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Synthesis, Spectroscopic Characterization, Thermal Stability andantimicrobial activity of Schiff base, β- lactam and Zn (II), Cu (II) complexes derived from Sulfamerazine.

Jabbar S Hadi[a]*, Abdulelah A Almayah[b], and Ali G Swadi [a]

[a]College Of Education For Pure Science-Basrah University-Basrah-IRAQ
[b]College of Pharmacy-Basrah University-Basrah-IRAQ

ABSTRACT

Schiff base derived from *o*-vanillin and Sulfamerazine, β – lactam and Zn(II) , Cu(I) complexes have been Synthesized and characterized by IR , ^{1}HNMR , MASS spectrometry , molar condactance 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 condactane measurments 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 β-lactam emerged as laed with MIC (mg/ml) values with mentioned gram +Ve,While the complexes-schiff base showed highly antimicrobial activity toward mentionded gram –Ve bacterai. These results compared with standard drugs (Cephalexin;30µg/disc, Ciprofloxacin;5µg/disc, Oxacillin;1µg/disc, Cloxacillin;1µg/disc, Aztreonam;30µg/disc, Ampicillin;10µg/disc, Clarithromycin;15µg/disc , Novobiocin;30µg/disc)

Keywords: Sulfamerazine , antimicrobial activity , β – lactam , Thermal stability.

Corresponding author

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INTRODUCTION

Schiff bases have recieved 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 base derived from sulfa drugs have gained considerable importance due to their pronounced biological activities .Schiff base derived from sulfa drugs are good ligands due to the presence of different active site such as heterocyclic moieties NH, OH and SO2 groups, this led to the formation a stable complexes with d-block transition metals with a wide range of applications. β – lactam 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 stained β – lactam nucleus is stabilized by means of the fusion of a variety of either 5-membered or 6-membered hetetocyclic moieties to give rise to a wide spectrum of newer antibiotics [12].

EXPERIMENTAL

Materials

Analar grade Cu $(CH_3COO)_2.H_2O$ and $Zn(CH_3COO)_2.2H_2O$ as well as o.vanillin were obtained from fluka. Sulfamerazine and chloroacetylchloride were obtained from sigma and used as recieved. Muller-Hinton agar, Nutrient agar, Mckonky agar and Nutrient Brouth were obtained from HiMEDIA . Standard drugs were obtained from Bioanalyse .

Instrumentation and spectral measurments

Meliting points were recorded by using a Fisher Johus melting pointapparatuse IR spectra were recorded by using shimadzu FTIR – 8300 spectrophotometer in the region 4000-400cm^{-1} in KBr pellet. The Mass spectra were scanned by EI technique at 70eV with an Agilent technologies 5975C spectrometer. ^{1}HNMR spectra were scanned on a Bruker 400MHz spectrometer TMS as the internal standard and DIMSO-d_6 was used as solvent. The molar condactance of complexes was measured using conductometer coming model 441at room temperature using DMF as a solvent. The thermal analysis (TG and DTG) were carried out in dynamic Argone atmospher (20ml/min) witha heating rate of 10°C/min using a Rheometric scintific and TGAQ50(USA) .Sterlizer Aesthtica UV.Ray (USA) .Incubator (HI 900 D) at 37°C. Autoclave (HIRAYAMA) at 120°C.

