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ANTI-MICROBIAL AND ANTI-TUBERCULAR ACTIVITY EVALUATION OF NEWLY SYNTHESIZED ZINC COMPLEXES OF AMINOTHIOPHENE SCHIFF BASES

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ABSTRACT: Tridentate Schiff bases were obtained in succession from the condensation of ethyl 2-amino-5,6-dihydro-4*H*-cyclopenta [*b*] thiophene-3-carboxylate and different aryl aldehydes in 1:1 molar ratio. Zinc chloride complexes of these new aminothiophene Schiff bases were prepared in 1:1 metal: ligand stoichiometry. These complexes were characterized using IR, UV-Visible, elemental analysis and molar conductivity. Schiff bases displayed monobasic tridentate coordination with central Zn (II) ion and formed tetrahedral complexes. These complexes were evaluated for their antimicrobial activity against gram-negative, multidrug-resistant Metallo-β-lactamase (MBL) and Extended Spectrum β-lactamase (ESBL) producing uropathogens and for their antitubercular activity against *Mycobacterium tuberculosis* (H37Rv strain) to observe improved biological activity compared to parent Schiff bases.

INTRODUCTION: The condensation reaction between primary amine and carbonyl compound was first reported by Hugo Schiff and the products possessing the characteristic azomethine group are often referred as Schiff bases ¹. Over last few decades, a tremendous upsurge has been observed in the study of Schiff bases and their metal complexes due to their variety in reactions, diversified industrial applications and a broad spectrum of biological activity coupled with ease of preparation ^{2, 3}. The azomethine linkage and the presence of N, S and O donor atoms play important role in the biological properties as well as in the coordination of the metal complex. Bonding of metal ions to such biologically active compounds may enhance their biological activities ⁴.

Zinc is the most abundant essential trace mineral found in the human body after iron ⁵. Zinc is necessary for the physiological growth, reproduction and normal functioning of the brain and central nervous system and plays a key role in the metabolism of RNA and DNA and for gene expression ^{6, 7}.

Urinary tract infections (UTI) are one of the most common bacterial infections affecting millions of populace every year accounting for significant morbidity and high medical costs. Studies reveal that Women are more prone to UTIs than men and every fifth woman has a chance of developing a UTI during her lifetime. Approximately 25 % of women with an acute UTI reported with recurrent UTIs (RUTIs). Gram-negative bacteria *Escherichia coli* and *Klebsiella pneumoniae* are the most predominant uropathogens causing UTIs ^{8 - 10}. Infections caused by drug-resistant bacteria producing ESBLs (Extended spectrum β-lactamase) and MBL (Metallo-β-lactamase) have become a serious problem for the healthcare industry ¹¹. Another drug-resistant disease posing a major

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global health hazard to mankind is Tuberculosis. WHO reports 46.5 % TB burden in South Asia region of which India alone accounts for 23 % of World TB cases. In 2015, 37 % multi-drug resistance TB cases were detected worldwide accounting for 250000 deaths. The evolution of drug-resistant strains combined with co-infection from HIV creates a major hurdle in the treatment of TB¹². Such situations demand a substantial need for the discovery of new compounds with enhanced activities to overcome the drug-resistant bacterial strains.

Present work is focused on the synthesis and characterization of Zn(II) complexes of our previously reported¹³ aminothiophene Schiff bases derived from ethyl 2-amino-5,6-dihydro-4H-cyclopenta [b] thiophene-3-carboxylate and biological activity evaluation against gram-negative uropathogens and *Mycobacterium tuberculosis*.

MATERIALS AND METHODS: Analytical grade chemicals with purified and distilled solvents were used for synthesis and analysis. The elemental analysis was done by standard methods¹⁴. ELICO Digital conductivity meter was used for molar conductance measurement with DMF (10^{-3} M solution) as a solvent. The IR spectra were obtained from Perkin Elmer Model 1600 FTIR Spectrophotometer using KBr disc. Electronic spectra were obtained from a UV-Vis Jasco Spectrophotometer Model V-630. For calculation of magnetic susceptibility, Gouy balance was used along with Hg[Co(SCN)₄] as a calibrant.

