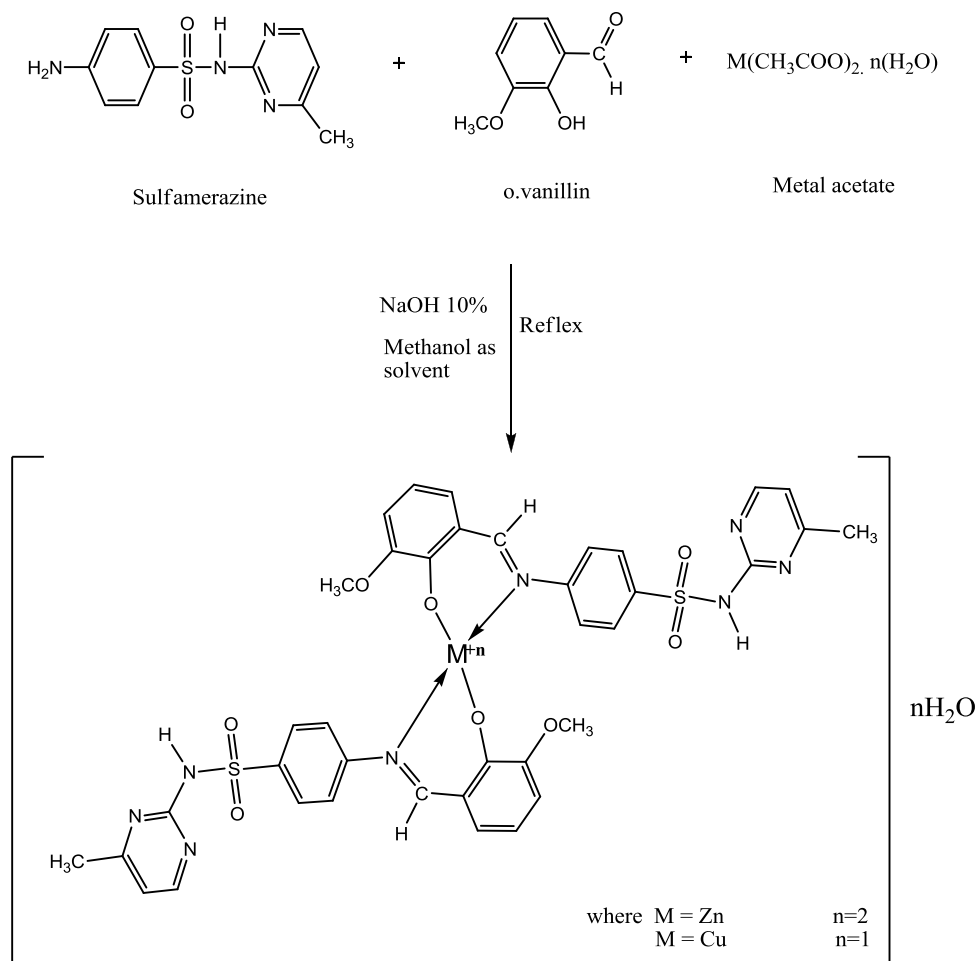
1mmole of (A<sub>1</sub>) dissolved in 10ml of Dioxane and the solution was kept at 0-5°C in ice water. Then, 1mmole (0.14ml) of triethylamine was added and the mixture stirred for 5 min. Then, 2mmole (0.16ml) of chloroacetyl chloride was added dropwise and finally the mixture was stirring for 4hrs and left at room temperature over night. The mixture was poured into crush ice and the white solid precipitate was filtered and washed with ethanol, dried at 70°C .yield 37% and m.p 190°d .



Scheme 1: preparation of (A<sub>1</sub>) and (A<sub>2</sub>)

### Synthesis of Zn complexes (ZnA<sub>1</sub>) and Cu complexes (CuA<sub>1</sub>):

Metal complexes ZnA<sub>1</sub> and CuA<sub>1</sub> were prepared using template reaction as follow. In methanolic solutions of each sulfamerazine (2mmole), o.vanillin (2mmole).The pH of the mixture was adjusted to 7.8-8 bu adding drops from 10%NaOH. Methanolic solution of metal acetate (1mmole in 10ml) then added to the reaction mixture drop wise with constant sterring and finally heated under reflex for 3hrs.The mixture was concentrated by evaporation. The precipitated metal complexes were filtered off, then washed with ether and preserved in desicator over anhydrous Calcium Chloride



