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SYNTHESIS OF AROMATIC HETEROCYCLIC KETIMINES: PART-III. SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL STUDIES OF ZINC COMPLEX OF BIOMOLECULE

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Abstract: The Zn-complex of Biomolecule or Ketimine was synthesized by reacting the biomolecule and metal salt by conventional method. Further the synthesized Zn-complex was characterized by colour, TLC, physical constant and UV-Vis spectra and FTIR spectral method. The Zn-biomolecule complex was also tested for the *in-vitro* biological activity and the results obtained were compared with biomolecule itself as well as Ciprofloxacin as standard drug.

Key Words: Biomolecule (BM), Ketimines, 3-Acetyl-pyridine, 2-Amino-6-(1-pyridin-3-yl-ethylideneamino)-hexanoic acid(BM), Zn-complex, Conventional method, TLC and UV-Vis, FTIR, and Biological activity studies.

Introduction: Schiff bases were first discovered by Hugo Schiff[1] and hence they are referred as Schiff bases or ketimines. A compound formed by the reaction between an aliphatic or aromatic amino compound and an aldehyde or ketone is known as Schiff base or Ketimine[2a]. It is a intermediate in Strecker degradation reaction where amino group of α-amino acid is transferred to a carbonyl group via schiff base formation[2b]. Many types of reaction Ketimines or Biomolecule are involved in metal complex formation[3] oxidations[4], hydrolysis[5] and reduction[6] have been studied with ketimines. Literature survey shows report[7] on synthesis, crystal structure and anticancer activities of transition metal complex with Ketimine derived from 2-acetylpyridine and L-tryptophan.

L + Metal salt ------> L-Metal complex

Recently we have reported the preparation of 2-Amino-6-(1-pyridin-3-ylethylideneamino)-hexanoic acid(as BM) from 3-Acetylpyridine with L-Lysine monohydrochloride and its copper complex[8] as biomoleculer complex. Herein in continuation we report the preparation of 2-Amino-6-(1-pyridin-3-ylethylideneamino)-hexanoic acid(as BM) from 3-

Acetylpyridine with L-Lysine monohydrochloride and its zinc complex as biomoleculer complex: It is also studied for its biological activity.

Experimental:

Preparation of Schiff base metal complex: The complexation of the ketimine is performed as per reported methods[8-10]. The Ketimine, synthesized. 2-Amino-6-(1-pyridin-3-ylethylideneamino)-hexanoic acid(**BM**) and reported[2c] from our lab, was complexed with a calculated quantity of metal salt to form the complex.

Preparation of Ketimine-metal Complex: The ketimine ligand (2.5 mmol) solution of $ZnSO_4 \cdot 7H_2O$ dissolved in ethanol is mixed with 50 ml, 5 mM solution of Ligand, **BM** in (2:1) Ligand: Metal ratio. The reaction mixture was refluxed for 4–5 hrs. The coloured powdered product appeared on standing and cooling the solution. The precipitated compound is filtered, washed with ethanol and dried under vacuum to a constant weight at 60°C. Record its dried weight and the physical constant. The purity of the ketimine-complex were ascertained by recording the melting point(uncorrected) and by analyzing them for carbon, hydrogen, nitrogen and the metal content. The elemental analysis indicates that all the metal complex have 2:1 (L:M) ratio. Calculate the yield of complex obtained. The complex, **BM-Zn,** thus obtained is listed in **Table-1**.

Safety measures : During the above type of work always wear personal safety protective equipments including safety goggles, gloves and the lab-coat made of cotton must be used at all times during performing the experiment. Also, the long pants should be worn along with close-toed shoes. No food or drink is allowed in the laboratory. Always work using the fume-hood. Be careful when handling the products, they are deeply coloured and it may stain your skin and cloth on exposure for a long period of time. Do not wipe gloves on the lab-coat.

Antibacterial Activity - Experimental Procedure:

To study the in-vitro antibacterial activity of Schiff Base following setup will be required. The following experimental procedure will be adopted.

Newly synthesized compounds were screened for their antibacterial activities against four strain of bacteria *E. coli, B. subtilis, P. aeruginosa* and *S. aureus* using disk diffusion method [11-12]. Activity of each compound was compared with that of standard drug. All the following procedure steps were performed aseptically. The test bacterial suspension was heavily inoculated on the surface of sterile nutrient agar medium by spreading which was then allowed to dry. The paper discs soaked with compound (100 and 500 mg/ml) were placed in the inoculated plates and the plates were kept in refrigerator for 10 min for diffusion of compound in the medium. Then incubate the plates at 37°C for 24 hrs[13]. After 24 hrs incubation the diameter of zone of inhibition was measured and recorded in the **Table 6**.