Synthesis

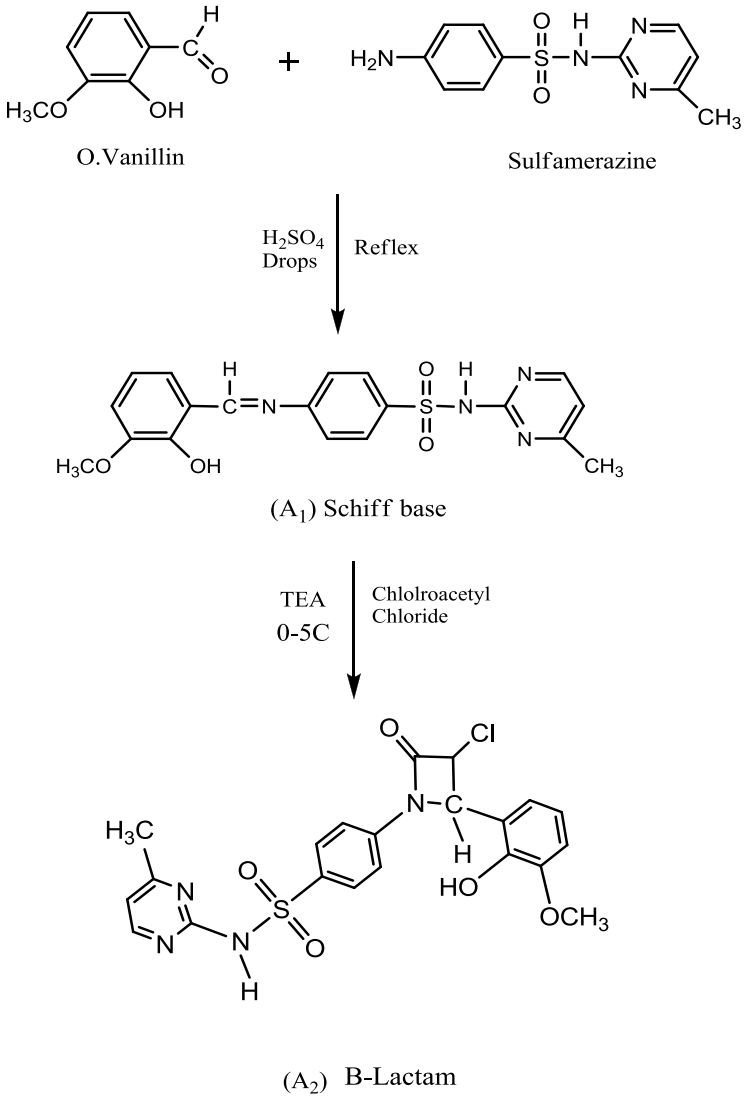
Synthesis of schiff base ligand (A_1):4-(2-hydroxy-3-methoxybenzylideneamino)-N-(4-methylpyrimidin-2-yl) benzenesulfonamide.

2 mmole(0.538g) of sulfamerazine (S.M) dissolved in 10ml ethyl actate was mixed with 2mmole (0.304) of o.vanillin dissolved in 10ml absolute ethanol.2 drops ofH_2SO_4 was

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

Synthesis of β-lactam (A_2) :4-(3-chloro-2-(2-hydroxy-3-methoxyphenyl)-4-oxoazetidin-1-yl)-N-(4-methylpyrimidin-2-yl)benzenesulfonamide.

1mmole of (A_1) 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 chloroacetyle chloride was added dropwise and finally the mixture was stirring for 4hrs and left at room temprature over night. The mixture was poured into crush ice and the white solid preciptate was filtered and washed with ethanol,dried at 70°C .yield 37% and m.p 190°d .



Scheme 1: preparation of (A_1)and(A_2)