Scheme 2: preparation of ZnA<sub>1</sub> and CuA<sub>1</sub>

### Bacterial Isolates

Four bacterial clinical isolates were obtained from laboratory research/Clinical laboratory science branch/Collage of Pharmacy/Basrah University, and they are: (*Staphylococcus aureu* , *Escherichia coli* , *Pseudomonas aeruginosa* and *Proteus spp.*)

### Antimicrobial activity investigation

Disc diffusion by Baueyer-Kirby method [13] were applied .Circular disc of watman No.1 were sterilized by Ultra-Violet Ray (U.V). The discs papers were imprgnated into prepared chemical compounds (S.M ,A<sub>1</sub> , A<sub>2</sub> ,ZnA<sub>1</sub> and CuA<sub>1</sub> ) in one (1ml) DMSO as solvent with differant concetratation (1mg ,10mg ,50mg ,100mg) .The clinical isolates were cultured and spreading on Mullar-Hinton agar,then discs were applied in each bacterial isolates .

### Standard antimicrobial investigation

Standard antibiotic discs (Cephalexin, Ciprofloxacin, Oxacillin, Cloxacillin, Aztreonam, Ampicillin, Clarithromycin and Novobiocin) were used to comparison with prepared chemical compounds.

### Statistical Analysis

Analysis of variance(ANOVA) [14] were used to explain the significancy differences of antimicribial activity among bacterial isolates.

## RESULTS AND DISCUSSION

Schiff base (A<sub>1</sub>) was prepared from condensation of Sulphamerazine(S.M) with o.vanillin and then covered to β-lactam (A<sub>2</sub>) by cycloaddition of chloroacetyle chloride with (A<sub>1</sub>) (scheme 1). Two complexes( Zn complex) and (Cu complex) were prepared from A<sub>1</sub> . The observed physical properties of compounds were collected in ( Table 1).

**Table 1 : Physical properties of prepared compounds**

compound	Chemical formula MW. g/mole	m.p °c or °d	physical state	molar conductance	Yield %
A <sub>1</sub>	C <sub>19</sub> H <sub>18</sub> N <sub>4</sub> O <sub>4</sub> S 398	202-203.5	orange crystal	.....	75
A <sub>2</sub>	C <sub>21</sub> H <sub>19</sub> N <sub>4</sub> O <sub>5</sub> SCI 474	190	white powder	.....	37
ZnA <sub>1</sub>	C <sub>38</sub> H <sub>38</sub> N <sub>8</sub> O <sub>10</sub> S <sub>2</sub> Zn 896	>300	yellow powder	11	67
CuA <sub>1</sub>	C <sub>38</sub> H <sub>36</sub> N <sub>8</sub> O <sub>9</sub> S <sub>2</sub> Cu 876	>300	brown powder	3	63

Compound A<sub>1</sub> orange in colour, A<sub>2</sub> white colour ,air stable ,having sharp melting points >200°C . (A<sub>1</sub>) soluble in dioxane,DMF and DMSO but, insoluble in common organic solvents. (A<sub>2</sub>) soluble in acetonitrile,DMFandDMSO but, insoluble in most common organic solvents. Complexes (ZnA<sub>1</sub>) and (CuA<sub>2</sub>) are stable and decompose >300°C,they were insoluble in most common organic solvents but soluble in DMSO and DMF .

## IR Spectra analysis

The IR spectrum of the free ligand  $A_1$  ( Fig 1) shows broad band at  $3462\text{cm}^{-1}$  that attributed to the hydrogen bonded(OH) group . A strong band is observed at  $1616\text{cm}^{-1}$  which is assigned to(  $\text{C}=\text{N}_{\text{azo}}$ .), this band is shifted to a lower wave number side  $\Delta V= 4\text{cm}^{-1}$  in case  $\text{Zn(II)}$  complexe and  $\Delta V= 17\text{cm}^{-1}$  in case of  $\text{Cu(II)}$  complexe (Fig2), which indicates the participation of the azomethine groups in coordination to the metal ions through the lone pair of electrons on the nitrogen[2,4]. In addition the involvement of phenolic OH group in bonding with metal ions can be examined by the(C-O) band which is shifted to lower wave number side  $\Delta V=23\text{cm}^{-1}$  in case  $\text{Zn(II)}$  complexe and  $\Delta V=48\text{cm}^{-1}$  in case of  $\text{Cu(II)}$  complexe. The IR spectrum of  $A_2$  (Fig 3) shows a new band at  $1687\text{cm}^{-1}$  that corresponding to ( $\text{C}=\text{O}_{\beta\text{-Lactam}}$ ) [15,16] of lactam moiety, this gives information regarding lactam ring formation as well the disappearance of  $\text{C}=\text{N}_{\text{azo}}$ . Group.

