



## Original Article

# Synthesis, Characterization and Antimicrobial Screening Studies of Some Metal Complexes of Novel Schiff Base of 2, 6-Diaminopyridine and 2, 5-Thiophenedicarboxylic acid

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Synthesis of Cu(II), Co(II), Zn(II), Pb(II) and Hg(II) complexes with Schiff base 2, 6-diaminopyridine and 2,5-thiophenedicarboxylic acid. Schiff base and its complexes were characterized by analytical data, elemental analysis, molar conductance. The structural investigation performed by UV-Vis, IR and <sup>1</sup>HNMR, <sup>13</sup>CNMR spectral techniques. Interpretation of elemental analysis shown 1:1 molar ratio of Schiff base with metal complexes. Molar conductance values indicate neutral nature of metal complexes. Antimicrobial activity studied at different concentration (MIC method) by disc diffusion technique. Schiff base and the metal complexes found to be significant antibacterial and antifungal agents.

**Keywords:** Schiff base, Antimicrobial activity, Metal complexes, 2, 6-diaminopyridine and 2, 5-thiophenedicarboxylic acid

## 1. INTRODUCTION

The increase in drug resistance to clinically used anti-infective agents reveals that there is an urgent need of the search for new antimicrobial compounds to treat multi-resistant infections with different mechanism of action [1]. The Schiff bases also exhibit a broad range of biological activities like antibacterial, antifungal, antimalarial, antituberculosis, antipyretic, anti-inflammatory and antiviral properties [2]. The imines are present in various natural and synthetic compounds. The imine group from Schiff base has been shown to be critical towards biological activities

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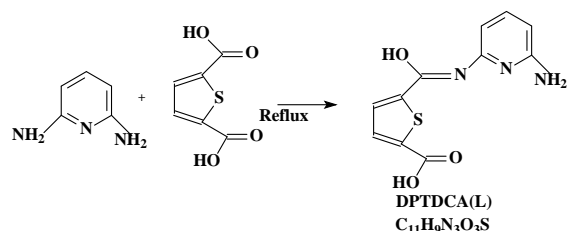
[3]. Schiff bases obtained by the condensation of amino and carbonyl compounds are intrinsic class of ligand that coordinate to metal ions via azomethine nitrogen and have been extensively for its wide range of medicinal applications. Thiophene derivatives attract chemist due to various medicinal applications such as antimicrobial, antifungal, antimycobacterial, antiallergenic, antihypertensive, anticonvulsant, anticancer, antioxidant, antiinflammatory, protein kinase C inhibitors and mushroom tyrosinase inhibition activities [4-8]. Chemists pay more attention on Schiff base obtained thiophene and its derivatives. In the present work, we have synthesized novel ligand from base 2, 6-diaminopyridine and 2, 5-thiophenedicarboxylic acid and its complexes with Cu (II), Co (II) Zn (II), Pb(II) and Hg (II). Ligand and its complexes are characterized by IR, NMR, UV, elemental analysis. Emphasis has been put on biological evaluation of the complexes.

## 2. MATERIALS AND METHODS

**Materials:** All chemicals employed in synthesis were used as analytical extra-pure grade and solvents are purified according to the literature methods [9]. The melting points of newly synthesized compounds are determined in open glass capillary tubes. Purity of the compounds was checked by TLC and the spots observed in iodine vapours. The presence of metal contents was determined according to standard procedure, [9] compound, 2, 6-diaminopyridine and 2, 5-thiophenedicarboxylic acid were prepared according to reported method [10].

### Physical Measurements

IR spectra of the new synthesized Schiff base and its metal complexes were recorded in KBr pellets on a Perkin-Elmer FT-IR instrument in the range 4000-400  $\text{cm}^{-1}$ .  $^1\text{H}$  NMR spectra were recorded on Bruker Avance II 400 MHz NMR spectrometer in DMSO- $d_6$  using TMS as an internal standard. UV Spectra were recorded on Jasco V-650.



Scheme 1: Synthesis of Schiff base ligand

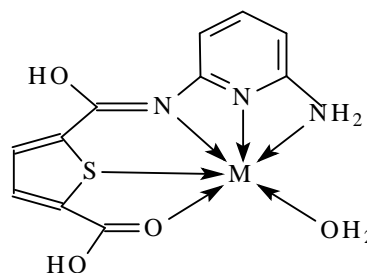
### Synthesis of Schiff base ligand

Synthesis of Schiff base ligand was summarized in **scheme 1**. Molar conductance was measured on the ELICO (CM-185) conductivity bridge using  $10^{-3}$  M solution in dry DMF by dip type conductivity cell fitted with a platinum electrode. An equimolar mixture of 2, 6-diaminopyridine (0.01 mole) and 2,5-thiophenedicarboxylic acid (0.01 mole) with a catalytic amount of glacial acetic acid (1-2 drops) in ethanol (25 ml) was refluxed for about 6-8 hrs. The reaction was

monitored by thin-layer chromatography. The black solid separates were filtered, washed with little ethanol, dried and recrystallized from alcohol. Melting point was (180-190°C) yield 72%; molecular weight (263) g per mole  $\text{C}_{11}\text{H}_9\text{N}_3\text{O}_3\text{S}$ . Elemental analysis: found (calc.): C 50.10(50.05), H 3.40(3.70), and N 16.57 (16.60).