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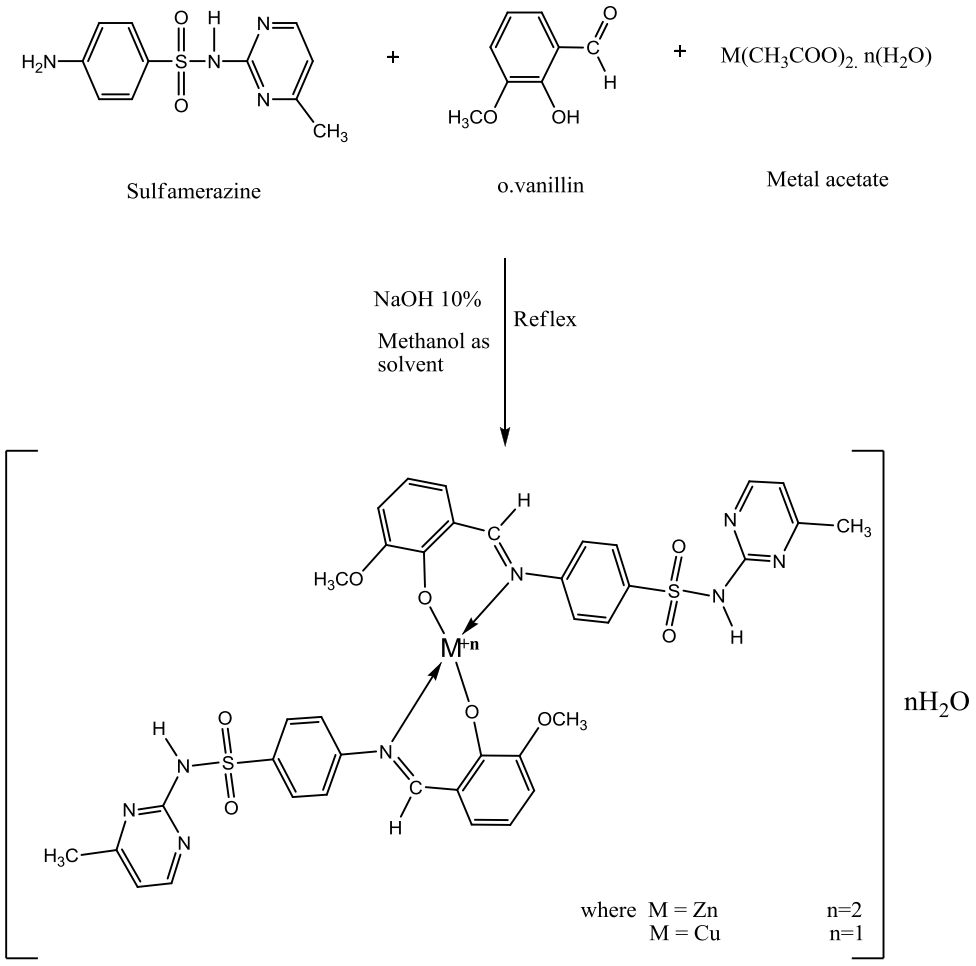
Synthesis of Zn complexes (ZnA_1) and Cu complexes (CuA_1):

Metal complexes ZnA_1 and CuA_1 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_1 and CuA_1

Bacterial Isolates

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

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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 imprignated into prepared chemical compounds (S.M ,A_1 , A_2 ,ZnA_1 and CuA_1) in one (1ml) DMSO as solvent with differant concetration (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_1) was prepared from condensation of Sulphamerazine(S.M) with o.vanillin and then coverted to β-lactam (A_2) by cycloaddition of chloroacetyle chloride with (A_1) (scheme 1). Two complexes(Zn complex) and (Cu complex) were prepared from A_1 . 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_1	$C_{19}H_{18}N_4O_4S$ 398	202-203.5	orange crystal	.....	75
A_2	$C_{21}H_{19}N_4O_5SCl$ 474	190	white powder	.....	37
ZnA_1	$C_{38}H_{38}N_8O_{10}S_2Zn$ 896	>300	yellow powder	11	67
CuA_1	$C_{38}H_{36}N_8O_9S_2Cu$ 876	>300	brown powder	3	63

Compound A_1 orange in colour, A_2 white colour ,air stable ,having sharp melting points >200°C . (A_1) soluble in dioxane,DMF and DMSO but, insoluble in common organic solvents. (A_2) soluble in acetonitrile,DMFandDMSO but, insoluble in most common organic solvents. Complexes (ZnA_1) and (CuA_2) are stable and decompose >300°C,they were insoluble in most common organic solvents but soluble in DMSO and DMF .

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IR Spectra analysis

The IR spectrum of the free legand A_1 (Fig 1) shows broad band at 3462cm^{-1} that attributed to the hydrogen bonded(OH) group . A strong band is observed at 1616cm^{-1} which is assigned to(C=$N_{azo.}$), this band is shifted to a lower wave number sideΔV= 4cm^{-1} in case Zn(ıı)complexe andΔV= 17cm^{-1} in case of Cu(ıı) 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 sideΔV=23cm^{-1} in case Zn(ıı) complexe and ΔV=48cm^{-1} in case of Cu(ıı) complexe. The IR spectrum of A_2 (Fig 3) shows a new band at 1687cm^{-1} that corresponding to (C=$O_{\beta\text{-Lactam}}$) [15,16] of lactam moity,this gives information regarding lactam ring formation as well the disappearance of C=$N_{azo.}$ Group.

