Microwave Synthesis, Characterization and Study of Biological Activities of Cefixime Schiff base Silver Complexes

Shilpa Kodge[1], K. H. Shivprasad[2], Vijayakumar Durg*[3] and Anilkumar Kodge[4]

[1, 4] Department of Chemistry, Guru Nanak Dev Engineering College, Bidar-585403, Karnataka, India,
[2]Department of Studies in Chemistry, Vijayanagara Sri Krishnadevaraya University, Bellary-585104, Karnataka, India,
[3]Department of Chemistry, Bheemanna Khandre Institute of Technology, Bhalki-585328, Karnataka, India.
[1]kodgeshilpa@rediffmail.com, [2]khsprasad60@gmail.com, *[3]vijayakumardurg@gmail.com, [4]kodgeanil@rediffmail.com

***Abstract*—** In continuation of our previous work, a series of novel silver complexes of Cefixime Schiff bases have been synthesised under microwave irradiation and they have been characterized by elemental analysis, FTIR & NMR spectroscopy. They also have been screened in vitro for their antibacterial and antifungal activities against two bacteria namely Escherichia coli & Staphylococcus aureus and two fungus Candida albicans & Aspergillus niger. One of the Schiff bases and some silver complexes were found to posses excellent antifungal activities against A. Niger than the reference drugs.

***Keywords*—** Microwave, irradiation, IR, Silver and Drug

I. INTRODUCTION

In continuation of our work [1] on the Schiff bases and their Silver complexes, in the present work, five Schiff bases of Cefixime and their Silver complexes have been synthesized. Many drugs in the form of silver complexes possess modified toxicological and pharmaceutical properties. This observation encouraged us to synthesise and screen the synthesized compounds for antimicrobial activities to study the changes in their biological activities of Cefixime after Schiff base and Silver Complex formation. IR, ^{1}HNMR spectroscopy and elemental analysis were used for confirmation of structures of synthesized silver complexes.

II. MATERIALS AND METHODS

All required chemicals were purchased from commercial shop and were used without further purification. Washing of all the glassware during the reaction was accomplished by using distilled water. The synthesis were carried out in a Scientific Microwave Synthesizer Model: CATA-2R of capacity 32 litre with a maximum power output of 850W and microwave frequency 2450MHz. Completion of reaction was monitored by performing TLC. Elemental composition was determined by elemental analyzer at SAIF, Cochin. For IR spectra, Perkin Elmer FT-IR spectrometer within 350-4000 cm^{-1} range was used by employing KBr disc method. Reports of Antibacterial and Antifungal activities of synthesized compounds were obtained from Bio-Genics, Research and Training Centre in Biotechnology, Hubli, and Karnataka as diameter of Inhibition Zones in mm.

A. Synthesis Of Schiff's Bases

The Schiff bases were synthesized by using methanol as a solvent **[2, 3]**. Initially the equimolar ratio of methanolic solutions of Cefixime drug and methanolic solution of Acetophenone/substituted Acetophenone were mixed thoroughly and few drops of glacial acetic acid were added.

The mixture was subjected to microwave irradiation at an interval of 1 min at 450 W for about 8-10 min. The progress of the reaction and purity of the products were monitored by TLC using silica gel. After the completion of the reaction, the obtained product was poured into ice cold distilled water and stirred well. Solid separated was filtered and recrystallized from suitable solvent. The crystalline products were dried under vacuum or reduced pressure under anhydrous $CaCl_2$ and kept in a desiccator till further use.

B. Synthesis of Silver Complexes

The syntheses of all silver complexes were conducted in the absence of light and the products were also stored in the dark at all times. The equimolar solutions of ligand and $AgNO_3$ in methanol were mixed thoroughly in 1:1 ratio and the resulting mixture was then irradiated in the microwave synthesizer at an interval of 1 min at 500 W for about 15-20 min. The progress of the reaction and purity of the products were monitored by TLC using silica gel. After the completion of the reaction, the obtained product was poured into cold distilled water and stirred well. The obtained product was filtered off, re-crystallized from methanol and finally washed with petroleum ether. The final product was dried under reduced pressure over anhydrous calcium chloride in a desiccator [4, 5].