### Syntheses of the Schiff base metal complexes

An ethanolic solution (25 ml, 0.2gm -0.00076 mol) of the appropriate metal chloride  $\text{MCl}_2$ , (M=Co, 0.22 gm; M=Pb, 0.25gm; M=Cu, 0.189gm, M=Zn, 0.2 gm, M=Hg, 0.206gm) was added to an ethanolic solution (30 ml) of the Schiff base. The reaction mixture was refluxed for about 8 hrs, during which no solid separated out. An aqueous alcoholic solution of sodium acetate (0.5gm) was added to the reaction mixture to maintain a pH about 6.0-7.0 and reflux was continued for about an hour. The reaction mixture was transferred into the distilled water. The separated solid complexes were collected by filtration, washed with minimum quantity of distilled water and dried in a vacuum over anhydrous calcium chloride in desiccators.



(DPTDCA) M=Cu, Zn, Pb, Co, Hg

**Antimicrobial studies:** The disc diffusion assay, standardised by NCCLS was performed with the ligands and the complexes to determine the antibacterial activity<sup>11</sup>. The zone of inhibition against gram negative bacteria species (E.Coli, Stigella, Klebsiella, Pseudomonas and Salmonella) and gram positive species (Staphylococcus Aureus) was measured. About 3ml of Mueller-Hinton Broth (MHB) was inoculated with 4-8 well isolated colonies of each strain. Each bacterial suspension was spread over the surface of Mueller-Hinton Agar (MHA) using sterile cotton swabs. The paper discs were soaked in 1mg of ligand /complex in 1ml DMSO and then placed on to the inoculated agar surface. After being kept at room temperature for 30 min, they were incubated at 37°C for 20hrs. Discs containing DMSO only were included as negative control. The results were expressed as diameter of inhibition zones. Inhibition of the fungal mycelial growth was determined on Potato Dextrose Agar medium (PDA) using poisoned substrate technique<sup>12</sup>. Radial mycelial growth in the presence of the ligand DPTDCA and complexes of the ligand with Zinc (II), Mercury (II), Lead (II), Cobalt (II) and Copper (II), at a concentration of 1mg/ml in DMSO was measured against *Aspergillus flavus*, a highly poisonous fungus found in spoiled food materials. Mycelial growth on PDA plate in

presence of DMSO alone was also measured as the control. Growth of the fungal colony was noted at two days interval for ten days.

### 3. RESULTS AND DISCUSSION

The results of elemental analysis of the ligand DPTDCA are in good agreement with the proposed molecular formula  $[C_{11}H_9N_3O_3S]$  (263) calculated: C, 50.10; N, 15.9; H, 4.79. Found: C, 61.27; N, 15.45; H, 3.4. S, 12.1; O, 18.2.

**FT-IR spectrum** showed bands at 3433,  $\nu$  (N-H)  $cm^{-1}$ , 2960  $cm^{-1}$ , (C-H aromatic), and 1646 (C=N), 1524  $cm^{-1}$  (C=C aromatic), 1321  $cm^{-1}$   $\nu$  (C-H),  $\nu$  (C=S)-1037  $cm^{-1}$  (N-H) bending 711  $cm^{-1}$  respectively.  $^1H$  NMR spectral data of the ligand in  $d_6$ -DMSO relative to TMS (Figure- 1) provide support for the keto form. The ligand was soluble in ethanol, methanol, acetone, DMF, DMSO and sparingly soluble in ether and insoluble in water.

UV-Visible spectra of the ligand DPTDCA were recorded in solvents of different polarities. Three main bands were observed in the spectra of the ligand. The absorption bands of the ligand  $\lambda_1$  and  $\lambda_2$  ( $\lambda_1 = 200-240nm$ ,  $\lambda_2 = 280-300nm$  in ethanol) were more solvent sensitive and can be ascribed to  $\pi \rightarrow \pi^*$  transitions of the aromatic system. The UV band at the longest wave length,  $\lambda_3$  ( $\lambda_3 = 330-354nm$  in ethanol) suggests its charge transfer nature and it gives black colour to the ligand. The intense charge transfer band may be hiding the weak  $n \rightarrow \pi^*$  transitions due to C=O and C=N. Most of the bands undergo red shift with increase in polarity of the solvents. Since the shifts in absorptions depend on several factors like polarity, acid base and donor acceptor properties of the solvent, exact correlation between solvent parameters and observed shifts is difficult. The chemical analysis data and physical properties of the complexes are given in table 2. Complexes obtained were all coloured, non hygroscopic solids stable in air. Complexes were only sparingly soluble in ethanol, methanol and chloroform and soluble in DMF and DMSO.