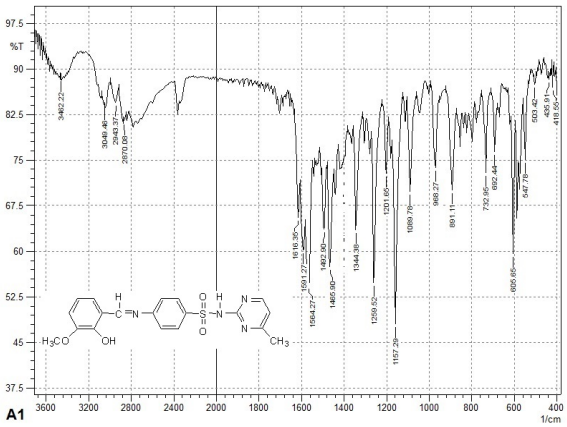

Figure 1 :IR Spectrum of A_1

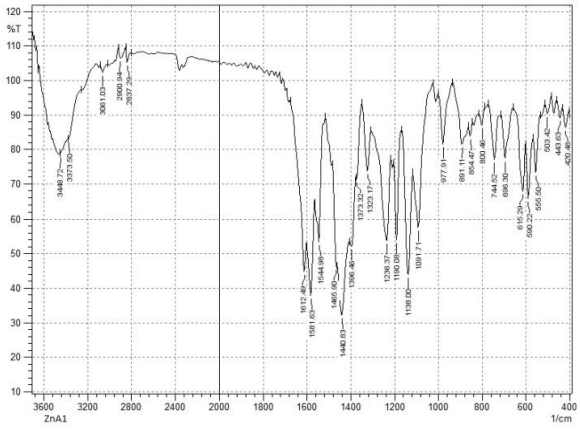

Figure 2: IR Spectrum of ZnA_1

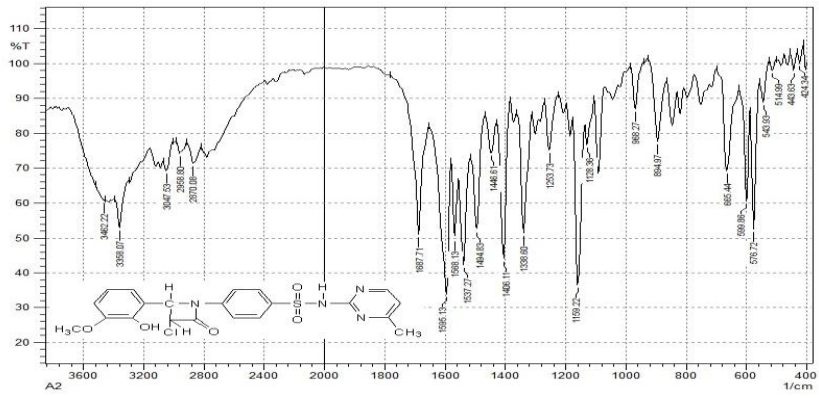

Figure 3: IR Spectrum of A_2

^{1}HNMR Spectra Analysis

The ^{1}HNMR spectral data of the schiff base ligand (A_1) confirms the proposed structural elucidation of the ligand, this ^{1}HNMR spectrum(Fig 4)shows the methyl protons as a singlet signal at σ2.4ppm, it also displays a singlet signal at σ 3.9ppm which is attributed to methoxy protons. The azomethine proton appear at σ 8.6ppm.The signal at σ10.7ppm is attributed to –

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NH proton [4] and the signal of –OH proton appear at σ13.07ppm. 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 σ 8.4 in complex spectrum, it supports the coordination of azomethine nitrogen to matal ion. β-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 β-lactam ring (Fig 6) .The chemical shift of proton HC-N appear at σ 3.57ppm [17] andHC-CI proton appear at σ 2.57ppm [18].The -OH proton signal which would have undergone very rapid exchange with the solvent appear as quit broad signal atσ 4ppm [19 ,20] .