Anti-microbial Activity: For the evaluation of the antibacterial activity, the agar well-diffusion method was employed. Total ten isolates, five ESBL and five MBL uropathogens comprising five of each of the genera, *Escherichia*, *Pseudomonas*, *Klebsiella*, *Proteus* and *Citrobacter* (**Table 2**) were used in the studies. The bacterial cultures were procured from local medical facilities in Mumbai and characterized for ESBL and MBL production in our previous studies¹⁵. The Zn(II) complexes were dissolved in HPLC grade DMSO. The final concentration of 25 µg/mL of the test compound was used in the experiment. All the bacterial strains were inoculated in 10 mL of Brain Heart Infusion (BHI) broth and kept for incubation at a temperature of 37 °C for one day.

The cooled, sterilized molten Mueller-Hinton agar (20 mL) was poured into sterilized Petri dish of 9 cm diameter under the aseptic condition and permitted to solidify. The plates were seeded with 0.4 mL test culture (0.1 O.D. at 540 nm). The inoculation was allowed to cool with gentle shaking. Wells were ditched in the medium using sterile cork borer of 8 mm diameter. Test compounds (50 µl) were added to these wells and the plates were kept for incubation at a temperature of 37 °C for one day to obtain the zones of inhibition. Control wells were set up using 50 µl of solvent (DMSO) for each test isolate. The antimicrobial activity was evaluated in triplicate set for each compound¹⁶.

Antituberculosis Activity: The entire test compounds were assessed for antitubercular activity against *M. tuberculosis* (H37Rv strain) by the MABA (Microplate Alamar Blue assay) method using pyrazinamide, ciprofloxacin and streptomycin as positive controls. The final drug concentration tested from 100 to 0.8 µg/mL. In the experiment, a 96 well plate covered with de-ionized water in outer perimeter was used. In each well, 100 µl of the Middlebrook 7H9 broth solution along with the serial concentration of test compound was added. These plates were sealed with paraffin and kept for incubation at a temperature of 37 °C for five days.

On the 6th day of incubation freshly prepared 25 µl of 1:1 mixture of Almar Blue reagent and Tween solution (10% and 80%) was added to each well and these plates were again kept for incubation for another one day. On 7th day plates were removed, to check the MIC (Minimum Inhibitory Concentration). The lowest concentration of the test compound which prevented blue colour change to pink was reported as MIC¹⁷.

Synthesis of Ligand: The Schiff bases were prepared from the condensation of ethyl 2-amino-5,6-dihydro-4H-cyclopenta[b]thiophene-3-carboxylate with o-hydroxyl aldehyde derivative in 1:1 molar proportion as per reported method¹³.

Synthesis of Metal Complexes: To an ethanolic solution of the Schiff base ligand (0.01 mol) the Zinc chloride (0.01 mol) dissolved in minimum quantity of ethanol was added dropwise with constant stirring. The pH was adjusted to 6-7 and

the mixture was heated under reflux for 4 - 5 h on a water bath. A fine yellow precipitate of the solid complex formed was filtered by suction filtration and purified by washing with aqueous ethanol and ether and dried in vacuum.

RESULTS AND DISCUSSION: Physicochemical analysis indicated 1:1 metal-ligand stoichiometry

of Zinc complexes. All the complexes are coloured, stable and are of good keeping quality. The complexes showed insolubility in common organic solvents apart from DMF and DMSO. The molar conductance values pointed towards the non-electrolyte nature of the complexes. The elemental analysis data is found in agreement with proposed molecular formula (**Table 1**).