**$^1H$  NMR &  $^{13}C$  NMR-DPTDCA:**  $^1H$  NMR spectrum of the ligand shows signal due to OH at (7.5). Singlet at (7.6) in the free ligand due to NH disappears in the complex and a signal at (8.678) observed in the spectrum of the free ligand had shifted to (8.18) indicating coordination through azomethine nitrogen. This downward shift may be due to the reduction of electron density at the azomethine C-H. The four protons of unsymmetrical the ligand appear at (3.3)  $^1H$ -NMR spectrum as shown in Fig-2. All data mention in table-1,  $^{13}C$  NMR spectrum of the ligand shows signal due to 166(s), 152(s), 131(s) and 94(s), 47(p) spectrum shows signal in figure 3.

**Mass Spectra DPTDCA:** The mass spectrum of ligand showed a peak recorded at  $m/z$  262 due to  $M^{+1}$  corresponding to the molecular weight of the ligand. Further, this molecular ion underwent fragmentation peak recorded at 213  $m/z$  value  $C_{10}H_8N_3OS$ , and  $m/z$  188 (40 %) followed by expulsion of

$C_6H_2S$   $m/z$  value 107(10%) molecule gave a fragment ion peak. The fragmentation of ligand may represent the  $C_3H_5N_3$  gives at  $m/z$  77 (10%) value and remaining fragmentations are in agreement with the molecular formula  $C_3H_5N_2$  base peak  $m/z$  value 60(100%), 173(80%), 145(15%), 101(10%), 89(18%), - spectrum shows signal in figure 4.

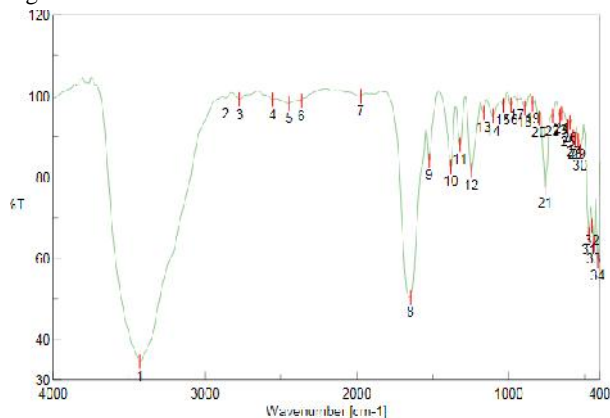


Fig 1: FT-IR- Spectra DPTDCA

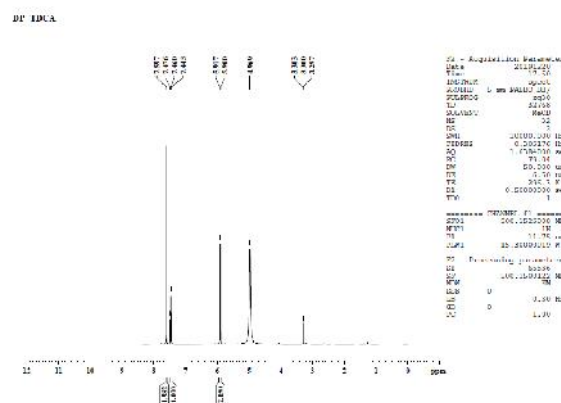


Fig 2:  $^1H$  NMR spectra- DPTDCA

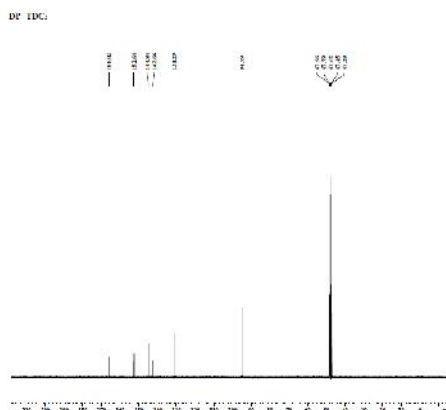


Fig 3:  $^{13}C$  NMR spectra- DPTDCA

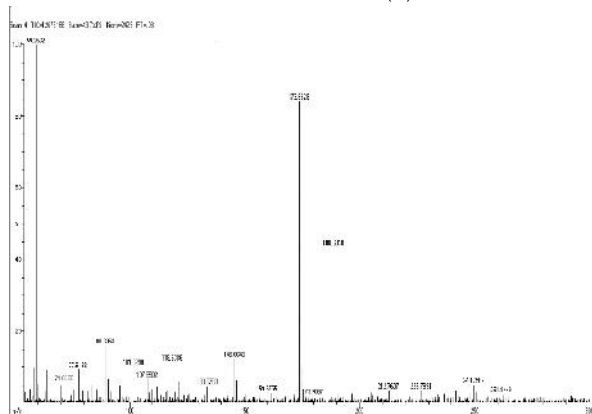


Fig 4: Mass Spectra DPTDCA

Table 1: <sup>1</sup>H-NMR, <sup>13</sup>C NMR, and DPTDCA of the ligand as depicted in Figure 2&3