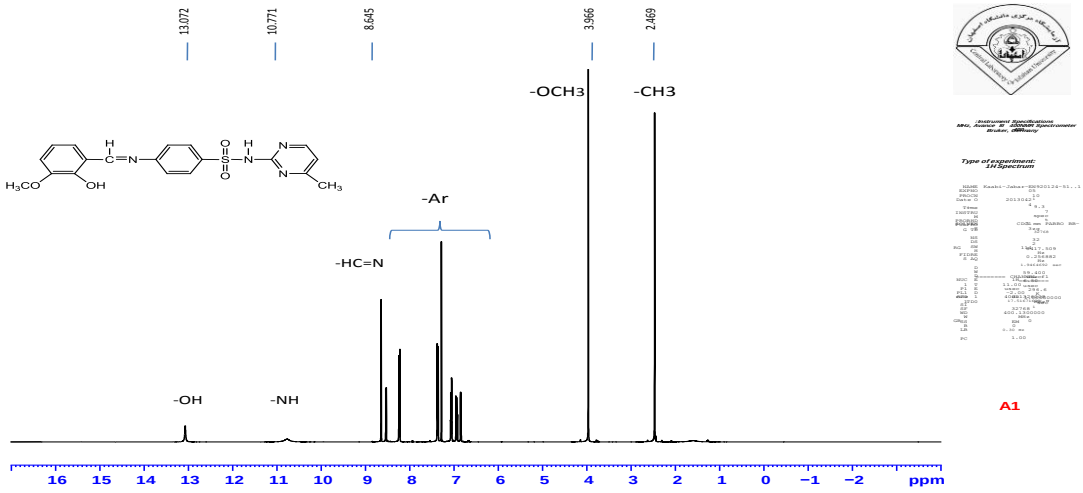

Fig 4:[1]HNMR Spectrum of A_1

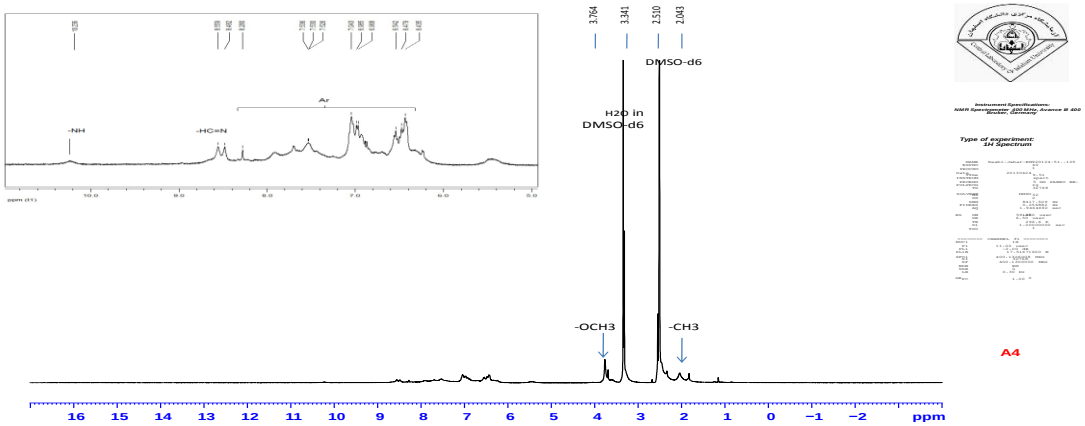

Figure 5: [1]HNMR Spectrum of ZnA_1

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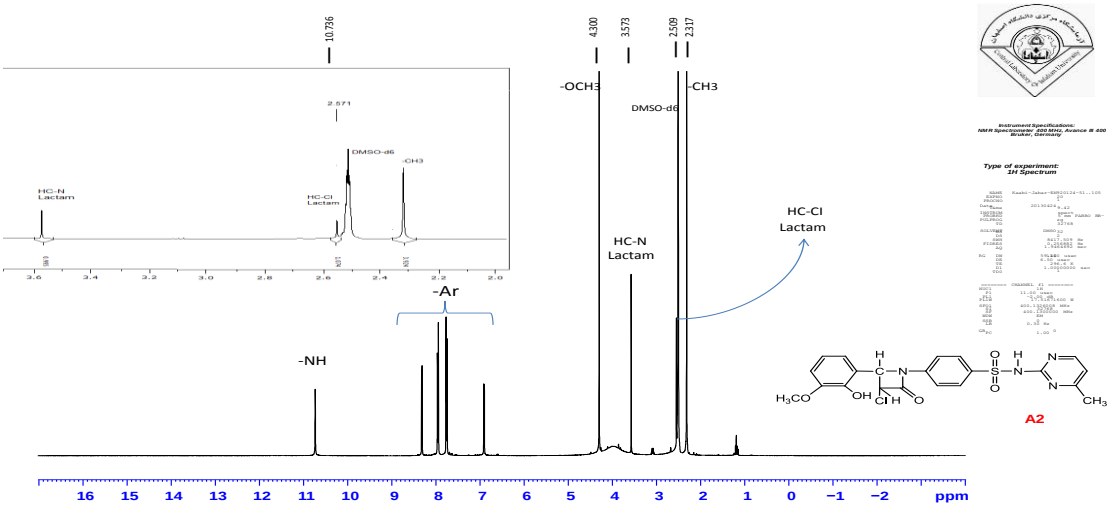

Figure 6: ^{1}HNMR Spectrumof A_2

Mass Spectrum analysis

The mass spectrum of A_1 (Fig 7) reveals the molecularion peak at m/z398 with relative abundance 21% which is agreement with the fromula $C_{19}H_{18}N_4O_4S$ and is equivalent to its molecular weight. The base peak at m/z199.2 this maybe attributed to unusual double chargeion m/2z[21]. The fragment pattern of some importantions are illustrated in scheme 3.