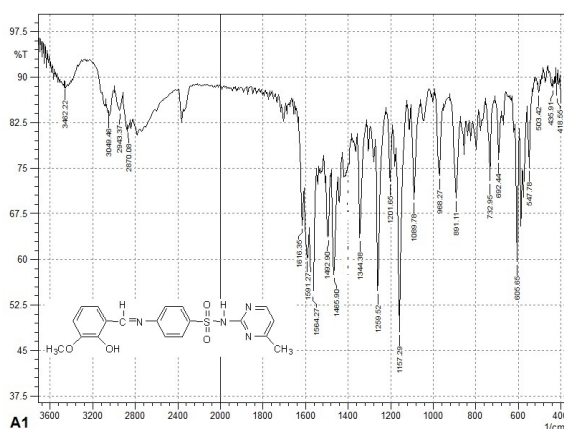


Figure 1 :IR Spectrum of  $A_1$

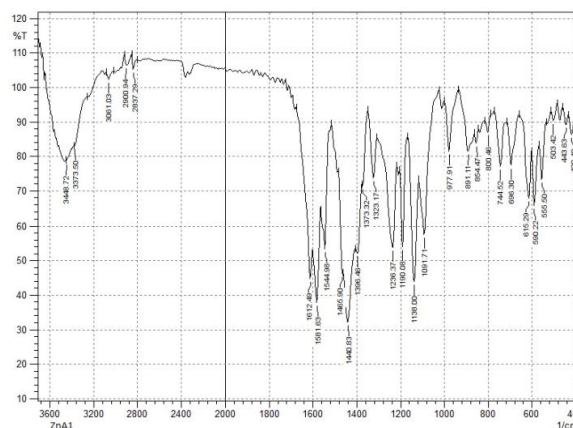


Figure 2: IR Spectrum of  $\text{ZnA}_1$

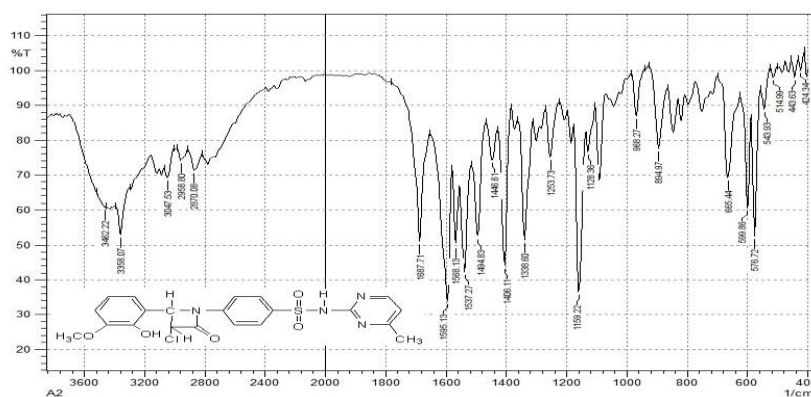


Figure 3: IR Spectrum of  $A_2$

## $^1\text{H}$ NMR Spectra Analysis

The  $^1\text{H}$ NMR spectral data of the schiff base ligand ( $A_1$ ) confirms the proposed structural elucidation of the ligand, this  $^1\text{H}$ NMR spectrum(Fig 4)shows the methyl protons as a singlet signal at  $\sigma 2.4\text{ppm}$ , it also displays a singlet signal at  $\sigma 3.9\text{ppm}$  which is attributed to methoxy protons. The azomethine proton appear at  $\sigma 8.6\text{ppm}$ .The signal at  $\sigma 10.7\text{ppm}$  is attributed to –

NH proton [4] and the signal of -OH proton appear at  $\sigma$ 13.07ppm. A comparison of the chemical shifts of ligand  $A_1$  with its Zn complex (Fig 5) indicates that the signal due to phenolic proton is absent in the complex spectrum, this can be attributed due to the deprotonation of the phenolic group and subsequently the replacement of the proton by metal [2 ,4]. The azomethine signal is observed up field at  $\sigma$  8.4 in complex spectrum, it supports the coordination of azomethine nitrogen to metal ion.  $\beta$ -lactam ( $A_2$ ) formation is characterized by the disappearance of the azomethine proton signal of the corresponding schiff base ( $A_1$ ) and appearance of the two new signal of the protons of  $\beta$ -lactam ring (Fig 6) .The chemical shift of proton HC-N appear at  $\sigma$  3.57ppm [17] and HC-Cl proton appear at  $\sigma$  2.57ppm [18].The -OH proton signal which would have undergone very rapid exchange with the solvent appear as quit broad signal at  $\sigma$  4ppm [19 ,20] .