TABLE 1: PHYSICO-CHEMICAL DATA OF Zn (II) COMPLEXES

Complex	Colour	F.W.	Elemental analysis (%) found (calc.)						Molar conductance in DMF ($\Omega^{-1} \text{cm}^2 \text{mol}^{-1}$)
			C	H	N	S	Cl	Zn	
[Zn(L ₁)Cl]	Yellow	415.24	48.77(49.13)	3.66(3.85)	3.21(3.37)	8.03(7.71)	9.02(8.54)	15.47(15.75)	5.20
[Zn(L ₂)Cl]	Yellow	449.69	45.90(45.37)	3.21(3.34)	3.01(3.11)	7.00(7.12)	15.42(15.77)	14.23(14.55)	1.10
[Zn(L ₃)Cl]	Yellow	494.14	41.71(41.28)	3.11(3.04)	2.80(2.83)	6.34(6.48)	6.98(7.18)	13.50(13.24)	1.30
[Zn(L ₄)Cl]	Yellow	465.30	53.78(54.16)	3.76(3.87)	2.96(3.01)	6.97(6.88)	7.49(7.62)	14.56(14.06)	6.30

The IR spectrum is an important tool for the identification of different functional groups present in the complex and their bonding mode with the central metal atom. The IR spectra of the free

ligands and the zinc complex were compared, in order to understand the bonding of Schiff base with the central zinc atom. The important IR bands and their assignments are depicted in **Table 2**.

TABLE 2: FTIR SPECTRAL DATA

Compound	$\nu(\text{O-H})$	$\nu(\text{C=O})$	$\nu(\text{C=N})$	$\nu(\text{C-O})$	$\nu(\text{C=S})$	$\nu(\text{M-O})$	$\nu(\text{M-N})$
	phenolic	ester	azomethine	phenolic	thiophene		
HL ₁	3200-3000	1682	1597	1310	616	-	-
HL ₂	3200-3000	1704	1598	1311	619	-	-
HL ₃	3200-3000	1708	1598	1311	626	-	-
HL ₄	3200-3000	1704	1598	1307	617	-	-
[Zn(L ₁)Cl]		1644	1570	1338	615	515	432
[Zn(L ₂)Cl]		1643	1579	1340	618	515	426
[Zn(L ₃)Cl]		1642	1577	1341	627	519	422
[Zn(L ₄)Cl]		1663	1577	1336	618	511	415

The phenolic -OH band at 3000-3200 cm^{-1} present in the Schiff base ligand spectra does not appear in metal complexes spectra indicating the participation of phenolic oxygen in the coordination with central Zn(II) atom.

This is confirmed by the upward shifting of phenolic $\nu(\text{C-O})$ band from the region 1307-1311 cm^{-1} in Schiff base spectra to higher wave number by 28-30 cm^{-1} in the metal complexes spectra.

The involvement of ester carbonyl group in the bonding is indicated by the downward shifting of the absorption band in the region 1682-1708 cm^{-1} in the Schiff base spectra due to $\nu(\text{C=O})$ to lower wave number region by 38-66 cm^{-1} in the metal complexes spectra.

Further prominent band due to azomethine group 1597-1598 cm^{-1} in the Schiff base spectra is shifted to lower frequency by 19-27 cm^{-1} in metal complexes

spectra confirming the involvement of azomethine nitrogen in coordination with zinc atom.

The stretching vibrations due to $\nu(\text{C=S})$ band showed no significant change in the complex spectra, indicating non-participation of thiophene sulfur in the coordination.

The presence of the two medium intensity non-ligand band around 511-519 cm^{-1} and 415-432 cm^{-1} due to $\nu(\text{M-O})$ and to $\nu(\text{M-N})$ stretching vibrations respectively confirms the coordination of phenolic oxygen and azomethine nitrogen atoms with the zinc atom.

The far IR spectra of [Zn(L1)Cl] complex exhibited a non-ligand peak due to $\nu(\text{M-Cl})$ at 366 cm^{-1} indicating coordination of chlorine atoms to the metal ion.

In the zinc complex electronic spectra, the $\pi \rightarrow \pi^*$ transitions were slightly red-shifted **Fig. 1**

compared to Schiff base spectra indicating the coordination of ligand to the zinc atom with no change in the structural arrangement of ligand¹⁸. The Zn(II) complexes are diamagnetic with d¹⁰ configuration and do not show any d-d transitions, hence the tetrahedral geometry of the ligand molecules around Zn(II) can be proposed¹⁹.