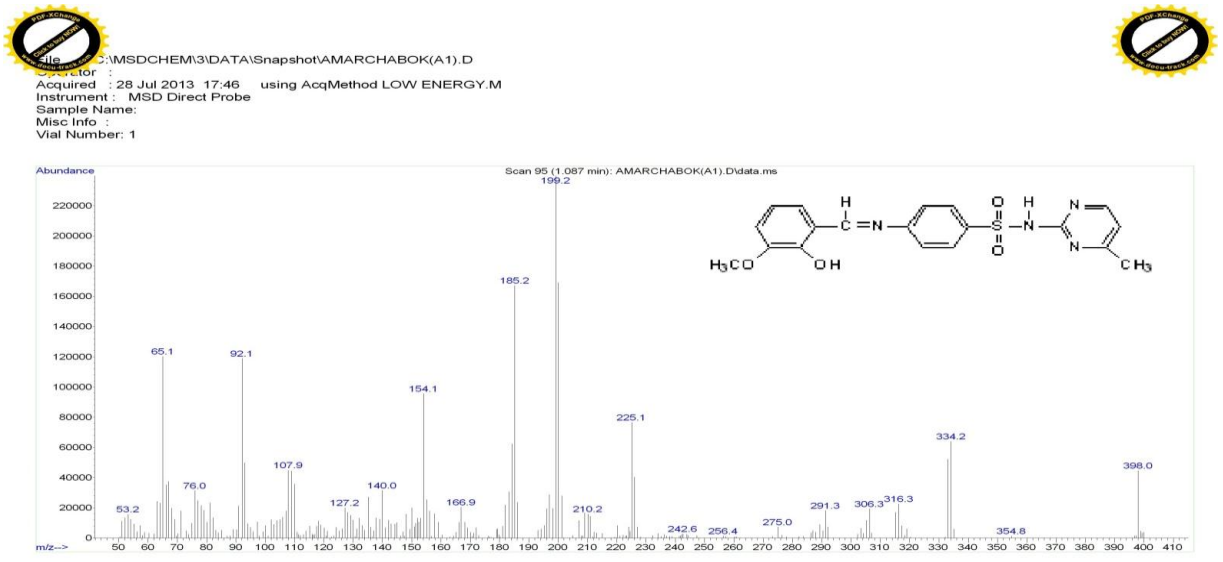

Figure 7: Mass spectrum of A_1

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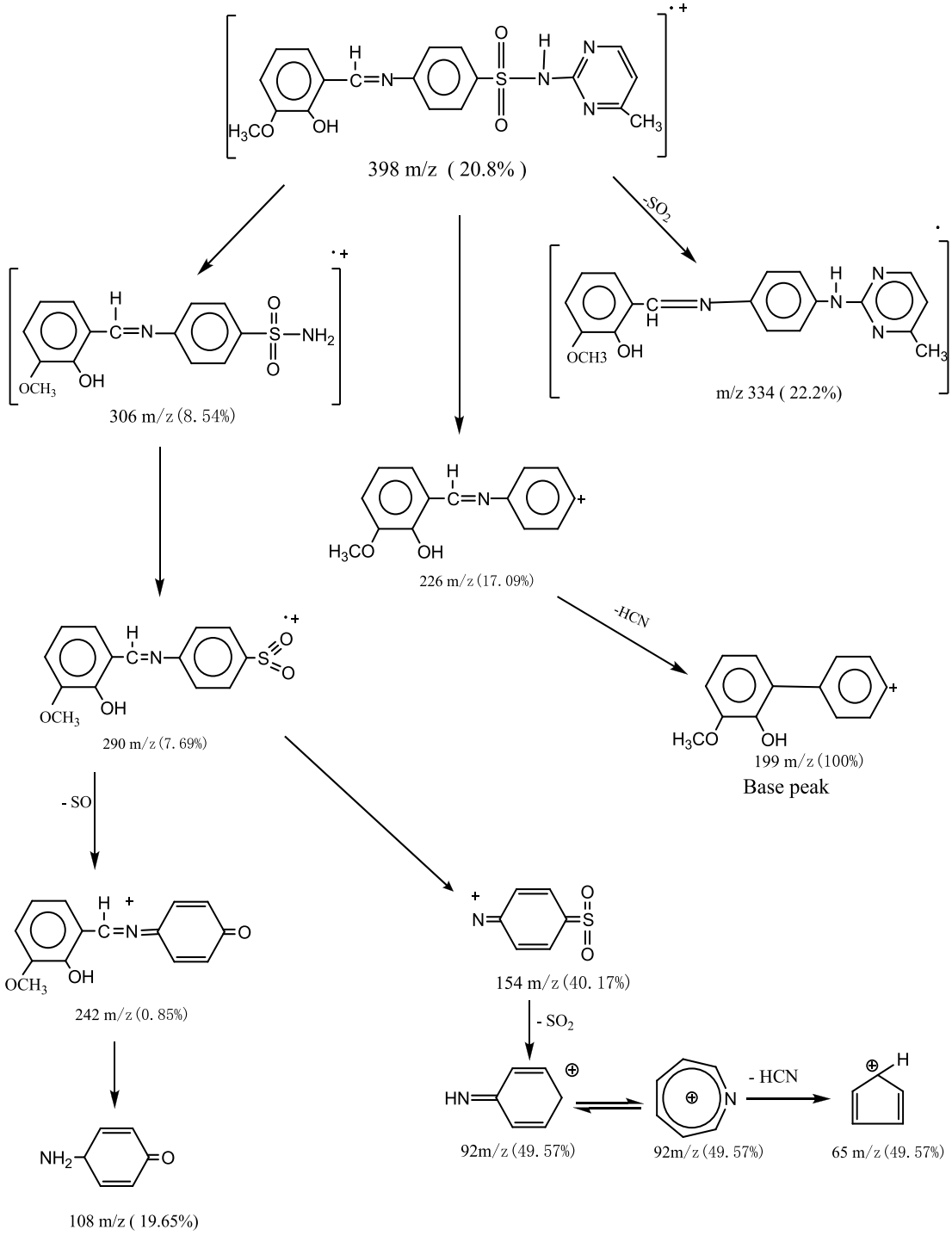

Scheme 3: Fragmentation mechanism of A_1

Molar conductance

The molar condactance of the complexes ZnA_1 and CuA_1 showing that they are non-electrolyte [2] . A value of (11ohm^{-1} cm^{2}mol^{-1}) for ZnA_1 and (3 ohm^{-1}cm^{2}mol^{-1}) for CuA_1.