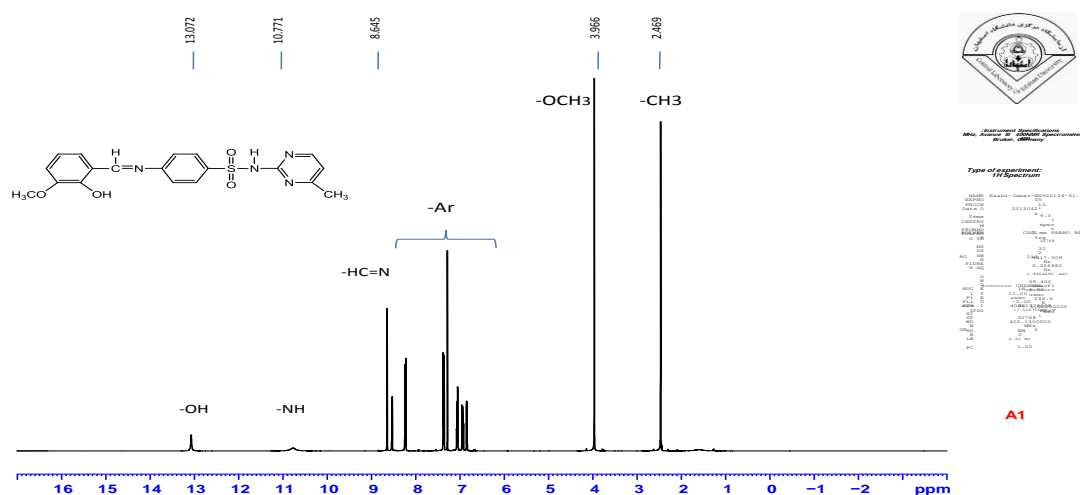


Fig 4: <sup>1</sup>H NMR Spectrum of  $A_1$

