WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 8.084
Volume 9, Issue 3, 1367-1380. Research Article ISSN 2277– 7105

SYNTHESIS, CHARACTERIZATION AND ANTIMICROBIAL ACTIVITY OF SOME METAL COMPLEXES DERIVED FROM THIAZOLE SCHIFF BASE (2-THIOPHENE CARBOXAMIDE-2, 6-DIAMINOPYRIDINE)

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Article Received on
14 Jan. 2020,
Revised on 03 Feb. 2020,
Accepted on 24 Feb. 2020,
DOI: 10.20959/wjpr20203-16963

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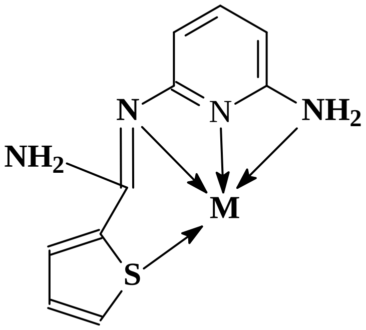
ABSTRACT

As multidrug resistant pathogens are emerging, the search for novel potent drug candidates is ever going. Heterocycles are known by their broad spectrum of biological activities, so a search for a new drug from heterocycles can elevate the chance of success. The aim of this study was to obtain novel potent antimicrobial compounds. In line with this 2-Thiophene Carboxamide-2,6-DiaminoPyridine and its complexes (Cu(II), Co(II) Zn(II), Pb(II) and Hg(II)) were synthesized, characterized, and evaluated against bacterial strains. The compounds were characterized by elemental analyses (C, H, N, and S), FT-IR, ^{1}H-NMR, ^{13}C-NMR, UV-Vis spectra, and molar conductivity measurement. The results showed that the ligand is tetradendate, and the molar conductivity measurement indicates that complexes are electrolytic. Electronic spectral study showed octahedral and distorted octahedral geometry for the (Cu (II), Co (II) Zn (II), Pb (II) and Hg (II)) complex, respectively. The ligand and its complexes were screened against four bacterial strains using disk diffusion method. The result revealed that the Hg (II) complex showed more bioactivity than gentamicin against *Staphylococcus aureus* and *Escherichia coli*, while the Cu (II) complex is more active than the Zn (II) complex against *Bacillus subtilis*. Both (Cu (II), Co (II) Zn (II), Pb (II) and Hg (II)) complexes exhibit higher antibacterial activities than the free ligand.

KEYWORDS: Schiff base, antimicrobial activity, 2-Thiophene Carboxamide-2, 6 Diamino Pyridine, Metal complexes.

INTRODUCTION

The chemistry of the Schiff base ligands and their metal complexes evoke much current interest and encompasses a vast area of organometallic compounds and various aspects of bioinorganic chemistry.[1] Schiff bases are considered as privileged ligands because they are easily prepared by condensation of aldehydes or ketones with amines and are able to stabilize different metals in various oxidation states.[2,3] The importance of Schiff base complexes for bioinorganic chemistry, biomedical applications, supramolecular chemistry, catalysis and material science, separation and encapsulation processes, and formation of compounds with unusual properties and structures has been well recognized and reviewed.[4] Large numbers of Schiff bases have shown to exhibit a wide range of biological activities, including antitumor[5] anti-bacterial[6,7] fungicidal,[8] and anticarcinogenic.[9] properties. On the other hand, coordination compounds with heterocyclic Schiff base ligand has attracted much attention of the chemist in current years to find applications as potential drugs,[10,11] due to the presence of multifunctional groups.[12,13] The excessive attention of synthesizing determined broad range S chelating ligands as thiazole molecule have attracted significant interest and gained special attention not only in structural chemistry of their multifunctional coordination modes but also showed importance in medicinal and pharmaceutical field.[14] This is because thiazoles have a great pharmacological activity. Many thaizole derivatives such as sulfathiazole, ritonvir, abafungi, blemycin and tiazofurin are well known as potent biologically active compounds.[15,16] Moreover, thiazoles are very important building blocks in medicinal chemistry and can be found in numerous natural products and biologically important compounds including anti-microbial, anti-inflammatory, anti-hypertensive, anti-HIV, anticancer and cytotoxic activity that can be well illustrated by the large number drugs in the market containing the moiety.[17,18]