C. Detection of silver in synthesized complexes

Approximately 1g of each complex was dissolved in dilute nitric acid and boiled to expel brown fumes. It is then diluted with distilled water, heated & dil. HCl was added slowly with constant stirring until precipitation is complete. The presence of Silver in complexes is confirmed by the appearance of precipitate [6**].**

D. Biological evaluation.

The newly synthesized ligands and their silver complexes were screened in vitro for their antibacterial activity against Staphylococcus aureus & Escherichia coli and antifungal activity against Aspergillus niger & Candida albicans at 250, 500, and 1000 μg/ml of concentrations in DMSO solvent by Agar diffusion method and are compared with Ciprofloxacin and Amphotericin respectively.

E. Antibacterial and antifungal analysis

Composition of media used for antibacterial analysis is peptone-10g, sodium chloride 10g, yeast extract 5g, Agar 20g in 1000 ml of distilled water. Media used for antifungal analysis is sucrose 30g, sodium nitrate 2g, K_2HPO_4 1g, $MgSO_4.7H_2O$ 0.5g, KCl 0.5g, $FeSO_4$ 0.01g, Agar 20g. Initially, the stock cultures of bacteria were revived by inoculating in broth media and grown at 37°C for 18 hrs. The agar plates of above media were prepared and wells were made by using sterile cork borer of 6mm diameter in the plate. Each plate was inoculated with 18 hrs old cultures and spread evenly on the plate. After 20 min, the wells were filled with compound and antibiotic at the above said concentrations. All the plates were incubated at 37°C for 24 hrs and the diameter of inhibition zone were noted. For antifungal activity the plates were incubated at 27°C for 96 hrs [7, 8].

III. RESULTS AND DISCUSSION

A. Elemental Analysis

Micro analytical data of Silver complexes with proposed molecular formula are given in the following **Table I.** The results obtained from elemental analytical measurements are in good agreement with calculated results from the empirical formula of each compound and confirms that the composition of the metal complexes corresponds to 2:1 (metal - ligand) stoichiometry.

TABLE I

ELEMENTAL ANALYSIS OF THE LIGANDS AND COMPLEXES

Complex	Molecular Formula	Elemental analysis observed (calculated)			
		C%	H%	N%	S%
CA1	$C_{24}H_{27}O_{17}N_7S_2Ag_2$	29.33 (29.8)	2.63 (2.79)	10.65 (10.15)	6.88 (6.63)
CA2	$C_{24}H_{25}O_{17}N_7\ S_2Ag_2$	28.24 (29.9)	2.49 (2.6)	10.24 (10.17)	7.03 (6.65)
CA3	$C_{25}H_{29}O_{18}N_7\ S_2Ag_2$	29.8 (29.8)	2.84 (2.79)	10.35 (10.15)	7.24 (6.63)
CA4	$C_{24}H_{26}O_{17}N_7\ S_2Ag_2$	28.30 (28.81)	2.79 (2.60)	9.94 (9.80)	6.83 (6.40)
CA5	$C_{24}H_{26}O_{17}N_8\ S_2Ag_2$	29.86 (30.37)	2.75 (2.74)	10.31 (10.33)	6.66 (6.75)

B. Infra-Red Spectral Analysis

All the observed characteristic peaks (in cm-) in the Infra Red spectra of Schiff bases & their silver complexes are cited in the **Table I1**. To ascertain the bonding mode of Schiff base to Silver metal ion in the complex, the IR spectrum of each Schiff base of cefixime was compared with that of their silver complex. The bands at 1735 and 3420 cm^{-1} due to stretching vibrations of >C=O and $–NH_2$ groups appeared in the IR spectra of cefixime are disappeared in the IR spectra of all the Schiff Base Ligands and a new band appeared in the range of 1580-1614 cm^{-1} due to formation of azomethine (>C=N-) group which confirms the formation of Schiff bases. There is no appreciable shift in the Lactum C=O band in the IR spectrum of silver complex indicating that this group is not involved in co-ordination. The Azomethine nitrogen (>C=N) band appeared in the range of 1577-1597 cm^{-1} in Schiff base ligands is disappeared in the IR spectrum of their silver complexes indicating that co-ordination occurred through the nitrogen atom of azomethine group. A strong band in the region 1675—1691 cm^{-1} in Schiff base ligands is shifted to lower frequency in the IR spectrum of their silver complex indicating that co-ordination has occurred through the nitrogen atom of amide group. The symmetrical stretching bands of Carboxylate groups are observed at longer wavelength in the IR spectrum of complexes due to co-ordination through oxygen of carboxylic acid groups. In the spectra of silver complexes, a broad band in the region 3398—3457cm^{-1} indicates the presence of coordinated water molecules. Further the numbers of water molecules coordinated are confirmed by TGA and DTA. The new bands appeared in the IR spectrum of complexes in the regions 556—565 and 716—737cm^{-1} are assigned to Ag—N and Ag—O respectively [6].