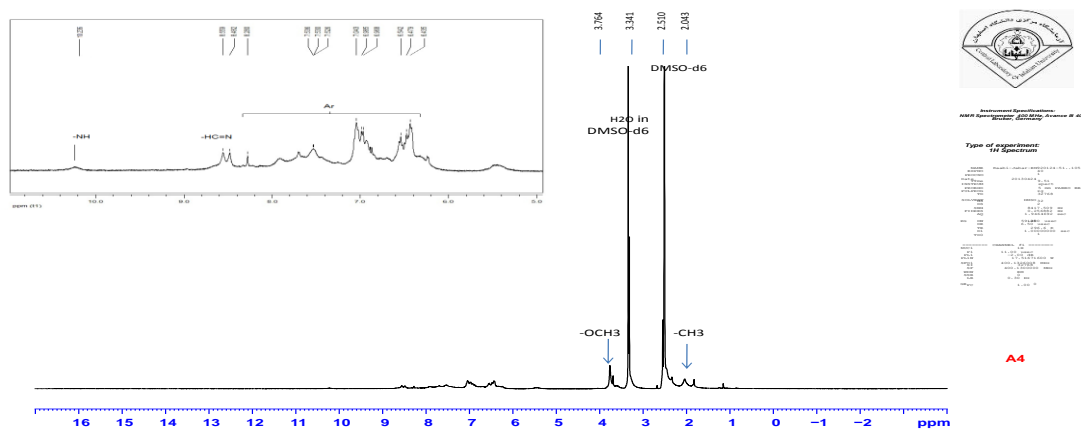
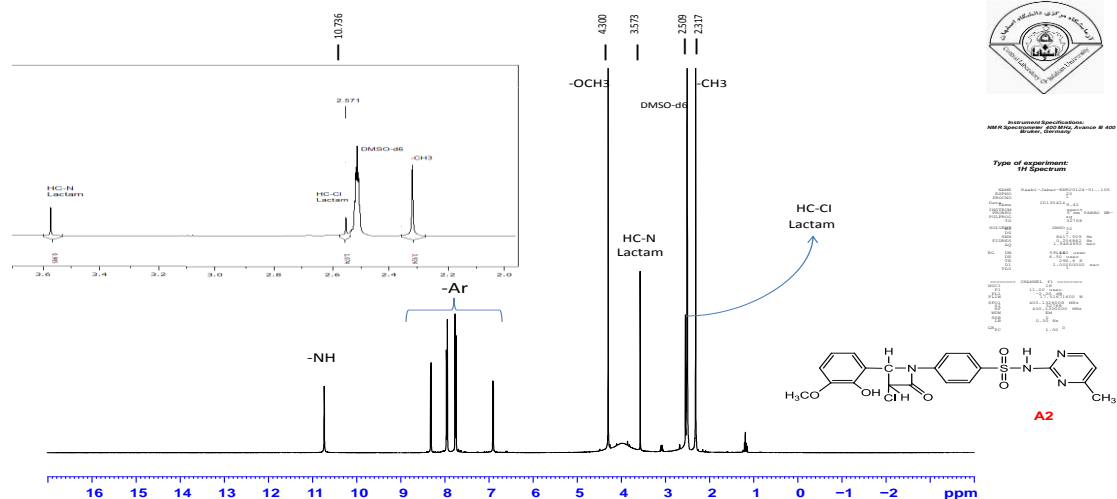
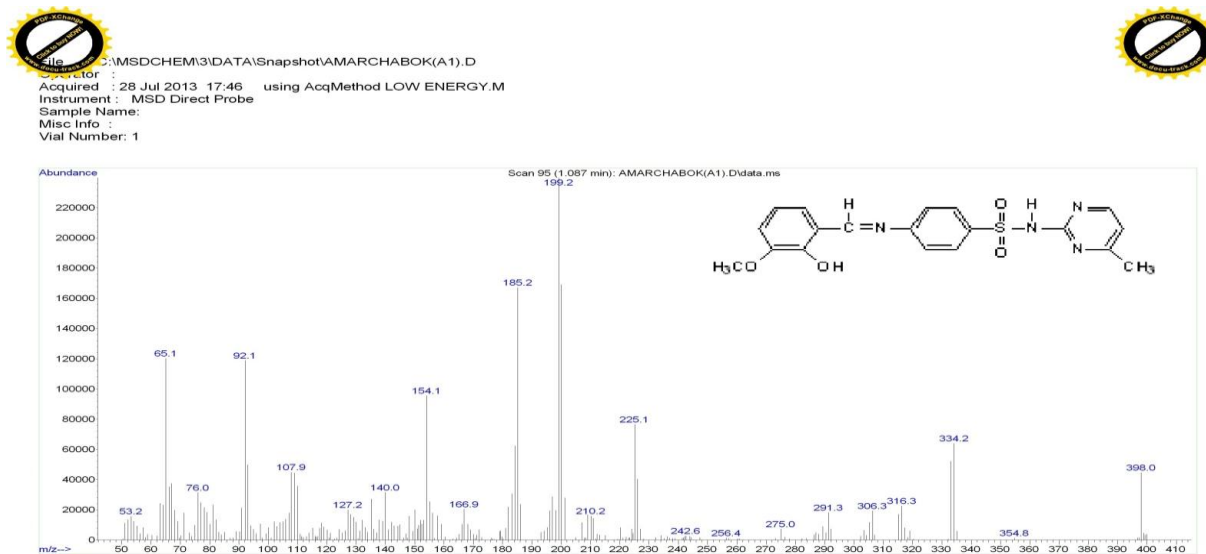