Results and Discussion:

The complexation of the ketimine is performed as per reported methods[8-10]. The Ketimine, synthesized, 2-Amino-6-(1-pyridin-3-ylethylidene-amino)-hexanoic acid(**BM**), was complexed with a calculated quantity of metal salt to form the complex.

The abbreviation of ketimine-complex, **BM-Zn**, colour, nature and melting point of the ketimine-complex were summarized in **Table-1**.

TABLE-1: The data for Analytical and Physical Characterization of the Ketimine-complex, **BM-Zn**.

ID No.	Mol. For. (Mol. Wt.)	Colour	Nature	Melting Point °C	Conductance, (mg)
BM-Zn	$[Zn(BM)_2](H_2O)_2$ (577.38)	Light yellow to brown	Powdery	> 290°C	1.67

The yield and elemental analysis data for the ketimine-Zn complex were depicted in **Table-2**.

TABLE-2: The Data for yield and elemental analysis of the Ketimine-Zn-complex, **BM-Zn**.

ID No.	Metal salt Used	Wt of complex, % Yield	Elemental analysis of ketimine-Zn complex							
			% C		% H		% N		% Metal	
			obs.	cal.	obs.	cal.	obs.	cal.	obs.	cal.
BM-Zn	**$ZnSO_4.7H_2O$**	1.58 gm	49.25	49.73	6.02	6.10	13.18	13.38	10.32	10.41

The above complex was also analyzed by Colour and UV-Vis spectral measurement. The data obtain is shown in following **Table-3**.

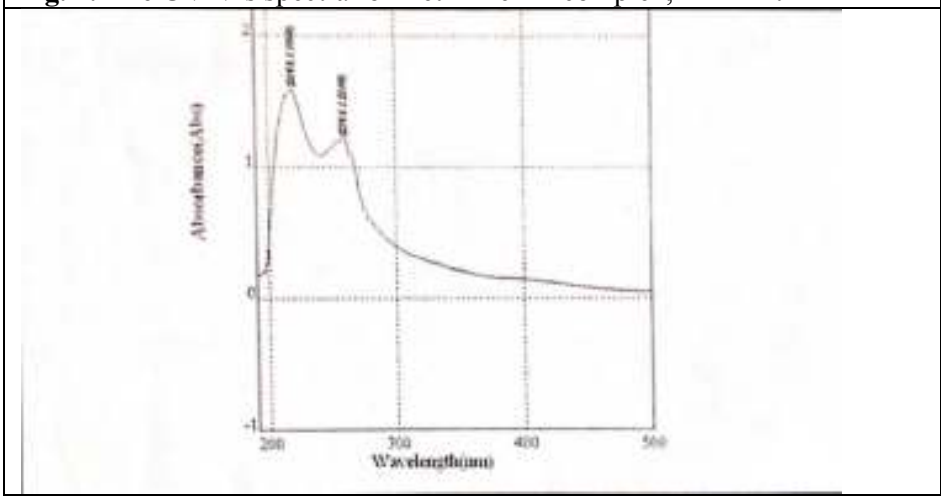
TABLE-3: The Analytical for Colour and UV-Vis Spectral Data for the Ketimine-complex, **BM-Zn**.

ID	Colour	UV-Vis ($\lambda_{max.}$)
BM-Zn	Light yellow to brown	338.0 w, 259.0 and 219.0

w = weak peak

The UV-Vis spectra for Bis[2-amino-6-(1-pyridin-3-ylethylideneamino)-hexanoic acid]-Zn.$(H_2O)_2$ (**BM-Zn,** $C_{26}H_{38}O_8N_6Zn$) is shown in the **Fig. 1.**

Fig. 1.-The UV-Vis spectra for Ketimine-Zn complex, **BM-Zn.**



The above complex was also analyzed for FTIR. The FTIR spectra are reported in the **Fig. 2**. The data obtain is shown in following **Table-4**.

Fig. 2 The FTIR spectra for Ketimine-Zn complex, **BM-Zn**.