TABLE II

CHARACTERISTIC IR SPECTRAL BANDS OF LIGANDS AND THEIR SILVER COMPLEXES (IN CM^{-1})

Ligand/ Complex	ν (H_2O)	ν (-OH)	ν (C=O) (Lactonyl)	ν(NHC=O) (Amide)	Symm ν (COO) (Carboxyl)	ν (>C=N-) Azomethine group
LA1			1758	1681	1359	1597
CA1	3408		1763	1670	1392	-
LA2		3212	1754	1684	1365	1580
CA2	3457		1762	1668	1384	-
LA3			1758	1685	1358	1598
CA3	3398		1763	1670	1392	-
LA4			1755	1681	1385	1588
CA4	3423		1763	1670	1396	-
LA5			1755	1691	1350	1577
CA5	3407		1765	1672	1392	-

Where LA1- Schiff base of cefixime & acetophenone CA1- Silver complex of LA1
LA2- Schiff base of cefixime & o-hydroxy acetophenone CA2- Silver complex of LA2
LA3-Schiff base of cefixime & p-methoxy acetophenone CA3- Silver complex of LA3
LA4- Schiff base of cefixime & p-chloro acetophenone CA4- Silver complex of LA4
LA5- Schiff base of cefixime & m-nitro acetophenone CA5- Silver complex of LA5

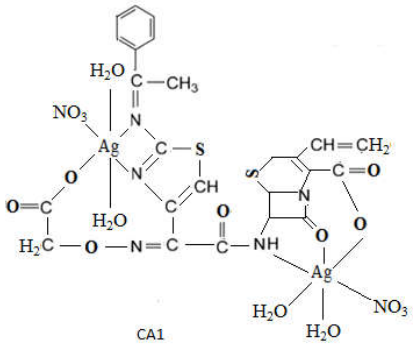
C. NMR Analysis

The 1HNMR spectral data of ligands and their silver complexes are shown in the **Table III**. The involvement of Carboxylic group in co-ordination via de-protonation is further supported by the disappearance of the singlet in the ^{1}HNMR spectrum of the complexes which was appeared at ≈ 9.6 ppm in the ^{1}HNMR spectrum of Ligands [9]. The involvement of Aromatic hydroxyl group in co-ordination via de-protonation in CA2 complex is further supported by the disappearance of the singlets in the ^{1}HNMR spectrum of complex which was appeared at 12 ppm in the ^{1}HNMR spectrum of Ligands. Retention of singlet for amide proton in complexes indicates the involvement of this group in co-ordination without de-protonation and also supports that amide group is co-ordinated with metal ion through nitrogen atom.

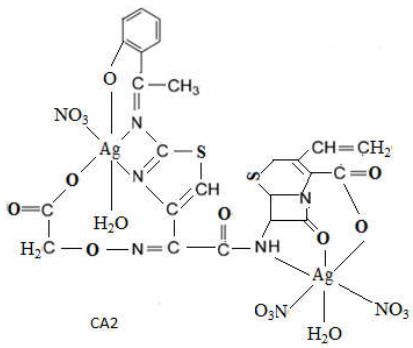
TABLE III
NMR SPECTRAL DATA OF LIGANDS AND THEIR SILVER COMPLEXES

Ligand/Complex	**Amide Proton (ppm)**	**Carboxyl Proton (ppm)**	Aromatic hydroxyl proton**(ppm)**
LA1	8.3	9.6	-
CA1	8.3	-	-
LA2	8.3	9.6	12
CA2	8.3	-	Disappeared
LA3	8.3	9.6	-
CA3	8.3	-	-
LA4	8.3	9.6	-
CA4	8.3	-	-
LA5	8.3	9.6	-
CA5	8.3		