Figure 5: <sup>1</sup>H NMR Spectrum of  $ZnA_1$

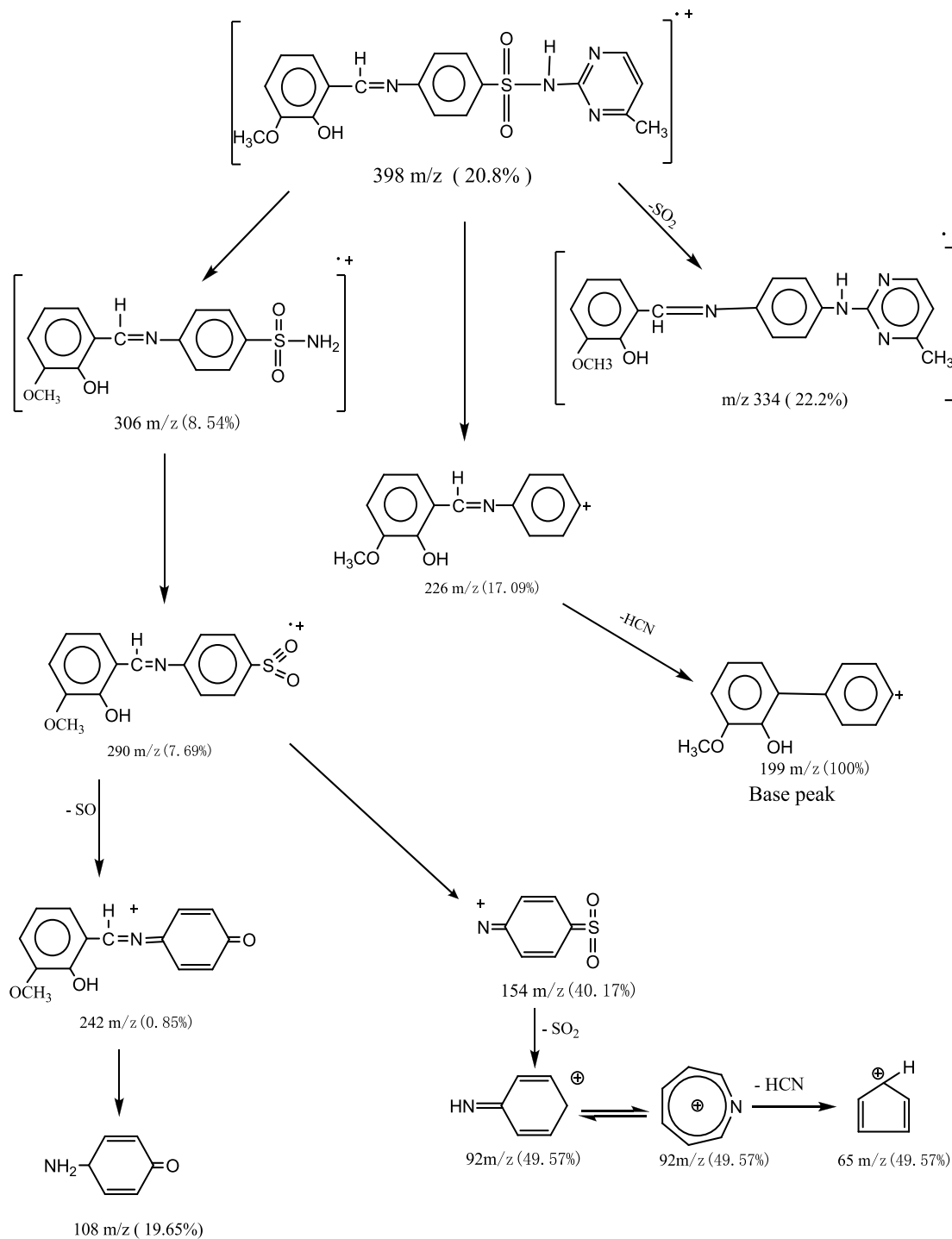

 Figure 6: <sup>1</sup>H NMR Spectrum of A<sub>2</sub>

### Mass Spectrum analysis

The mass spectrum of A<sub>1</sub> (Fig 7) reveals the molecular ion peak at m/z 398 with relative abundance 21% which is in agreement with the formula C<sub>19</sub>H<sub>18</sub>N<sub>4</sub>O<sub>4</sub>S and is equivalent to its molecular weight. The base peak at m/z 199.2 is attributed to an unusual double charge ion m/2z [21]. The fragment pattern of some important ions is illustrated in scheme 3.


 Figure 7: Mass spectrum of A<sub>1</sub>




 Scheme 3: Fragmentation mechanism of  $A_1$ 

### Molar conductance

The molar conductance of the complexes  $ZnA_1$  and  $CuA_1$  showing that they are non-electrolyte [2]. A value of ( $110 \text{ ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$ ) for  $ZnA_1$  and ( $3 \text{ ohm}^{-1} \text{ cm}^2 \text{ mol}^{-1}$ ) for  $CuA_1$ .