TCADAP —M

M=Cu^{2+},Zn^{2+},Co^{2+}, Pb^{2+}, Hg^{2+}

Thiazole ring also found applications in polymer, liquid crystals, photo-nucleases, fluorescent dyes, insecticides and antioxidants.[19,20] Their transition metal complexes have attracted a great deal of interest largely due to their ability to interact with DNA molecule.[21] Intrigued by the above observations and in continuation of our ongoing research work on synthesis and characterization of Schiff base ligands and their metal complexes, hereby we report the synthesis of a novel Schiff base ligand 2,6-DiaminoPyridine -2-Thiophenecarboxamide and its metal complexes, their characterization by different spectroscopic techniques and their antibacterial, antifungal, *in vitro* cytotoxicity property.

MATERIALS AND METHODS

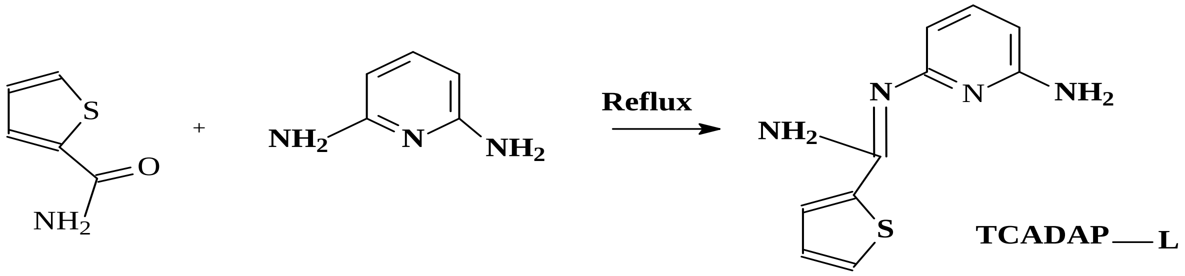
All chemicals employed in synthesis were used as analytical extra-pure grade and solvents are purified according to the literature methods.[22] The melting points of newly synthesized compounds are determined in open glass capillary tubes and are uncorrected. Purity of the compounds was checked by TLC and the spots observed in iodine vapours. The presence of metal and chlorides contents was determined according to standard procedure.[22] Compound, 2-Thiophene Carboxamide-2, 6-DiaminoPyridine were prepared according to reported method.[23]

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 cm^{-1}. ^{1}H NMR spectra were recorded on Bruker Avance II 400 MHz NMR spectrometer in DMSO-d6 using TMS as an internal standard.UV Spectra wear recorded Jasco V-650. 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.

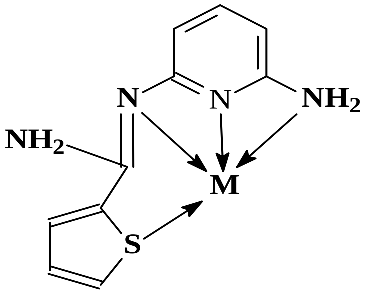
Synthesis of Schiff base ligand

Synthesis of Schiff base ligand was summarized in **Scheme 1**. An equimolar mixture of 2-Thiophene Carboxamide (0.01 mole) and 2, 6-DiaminoPyridine (0.01 mole) with a catalytic amount of glacial acetic acid (1-2 drops) in ethanol (25 ml) was refluxed on a water bath for about 6-8 hrs. The reaction was monitored by thin-layer chromatography. The brown solid separates were filtered, washed with little ethanol, dried and recrystallized from alcohol. Melting point was (76^{0}C) yield 72%; molecular weight (218) per mole $C_{10}H_{10}N_4S$, Elemental analysis: found (calc.): C 55 (55.15), H 45(45.1), and N 25 (25.2), S 14.0(14.2).



Scheme-1: syntheses of the schiff base metal complexes.