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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 spher. The mass losses obtained from TG curves are in a good agreement with the therotical values. Cu complex undergoes decomposition in four steps. The first decomposition step within the range 25-138°C (DTG 11O°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_{MAX}282°C) which is due to loss $C_5H_5N_2$ (obs 10.16%.cal10.41%) The third decomposition step began at 330 and end at 500°C (DTG_{max} 374°C) and represents the decomposition of the sulfa moieties. The residue after the fourth step until 800°C could be identified as $C_{26}H_{20}N_2O_2Cu$ (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_{max} 344) correspods to the loos of $C_{10}H_{12}N_6S_2O_4$. (obs 34.63% cal 32.43%). The third step began within 420°C and the (DTG_{max} 521°C) .where the ligand decompose in fast rate until 800°C. Fig (8)

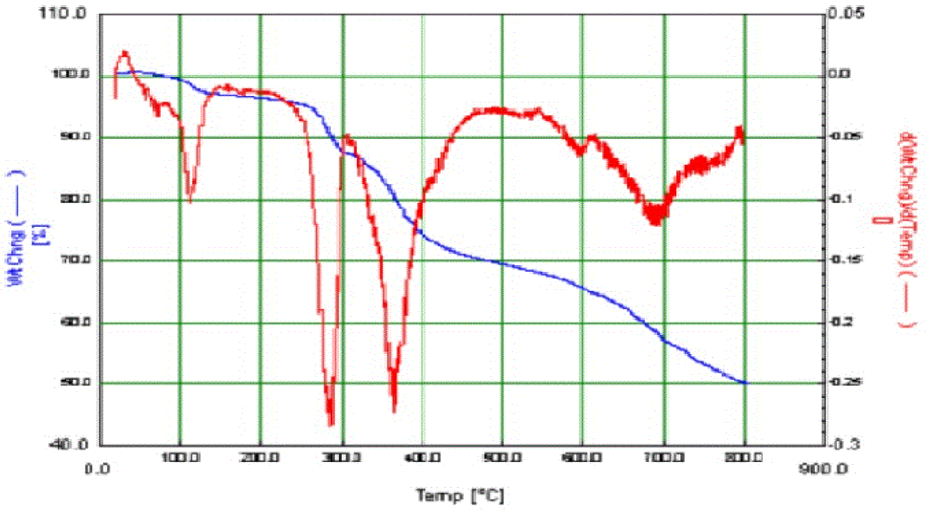

Fig 8 : TG and DTG curve of complex CuA_1

Antimicrobial activity

Bacterial sensitivity of(*staphylococeus aureus*) against perpared chemical compounds (S.M. ,A_1 , A_2 , ZnA_1 and CuA_1) were clarified in Table (2 , The maximum inhibition zone was recorded with β – lactam prepared compound (A_2). It was seventeen milimeter (17mm) with concetration (100mg) and eight milimter (8mm) with concentration (1mg) per1 ml DMSO. While others prepared compound (S.M. A_1 , ZnA_1 and CuA_1) were resist with concentration (1mg) and moderate sensitivity with elevated concentration (10mg, 50mg, 100mg) per 1ml DMSO. The results could be explained that β – lactam compound have active to block termination peptido-glycan crosslinking in G+Ve bacteria like *staphylococcus aureus* [22].

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hence, It is possible and use it as synthitic product for antimicrobial activity after cytotoxicity study proff 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 ohers three G-Ve clinical bacterial isolates (*E.coli , Ps.aerugiosa and pr.spp*.) were highly sinsetive toward shiff-complexes . The maximam inhibition zone were showed by (ZnA_1 , CuA_1) against these bacteria in 100mg concentrationper(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 whith 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[(,)],especially Zn(II) complexes . Tabl : 4 , Table : 5 , Table : 6

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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

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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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