## Thermal analysis

Thermal analysis (TG and DTG) of the complexes were used to get information about the thermal stability as well as to verify the status of water molecules into inside or outside the inner coordination sphere. The mass losses obtained from TG curves are in a good agreement with the theoretical values. Cu complex undergoes decomposition in four steps. The first decomposition step within the range 25-138°C (DTG 110°C) is accompanied by weight loss (obs 1.977%. cal 1.975%) with assigned to the loss one lattice water molecule [2,10]. The second step shows loss in weight within the temperature range of 220-325°C (DTG<sub>MAX</sub>282°C) which is due to loss C<sub>5</sub>H<sub>5</sub>N<sub>2</sub> (obs 10.16%.cal10.41%) The third decomposition step began at 330 and end at 500°C (DTG<sub>max</sub> 374°C) and represents the decomposition of the sulfa moieties. The residue after the fourth step until 800°C could be identified as C<sub>26</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>Cu (obs 51% cal.50.72%). TG curve of Zn(II) complex shows a three decomposition steps, the first step at 25-110°C (DTG 77°C) assigned to the loss of two water lattice molecules (obs 4.12 % cal 4.01 %) The second step lies in the temperature rang 250-410°C (DTG<sub>max</sub> 344) corresponds to the loss of C<sub>10</sub>H<sub>12</sub>N<sub>6</sub>S<sub>2</sub>O<sub>4</sub>. (obs 34.63% cal 32.43%). The third step began within 420°C and the (DTG<sub>max</sub> 521°C) .where the ligand decompose in fast rate until 800°C. Fig ( 8 )

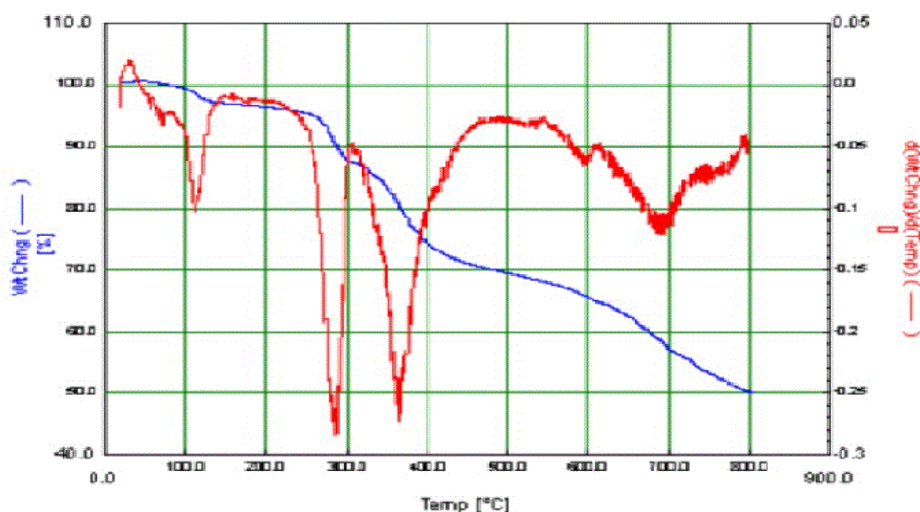


Fig 8 : TG and DTG curve of complex CuA<sub>1</sub>

## Antimicrobial activity

Bacterial sensitivity of (*staphylococcus aureus*) against prepared chemical compounds (S.M. A<sub>1</sub> , A<sub>2</sub> , ZnA<sub>1</sub> and CuA<sub>1</sub>) were clarified in Table (2 , The maximum inhibition zone was recorded with  $\beta$  – lactam prepared compound (A<sub>2</sub>). It was seventeen millimeter (17mm) with concentration (100mg) and eight millimeter (8mm) with concentration (1mg) per 1 ml DMSO. While others prepared compound (S.M. A<sub>1</sub> , ZnA<sub>1</sub> and CuA<sub>1</sub>) were resist with concentration (1mg) and moderate sensitivity with elevated concentration (10mg, 50mg, 100mg) per 1ml DMSO. The results could be explained that  $\beta$  – lactam compound have active to block termination peptido-glycan crosslinking in G+Ve bacteria like *staphylococcus aureus* [22].

hence, It is possible and use it as synthetic product for antimicrobial activity after cytotoxicity study proof or may be used as external use.

**Table 2: Antimicrobial activity of chemical compound toward *S. aureus***

Compound	Diameter of inhibition zone ( mm ) S.aureus Per 1ml DMSO			
	1 mg	10 mg	50 mg	100 mg
S . M	R	7	9	10
A1	R	8	11	15
A2	8	12	15	17
ZnA1	6	9	12	15
CuA1	6	9	10	14

As for the standard antibiotics, The drugs (Ciprofloxacin, Clarithromycin and Novobiocin) were showed results concrete inhibition zone , while other drugs will be never. Table (3).