On the basis of FTIR, elemental analysis, molar conductance, electronic spectra and magnetic susceptibility, the proposed structures of the Zn(II) complexes are shown in **Fig 2**.

Antimicrobial Activity: For the evaluation of the antimicrobial activity of all zinc complexes, Kirby Bauer method was employed using gram-negative uropathogens [ESBL and MBL strains]. Multidrug resistance profile of these uropathogens against mainline drugs is shown in **Table 3**. The antimicrobial activity against these multidrug resistance uropathogens using DMSO as a solvent is depicted in **Table 4**. Schiff bases exhibited antimicrobial activity against isolate Ec-10, Kp-7

85 and 135, while zinc complexes showed antimicrobial activity against all the test isolates. In addition, Schiff bases HL₂ and HL₃ were also sensitive against uropathogen 220 and Schiff base HL₃ against isolate - 618. All other isolates were resistant to Schiff bases but their metal complexes exhibited higher zones of inhibition (10-18.5 mm). Schiff base HL₃ and HL₂ exhibited higher zones of inhibition while isolate-135 was the most sensitive (14-18.5 mm) among all test isolate. The higher antimicrobial activity of zinc complex can be linked to chelate formation. As per chelation theory and cell permeability concept, penetrating power of complex increases with the lipophilicity. In chelate, the positive charge of the metal ion is shared by ligand donor atoms, resulting in π electrons delocalization over the whole chelate ring. This delocalization favors lipophilicity of metal complex through lipid layer allowing easy binding and penetration of the complex in the cellular structure resulting in the growth inhibition or cell death of the pathogens²⁰.