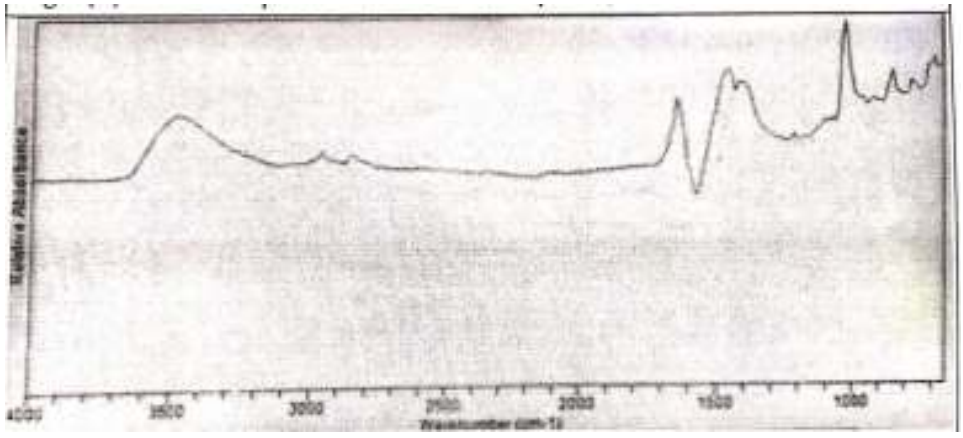


TABLE-4: The FTIR Spectral Data for the Ketimine-complex, **BM-Zn**.

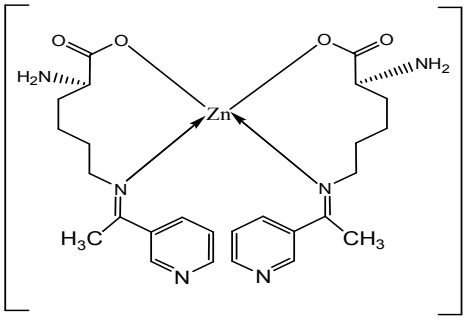
ID No.	FTIR absorption frequency (in cm^{-1})						
	ν_{-OH}	ν_{Ar-H}	$\nu_{Ar-C-CH3}$	$\nu_{>C=O-}$ of Carboxylic acid	$\nu_{>C=N-}$	$\nu_{>C-N-}$	ν_{Ar-CH-} bending
BM-Zn	3416 br	3028	2837	1693	1615	1190, 1265	1460 m

br = broad peak

The band at 1630 cm^{-1} are observed due to $\nu_{>(C=N)}$ which has been shifted towards lower region at around 1615 cm^{-1} in the complex indicated that there is participation of the azomethine group (N of >C=N-) in the metal-complex(M-N bond) formation [8-10], this shift is also due to the reduction of double bond character of carbon-nitrogen bond of azomethine group [14]. Also, the presence $-NH_2$ (amino group) ~ 3480 cm^{-1} in ligand. In complex it also overlaps the bans due to presence of water molecule co-ordinated to the metal[15]. FTIR of metal chelates shows a strong band in the higher frequency region 3500-3300 cm^{-1} revealed the presence of co-ordinated water in these metal complex[16].

From all the above characterization one arrives at the detailed structures and they are as shown in below **Table-5**.

Table-5: The proposed Structural and Molecular Formula, Molecular weight and the ID for the Ketimine or biomolecule-Complex, **BM-Zn**.

Sr. No.	Proposed Structural Formula of the complex	Mol. Formula of Ketimine - Complex (Mol. Wt.)	ID
1		$ZnC_{26}H_{38}O_8N_6$ (577.38)	**BM-Zn**

IN-VITRO ANTIMICROBIAL STUDY OF THE BIOMOLECULE(Ketimine) AND ITS COMPLEX:

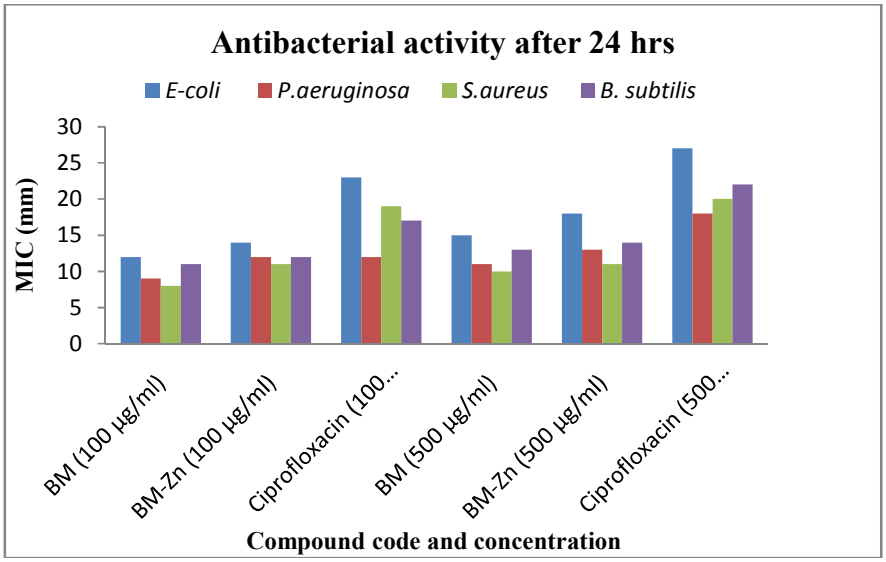
The in-vitro antimicrobial studies are performed for the biomolecule and its Zn-complex. The anti-bacterial activity against the strains(bacteria) like *E. coli, B. subtilis, P. aeruginosa and S. aureus* by disc diffusion method[11-12], and their results after 24 hrs. are depicted in **Table-6**.

Table-6. The Data showing the Antibacterial Activity of synthesized Biomolecule (Ketimine) and its Zn Complex, using different strains by disc diffusion method.

Sample Code	*E. coli*		*B. subtilis*		*P. aeruginosa*		*S. aureus*	
	Concn. (μg/ml)		Concn. (μg/ml)		Concn. (μg/ml)		Concn. (μg/ml)	
	100	500	100	500	100	500	100	500
After 24 hrs.								
BM	12	15	11	13	09	11	08	10
BM-Cu	13	16	11	14	10	10	09	10
Standard Drug (Ciprofloxacin)	23	27	19	22	12	18	17	20
+ ve control (Distilled water)	**+ ve**	**+ ve**	**+ ve**	**+ ve**	**+ ve**	**+ ve**	**+ ve**	**+ ve**
- ve Control (DMSO)	**- ve**	**- ve**	**- ve**	**- ve**	**- ve**	**- ve**	**- ve**	**- ve**

The antibacterial activities against four strain of bacteria *E. coli, B. subtilis, P. aeruginosa* and *S. aureus* by using disk diffusion method. The synthesized Cu-complex of Biomolecule (schiff base) was screened for the antibacterial activity. Inhibitory zone diameters for disks were measured in mm and compared with control disk used as controls. The graphical representation of the antibacterial activity is depicted in **Fig. 3** indicating the variation of zone of inhibition**.**

Fig. 3: The graphical representation of the antibacterial activity.



Glimpses of In-vitro Antibacterial Activity of Synthesized Biomolecules or Ketimines and their complex

1) Among the biomolecule or ketimine and the Zn complex studied for the MIC for bacterial strain, *E. coli* was found more than *B. subtilis, P. aeruginosa* and *S. aureus*.

2) BM-Zn complex exhibited more activity on studied bacterial strains.

3) The Biomolecule and its Zn complex showed less anti-bacterial activity than the standard drug.

4) A comparative study of inhibition values of the Biomolecule and their complex indicates that the complex exhibit higher antibacterial activity than the free Biomolecule. Zinc ions are proven to have antibacterial activity.

The overall and a comparative study of inhibition values of the ketimine or schiff base ligand and their complex indicate that the complex exhibit higher antimicrobial activity than the free ligand. Zinc ions are proven to be essential for the growth-inhibitor effect. The extent of inhibition appeared to be strongly dependent on the initial cell density and on the growth medium.

This study can be extended, for the study of similar varied biomolecule or intermediates, and their antimicrobial activities.

Conclusions:

The compounds were tested for the anti-bacterial and antifungal activities at the concentrations 100 and 500 (µg/ml) in DMSO and compared with known antibiotics *viz Ciprofloxacin* (**Table 6**), it is found that the inhibition by metal chelates is higher than that of a Biomolecule(**BM**) and results are in good agreement with previous findings in case of amino acid (Arginine and Glycine) schiff bases of 2-Hydroxy-naphthaldehyde with respect to comparative activity of free ligand and its complex[17].

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