An ethanolic solution (25 ml, 0.01 mol) of the appropriate metal (M=Co, 2.378 gm; M=Pb, 2.377 gm; M=Cu, 1.705gm, M=Zn, 1.363 gm, M=Hg, 2.013gm) was added to an ethanolic solution (30 ml) of the Schiff base. The reaction mixture was refluxed for about 6 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 was collected by filtration, washed with minimum quantity of distilled water and dried in a vacuum over anhydrous calcium chloride in desiccators Metal complexes indicate in **Scheme-2.**



TCADAP —M

M=Cu^{2+},Zn^{2+},Co^{2+}, Pb^{2+}, Hg^{2+}

Scheme-2

Antimicrobial activities

The biological activities of the synthesize Schiff base and its metal complexes were studied under antibacterial and antifungal activities by disc and well diffusion method respectively. The *In vitro* antibacterial activities of the compounds be tested against two Gram positive *Bacillus subtilis* and *Staphylococcus aureus* and two gram negative *Salmonella typhi* and *Escherichia coli* bacteria. The *In vitro* antifungal an activity was carried out against *Candida albicans, Cladosporium oxysporum* and *Aspergillus Niger* fungi.27, 28 The stock solution of the test chemicals (1 mg mL^{-1}) was prepared by dissolving 10 mg of the each test compound in 15 ml of distilled DMSO solvent. The different concentration of the test compounds (100, 75, 50, 25 and 12.5 µg mL^{-1}) prepared by diluting the stock solution with the required amount of freshly distilled DMSO. In addition a controlled experiment was carried out by using freshly distilled DMSO solvent alone.

Antibacterial Screening

Muller-Hinton agar media were used for the antibacterial studies. The dehydrated Muller-Hinton agar (38gm) was dissolved in 1000 mL distilled water. The pure culture of the bacterial strains *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and *Salmonella typhi* was sub cultured by inoculating in the nutrient broth and they were incubated at 37^0C for 18 hrs. The agar plates were prepared by using the above Muller-Hinton agar media, and wells were dug with the help of 6 mm sterile metallic cork bore. Each plate was inoculated with 18 hrs old bacterial culture using a micropipette and spreaded regularly using bent glass rod on each plate. The drug Gentamycin is used as per standard. Different concentrations of the test compounds was incorporated into the wells using micropipette and the plates were kept for incubation period, the diameter of the inhibition zone generate by each test compound against bacterial growth was measured using anti biogram zone measuring scale.

Antifungal screening

Potato dextrose agar (PDA) media was used for the antifungal studies. The following ingredients were used prepare the media: potatoes (sliced washed unpeeled) 200 g, dextrose 20 g, and agar 20 g in 1000 mL distilled water. The pure cultures *Cladosporium oxysporum*, *Candida albicans*, and *Aspergillus Niger* were inoculated on PDA slants. These slants were incubated at 32^0C for 7 days. To these 7 days old slants of fungal strains, 10 mL of 0.1% tween-80 solution were added, and the culture were scraped with sterilized inoculating loop to get uniform spore suspension. The agar plates were prepared through using the above Potato dextrose agar media and wells were dug with the help of 6 mm sterile metallic cork bore. Each plate was inoculated with 7 days old spore suspension of each fungal culture using a micropipette and spreaded regularly using bent glass rod on each plate. Next each well was incorporated with the test compound solution of different concentrations. The drug *Flucanozole* is used as standard. All the inoculated plates were incubated at 32^0C for 48 hrs. Soon after the completion of incubation period the diameter of the inhibition zone generated by each test compound against fungal growth is measured using antibiogram zone measuring scale.

RESULTS AND DISCUSSION

In our study, a new Schiff base based thiazole derivative has been synthesized by condensation of 2,6-DiaminoPyridine -2-Thiophenecarboxamide in the presence of catalytic amount of glacial acetic acid with good yield of 80% as shown in **Scheme-1**. The purity of the current Schiff base ligand was checked by running TLC on a silica gel coated plate using Cyclohexane- Ethyl acetate (80:20%) as the eluent. The complexes were synthesized by the reaction of thiazole ligand with metal chloride in 1:1 M ratio in ethanol shown in **Schemes-2**. The ligands and their complexes were found to be stable at room temperature and soluble in C_2H_5OH, in DMSO and DMF. Molar conductance data of the metal complex was measured in DMF at 10^{-3} M and all the complexes showed conductance in the range of 5-12.1ohm^{-1}cm^{2}mol^{-1} at ambient temperature indicating non-electrolytic in nature [24] and outside their coordination sphere there is no counter ion present.