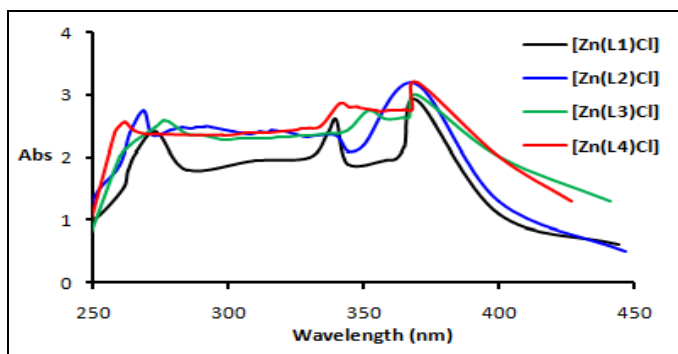


FIG. 1: ELECTRONIC SPECTRUM OF Zn(II) COMPLEXES

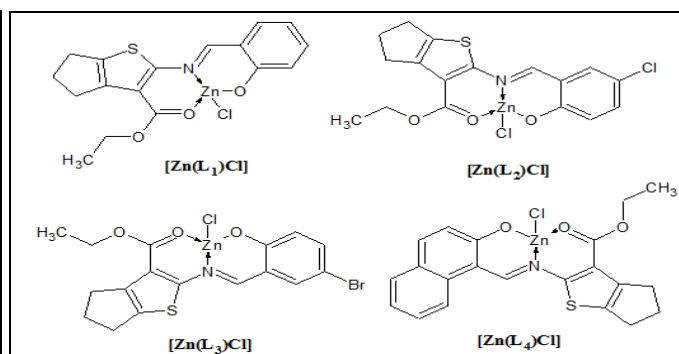


FIG. 2: PROPOSED STRUCTURES OF Zn(II) COMPLEXES

TABLE 3: ANTIBIOTIC RESISTANCE PROFILE OF ESBL AND MBL PRODUCERS

Cultures code name	Full form	Antibiotic sensitivity test		
		Sensitive	Intermediate	Resistant
ESBL Producers				
Citro-2	<i>Citrobacter diversus-2</i>	AS, BA, CH, RC, TE	CI, CF	CF, PC, ZN, GM, AK, GF, TT, OX, RP, ZX, CB, NA, NX, AG, CU, CP, FG, PB
Ec- 10	<i>Escherichia coli- 10</i>	CH, GM, AK, GF	PB, AS	BA, CF, PC, RC, CI, TE, ZN, TT, OX, RP, ZX, CB, NA, NX, AG, CU, CP, FG
Kp	<i>Klebsiella pneumoniae</i>	GM, AK	-	AS, BA, CF, PC, CH,RC, CI, TE, ZN, GF, TT, OX, RP, ZX, CB, NA, NX, AG, CU, CP, FG, PB
Kp-7	<i>Klebsiella pneumoniae- 7</i>	-	OX, TE	AS, BA, CF, PC, CH,RC, CI, ZN, GM, AK, GF, TT, RP, ZX, CB, NA, NX, AG, CU, CP, FG, PB
Pro- 7	<i>Proteus mirabilis- 7</i>	CP, AS, PC, AK	RP, NA, CF, RC, GM, GF	BA, CH, CI, TE, ZN, TT, OX, ZX, CB, NX, AG, CU, FG, PB
MBL Producers				
85	<i>Pseudomonas aeruginosa</i>	AK, GF, PB	RC, CF, PC	AS, CI, TE, ZN, GM, TT, OX, RP, ZX, CB, BA, CH, NA, NX, AG, CU, CP, FG,
135	<i>Citrobacter</i>	CH, PB	AK	AS, BA, CF, PC, RC, CI, TE, ZN, GM, GF,

	<i>amalonaticus</i>			TT, OX, RP, ZX, CB, NA, NX, AG, CU, CP, FG, CB, AS
220	<i>Escherichia coli</i>	CH, AK, GF	AS, ZN	BA, CF, PC, RC, CI, TE, GM, TT, OX, RP, ZX, CB, NA, NX, AG, CU, CP, FG, PB
607	<i>Proteus mirabilis</i>	-	CH, GM	AS, BA, CF, PC, RC, CI, TE, ZN, AK, GF, TT, OX, RP, ZX, CB, NA, NX, AG, CU, CP, FG, PB
618	<i>Klebsiella pneumoniae</i>	-	-	AS, BA, CF, PC, CH, RC, CI, TE, ZN, GM, AK, GF, TT, OX, RP, ZX, CB, NA, NX, AG, CU, CP, FG, PB

TT -Ticarcillin/clavulanic acid, OX- Oxytetracycline, RP – Ceftriaxone, ZX - Cefepime, CB - Cefuroxime, NA - Naladixic acid, NX- Norfloxacin, AG - Amoxicillin/clavulanic acid, CU - Cefadroxil, CP - Cefoperazone, FG - Ceftazidime, PB - Polymixin B, AS - Ampicillin, BA - Co-trimaxazole, CF - Cefotaxime, PC- Piperacillin, CH - Chloramphenicol, RC - Ciprofloxacin, CI - Ceftizoxime, TE - Tetracycline, ZN - Ofloxacin, GM - Gentamicin, AK -Amikacin, GF - Gatifoxacin

TABLE 4: ANTIMICROBIAL ACTIVITY

S. no.	Cultures used		Zone of Inhibition (mm) of Cu/Zn complex									
			DMSO	HL ₁	[Zn(L ₁)Cl]	HL ₂	[Zn(L ₂)Cl]	HL ₃	[Zn(L ₃)Cl]	HL ₄	[Zn(L ₄)Cl]	
1	Citro-2	E	0	0	13	0	11	0	11.5	0	11.5	
2	Ec- 10	S	0	11	14	11	10.5	10	11	10	13	
3	Kp	B	0	0	13.5	0	11.5	0	11	0	12	
4	Kp-7	L	0	11	12	12	12	10	13	12	12	
5	Pro- 7		0	0	13	0	9	0	10	0	12	
6	85	M	0	12	12	15	15.5	15	14.5	15	15	
7	135	B	0	16	18.5	17	17	15	18	15	15	
8	220	L	0	0	12.5	11	11	11	10	0	10	
9	607		0	0	14	0	10.5	0	11	0	10.5	
10	618		0	0	13	0	11	10	11	0	11	