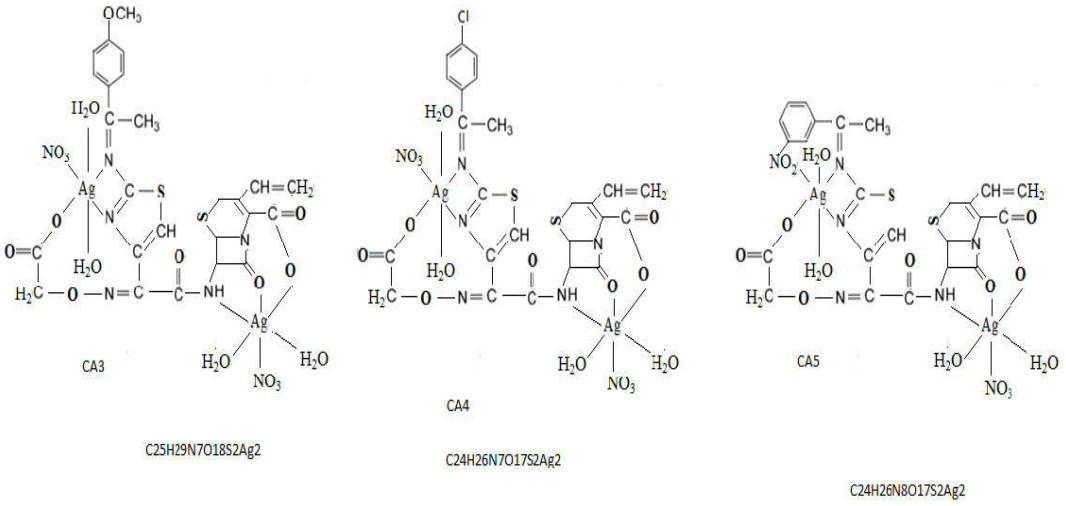
On the basis of Elemental & IR Spectral data analysis, the following structures have been proposed for synthesized complexes;



CA1
C24H27N7O17S2Ag2



CA2
C24H25N7O17S2Ag2



D. The antibacterial and antifungal activities

The antibacterial and antifungal activities of synthesized complexes were studied by agar and potato dextrose agar diffusion methods respectively at 250, 500, and 1000 μg/ml of concentrations in DMSO solvent by using bacteria S. Aureus & E. Coli and fungi A. Niger & C. Albicans as follows: The antibacterial and antifungal activities are recorded as diameter of inhibition zone in the **Tables IV and V**.

TABLE IV

DIAMETER OF INHIBITION ZONE INHIBITED BY LIGANDS AND COMPLEXES ON SELECTED BACTERIA

Pathogen→ **Conc.(μg/ml)→** **Sample ↓**	**Antibacterial activity**					
	S. aureus			**E. coli**		
	250	500	1000	250	500	1000
Cefixime	0	6	12	10	13	15
LA1	0	0	8	0	0	9
LA2	0	0	12	4	6	11
LA3	0	0	5	0	0	8
LA4	0	0	7	0	0	7
LA5	0	5	9	0	7	11
CA1	0	0	15	5	9	12
CA2	0	0	7	0	5	7
CA3	5	8	12	5	8	11
CA4	5	8	10	5	7	11
CA5	4	5	8	6	9	13
Ciprofloxacin	34	36	*	34	36	*

***Zone could not be measured due to merging**

TABLE V

DIAMETER OF INHIBITION ZONE INHIBITED BY LIGANDS AND COMPLEXES ON SELECTED FUNGI

Pathogen→ Conc.(μg/ml)→ Sample ↓	Antifungal activity					
	A. niger			C. albicans		
	250	500	1000	250	500	1000
Cefixime	0	0	0	0	0	0
LA1	5	7	8	0	0	0
LA2	0	0	0	0	0	0
LA3	0	0	0	0	0	0
LA4	0	0	0	0	0	0
LA5	0	0	0	0	0	0
CA1	0	0	0	0	0	0
CA2	0	0	0	0	0	0
CA3	3	5	7	5	8	10
CA4	5	7	8	3	5	6
CA5	5	10	11	3	5	7
Amphotericin	3	5	7	9	13	15

IV. CONCLUSION

The above results reveal that there is no much variations in the antibacterial activities of the ligand and complexes than the parent drug. But there is a drastic change in antifungal activities of **one of the prepared ligands and some complexes** against the selected fungi compared to the parent drug Cefixime. It is clear from the results that the synthesized schiff base ligands of Cefixime (LA1) and silver complexes (CA3,CA4 & CA5) are showing good antifungal activities against A. Niger, which is more than the standard drug Amphotericin and CA3,CA4 & CA5complexes are also showing moderate ant candida activity along with slightly suppressed antibacterial activity .