Proton (assigned)	<sup>1</sup> HNMR (ppm)	Carbon (assigned)	<sup>13</sup> CNMR (ppm)	Group (assigned)	DPTDCA (ppm)
=N-C-H	7.6	=N-C-H	152	C-H	152
-OH	7.5			C	-
-C-H	3.3	-C-H	131	C-H	132
	5.9		94	C	47

Table 2: Analytical data of DPTDCA and their metal complexes

Compound/complex colour	M.Pt°C	Mol Wt	Elemental Analysis Calculated				
			C%	H%	O%	N%	S%
DPTDCA-(Black)		263	50.1	3.4	18.2	15.9	12.1
DPTDCA-Cu(Black)	269	512	25	1.7	9.3	8.1	6.2
DPTDCA-Co(Black)	221	554	23.8	1.6	8.6	7.5	5.7
DPTDCA-Pb(Black)	227	594	22.2	1.5	8	7	5.3
DPTDCA-Zn(Black)	188	550	23	1.6	8.7	7.6	5.8
DPTDCA-Hg(Black)	242	534	24.6	1.68	8.9	7.8	5.9

Table 3: Molar conductance data of metal complexes of DPTDCA

DPTDCA-Complexes	Conductance (Ohm <sup>-1</sup> Cm <sup>2</sup> Mol <sup>-1</sup> )
DPTDCA-Cu	6.9
DPTDCA-Co	9.4
DPTDCA-Pb	10.2
DPTDCA-Zn	7.1
DPTDCA-Hg	5.5

**Antibacterial Activity Test.** newly synthesized ligand and its complexes (Cu<sup>2+</sup>, Co<sup>2+</sup>, Zn<sup>2+</sup>, Pb<sup>2+</sup> and Hg<sup>2+</sup>) were screened against four bacterial strains, namely, *Staphylococcus aureus* and *Bacillus subtilis* (Gram positive) and *Escherichia coli* and *Pseudomonas aeruginosa* (Gram negative) through disc diffusion method [13]. Antibacterial activities of the compounds were described measuring their inhibition zone (mm) in comparison with gentamicin (standard drugs). Results of antibacterial activity of the ligand and its complexes are found to be comparable with standard drugs, presented in table-4. Inhibition zone of metal complexes is higher than that of the ligand which indicates that complexes exhibit higher antibacterial activities than the free ligand due to chelation which enhance lipophilicity and

so favors permeation into the bacterial membrane to cause the death of the organisms [14]. Co (II) complex shows higher activity than standard drugs against *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, while the Cu (II) complex is more active than the Hg (II) complex against *Bacillus subtilis* evidence implies the Hg (II) towards *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Table 4: Antibacterial &amp; Anti-Fungal activity.

Compound	Zone of inhibition in diameter (mm)					
	Anti-Bacteria activity				Anti-Fungal activity	
	<i>S.aureus</i>	<i>B.subtilis</i>	<i>E. coli</i>	<i>P.aeruginosa</i>	<i>A.parasiticus</i>	<i>A.niger</i>
Ligand	12	16	17	13	12	13
Cu-L	18	24	19	16	14	15
Co-L	12.5	12.5	25	7	25	12.5
Zn-L	25	12.5	50	12.5	12.5	12.5
Pb-L	25	>50	25	12	12.5	12.5
Hg-L	50	25	50	50	12.5	6.25

#### 4. CONCLUSIONS

The ligand, PTDCA was synthesized from 2, 6 Diamino pyridine (DP) and 2, 5 Thiophene dicarboxylic acid (TDCA), and its complexes were synthesized by reacting the ligand with metal. The as-synthesized compounds were characterized by spectro-analytical techniques which suggested monodendate ligand. The result of conductivity measurement reveals electrolytic nature of both complexes. The analytical data showed that stoichiometry of both complexes to be 1: 1 metals to ligand ratio and formulated as Cu,Co,Pb,Zn,Hg-[C<sub>11</sub>H<sub>9</sub>N<sub>3</sub>O<sub>3</sub>S]. On the basis of the combined analytical results, distorted octahedral geometry of all metals complexes were achieved. Antibacterial activities of the prepared ligand and complexes have also been studied. From the comparative study, both metal complexes showed significantly enhanced antibacterial activity against selected bacterial strains in comparison to the free ligand.

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