Antitubercular Activity: Antitubercular activity against *M. tuberculosis* (H37Rv) was evaluated using Microplate Alamar Blue Assay (MABA) method. pyrazinamide, ciprofloxacin and streptomycin were used as standard drugs **Fig. 3** and **Table 5**. For the determination of MICs (Minimum Inhibitory Concentration) the lowest concentration at which a pink color was changed to blue color was considered.

All Schiff bases exhibited similar antimicrobial activity at MIC of 25.0 µg/mL. The activity of Schiff base may be attributed to the presence of

azomethine bridging with substituted aromatic aldehydes and thiophene heterocyclic ring in the ligand structure. Only two zinc complexes ([Zn(L₂)Cl] and [Zn(L₃)Cl]) have displayed enhanced anti-tubercular activity (MIC = 12.5 µg/mL) compared to parent ligand.

This improved activity of zinc complex is the effect of chelation due to which amphiphilic nature of the complex increases resulting in deeper penetration of metal complex into the test isolates leading to growth restriction²¹.

TABLE 5: ANTITUBERCULAR ACTIVITY OF SCHIFF BASES AND ZINC COMPLEXES

Test compound	MIC (µg/ml)							
	100	50	25	12.5	6.25	3.12	1.60	0.80
HL ₁	S	S	S	R	R	R	R	R
HL ₂	S	S	S	R	R	R	R	R
HL ₃	S	S	S	R	R	R	R	R
HL ₄	S	S	S	R	R	R	R	R
[Zn(L ₁)Cl]	S	S	S	R	R	R	R	R
[Zn(L ₂)Cl]	S	S	S	S	R	R	R	R
[Zn(L ₃)Cl]	S	S	S	S	R	R	R	R
[Zn(L ₄)Cl]	S	S	S	R	R	R	R	R
Streptomycin*	S	S	S	S	S	R	R	R
Pyrazinamide*	S	S	S	S	S	S	R	R
Ciprofloxacin*	S	S	S	S	S	S	R	R

* Standard, S - Sensitive, R – Resistant

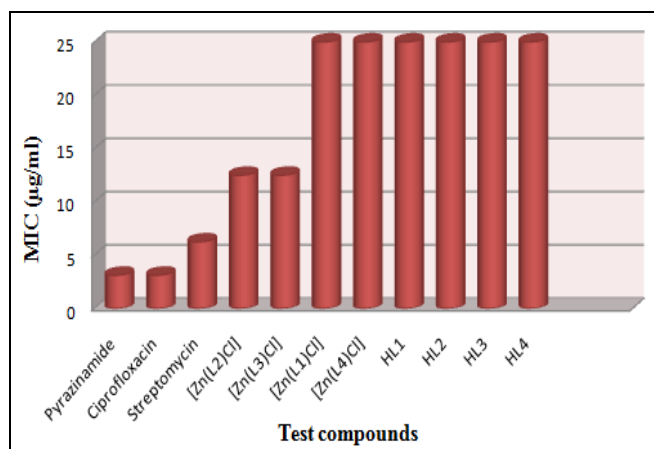


FIG. 3: ANTITUBERCULAR ACTIVITY PROFILE

CONCLUSION: Zinc (II) metal complexes from Schiff bases were synthesized and characterized. Schiff base ligand coordinated to central zinc atom through carboxylic oxygen, azomethine nitrogen and phenolic oxygen in tridentate fashion. On the basis of the spectroscopic investigation, tetrahedral geometry for Zn(II) complexes was assigned. Zinc complexes showed superior anti-bacterial and anti-tubercular activities compared to parent ligands.

This study may provide a stepping stone for the future development of potential zinc complex from the aminothiophene Schiff bases.

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CONFLICT OF INTERESTS: The authors declare that there is no conflict of interests regarding the publication of this paper.

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