ACKNOWLEDGMENT

We are very much thankful to The Principal, Bheemanna Khandre Institute of Technology, Bhalki, for providing laboratory facilities to carry out the synthetic part of our research work. One of the authors by name Vijayakumar Durg expresses his heartfelt thanks to VGST, Department of IT, BT, S&T Bengaluru, for the grant of fund to Chemistry Department, BKIT, Bhalki, for purchasing equipment, wide reference: No. VGST/P-6/K-FIST/ GRD -254/2013-14 Dated: 09-04-2014) which helped us to carry out this research work.

REFERENCES

[1] Shilpa Kodge, K. H. Shivprasad, Vijayakumar Durg and Anilkumar Kodge, "Microwave Assisted Synthesis, Characterization and Antimicrobial Screening of Drug Based Schiff Base Silver Complexes", International Journal of Pharma and Bio Sciences, 8(1), 183-188, 2017.

[2] Shilpa Kodge, K. H. Shivprasad and Anilkumar Kodge, "Microwave Aided Synthesis and Biological Screening of Drug Based Schiff Base Complexes of Silver", International Journal on Emerging Technologies, 6(2), 143-147, 2016.

[3] Shilpa Kodge, K. H. Shivprasad, Dr. Vijayakumar Durg, "Green Synthesis, Characterization and Biological Evaluation of Schiff Base of Drug and its Silver Complex", International Journal of Scientific Research, 6(5), 437-439, 2017.

[4] K .P. Srivastava, Anuradha Singh and Suresh Kumar Singh, "Microwave Assisted Synthesis, Characterization and Antibacterial study of Drug based Schiff Bases and their Zn(II) Complexes", American International Journal of Research in Science, Technology, Engineering & Mathematics, 6(3), 286-292, 2014.

[5] K. P. Srivastava, Anuradha Singh and Suresh Kumar Singh, "Eco-friendly and Efficient synthesis, Characterization and Anti-Bacterial Activity Of Schiff Base Ligand and their Copper(II) Complexes", International Journal of Advanced Research in Chemical Science, 1(2), 11-20, 2014,

[6] E. J. Threlfall, I. S. T. Fisher, L. Ward, H. Tschape & P. Gernersmidt "Harmonozation of antibiotic susceptibility testing for Salmonella: Results of a study by 18 national reference laboratories within the European Union-funded Inter-net group.Microb.Drug Resist, 5, 195-199, 1999.

[7] J. F. Prescott, J. D. Baggot, R. D. Walker, Antimicrobial susceptibility testing and interpretation of results. In: Antimicrobial Therapy in Veterinary Medicine, .Ames, I A, Iowa State University Press, 12-26, 2000.

[8] Muhammad Aslam, Itrat Anis, Nighat Afza, Ajaz Hussain, Shazia Yasmeen, Muhammad Safder, Asif Hanif Chaudhry, Mehroze Ahmed Khan And Muhammad Niaz, "Synthesis And Characterization Of Schiff Bases Derived From 7-{[2-(2-Amino1,3-Thiazol-4-Yl)-2-(Carboxymethoxyimino) Acetyl]Amino}-3-Ethenyl-8-Oxo- 5-Thia -1-Azabicyclo [4.2.0] Oct -2-Ene-2-Carboxylic Acid, International Journal of Current Pharmaceutical Research, 4(4), 51-53, 2012.

[9] K. S. Kashinath, and Vijayakumar Durg, Microwave Assisted Synthesis and Characterization of Mn(II), Fe(III), Co(II), Ni(II), Cu(II), Zn(II) Cd(II) and Hg(II) Complexes With 5-Methoxy- 2-(5-Methoxy-4, 6 D im ethylpyridin-2-Yl-Methanesulfinyl)-1H-Benzimidazole. International Journal on Emerging Technologies International Journal on Emerging Technologies (Special Issue on NCRIET-2016) 6(2), 105-111, 2016.