**Table 3: Antimicrobial activity of standard drug toward *S.aureus***

Standard Drug	Diameter of inhibition zone ( mm )		
	Con.	Abr.	S.aureus
Cephalexin	30mcg	CL	R
Ciprofloxacin	5mcg	Cip	20
Oxacillin	1mcg	Ox	R
Cloxacillin	1mcg	Cx	R
Aztreonam	30mcg	ATM	R
Ampicillin	10mcg	AM	R
Clarithromycin	15mcg	CLR	23
Novobiocin	30mcg	NV	24

On the other hand, The others three G-Ve clinical bacterial isolates ( *E.coli* , *Ps.aeruginosa* and *pr.spp.*) were highly sensitive toward Schiff-complexes . The maximum inhibition zone were showed by (ZnA<sub>1</sub> , CuA<sub>1</sub>) against these bacteria in 100mg concentration per ( 1ml DMSO) , but in the concentration 1mg per (1ml DMSO) the chemical compound had less antimicrobial activity and elevated with 10mg and 50mg concentration . As a result of various metal complexes which bi- and tridentate Schiff bases containing nitrogen and oxygen donor atoms play important role in biological system and represent interesting models for metalloenzymes ,which efficiently catalyze the reduction of dinitrogen and dioxygen ,In addition to the enhanced activity of the complexes may be ascribed to the increased lipophilic nature of the complexes arising due to chelation<sup>(1)</sup>, especially Zn(II) complexes . Table : 4 , Table : 5 , Table : 6

**Table 4: Antimicrobial activity of chemical compounds toward E.coli**

Compound	Diameter of inhibition zone ( mm ) E.coli Per 1ml DMSO			
	1 mg	10 mg	50 mg	100 mg
S . M	R	6	8	11
A1	6	10	12	14
A2	6	10	13	15
A4	7	10	14	17
A6	8	12	14	17

**Table 5: Antimicrobial activity of chemical compoundstoward Ps.aeruginosa**

Compound	Diameter of inhibition zone ( mm ) Ps.aeruginosa Per 1ml DMSO			
	1 mg	10 mg	50 mg	100 mg
S . M	R	6	7	9
A1	R	7	9	11
A2	6	10	12	12
A4	R	7	12	16
A6	R	8	11	13

**Table 6: Antimicrobial activity of chemical compounds toward Pr.ssp.**

Compound	Diameter of inhibition zone ( mm ) Pr.ssp. Per 1ml DMSO			
	1 mg	10 mg	50 mg	100 mg
S . M	R	6	7	9
A1	R	8	9	10
A2	R	7	10	12
A4	6	10	12	16
A6	6	9	11	13

The standard antibiotics were showed ineffective toward these bacteria except (Ciprofloxacin , Clarithromycin ,Novobiocin) in *E.coli* and (Ciprofloxacin ,Novobiocin) in *Ps.aeruginosa* as well as (Ciprofloxacin ,Aztreonam ,Novobiocin) in *Pr.spp*. Table : 7 , Table : 8 , Table : 9

**Table 7: Antimicrobial activity of standard drug toward E.coli**

Standard Drug	Diameter of inhibition zone ( mm )		
	Con.	Abr.	E.coli
Cephalexin	30mcg	CL	R
Ciprofloxacin	5mcg	Cip	28
Oxacillin	1mcg	Ox	R
Cloxacillin	1mcg	Cx	R
Aztreonam	30mcg	ATM	R
Ampicillin	10mcg	AM	R
Clarithromycin	15mcg	CLR	16
Novobiocin	30mcg	NV	7

**Table 8: Antimicrobial activity of standard drug toward Ps.aeruginosa**

Standard Drug	Diameter of inhibition zone ( mm )		
	Ps.aeruginosa	Abr.	Con.
	30mcg	CL	R
Cephalexin	5mcg	Cip	17
Ciprofloxacin	1mcg	Ox	R
Oxacillin	1mcg	Cx	R
Cloxacillin	30mcg	ATM	R
Aztreonam	10mcg	AM	R
Ampicillin	15mcg	CLR	R
Clarithromycin	30mcg	NV	14

**Table 9: Antimicrobial activity of standard drug toward Pr.ssp.**

Standard Drug	Diameter of inhibition zone ( mm )		
	Con.	Abr.	Pr.
Cephalexin	30mcg	CL	R
Ciprofloxacin	5mcg	Cip	26
Oxacillin	1mcg	Ox	R
Cloxacillin	1mcg	Cx	R
Aztreonam	30mcg	ATM	24
Ampicillin	10mcg	AM	R
Clarithromycin	15mcg	CLR	R
Novobiocin	30mcg	NV	20

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