Table 1: Analytical data of TCADAP and their metal complexes.

Compound/complex colour	**M.Pt^{O}C**	**Mol Wt**	**Elemental Analysis Calculated**				**Molar conduct. Ohm^{-1}cm^{2}mol^{-1}**
			C%	H%	N%	S%	
TCADAP-(Black)	76	218	55	45	25	14	NA
TCADAP-Cu(Black)	200	467	25	2	11	6.8	9.8
TCADAP -Co(Black)	210	437	27	2	12.8	7	9.4
TCADAP -Pb(Black)	179	549	21.8	1.82	10.1	5.8	10.2
TCADAP -Zn(Black)	201	505.54	23.7	1.9	11.0	6.32	12.1
TCADAP -Hg(Black)	204	489.50	24.5	2.0	11.4	6.5	5.5

Antimicrobial Results

In vitro Antimicrobial Activity

The *In-vitro* antimicrobial activity of all newly synthesized compounds were screened against *E.coli*, *S.aureus*, *B.subtilis* and *S.typi* bacteria and *C.albicans*, *C.oxysporum*, and *A.niger* fungal strains by minimum inhibitory concentration (MIC) method. The MIC profile of the entire compound against bacteria and fungi are summarized in Table 2. A comparative study of the ligand and their metal complexes indicates that complexes exhibit higher antimicrobial activity than free ligand and this activity enhanced on coordination with metal ions. This enhancement in the activity may be rationalized on the basis that ligands mainly possess C=N bond. The enhanced activity of the complexes over the ligand can be explained on the basis of chelation theory.[27,28] It is observed that in complex, the posi tive charge of the metal is moderately mutual with the donor atoms present in the ligand and there may be π-electron delocalization over the whole chelating. This increases the lipophilic character of the metal chelates and favors its permeation through the lipoid layer of the bacterial membranes and also other factors which increase the activity, namely solubility, conductivity, and bond length between the metal and the ligand.

FTIR spectral studies

The identification for the formation of thiazole Schiff base were obtained from the absence of IR characteristic band for amino group attached to thaizole ring and the carbonyl group of ketone. The IR spectrum of the Schiff base ligand clearly indicates sharp band at 1420 cm^{-1} is due to presence of C=C group (C=N) of amide and displayed one sharp band at 1667 cm^{-1} of Schiff base. One broad bands observed at 3174 cm^{-1} NH in Schiff base compound respectively **Figure 1**. To study the binding mode of Schiff base to the central metal ion in the complex. **Figure 2.** The complexation of meal ion with ligand were further confirmed by the appearance of new weak intensity, ligand bands in the region appears at C=S at resion1032 cm^{-1}, C-H at resion-2965 cm^{-1}, C-H bending region appears 716 c m^{-1} .The IR data (figure-1)of Ligand is presented in Table 2.

^{1}H NMR spectrum &^{13}C NMR Spectrum

The ^{1}H NMR data of Schiff base figure-2& figure-3. The proton spectrum of Schiff base display 7.6-7.75 ppm aromatic protons appears as multiplates .pyridine proton appears as 7.6(q), 7.2 (q)ppm, a proton appears at 5.9 ppm (d, thiophene 3H). The ^{1}H NMR complex displayed all aromatic protons as multiplates in region 7.65 to 7.7 (m, 3H Pyridine Ar-H).spectral data. The ^{13}C NMR data of Schiff base **figure-4**,Carbon spectrum of Schiff base display 165(s),158(s),139(d),127(s) and 96(s),47(q)for *^{13}C NMR* analysis.

Mass Analysis

The ESI mass spectra of Schiff base ligand performed to determine their molecular weight and study their fragmentation. The mass spectrum of ligand showed a peak recorded Kalshetty at m/z 218($C_{10}H_{10}N_4$ S) due to M^{+1} corresponding to the molecular weight of the ligand. Further, this molecular ion underwent fragmentation peak recorded at m/z 135 (38 %) followed by expulsion of $C_6H_7N_4$ molecule gave a fragment ion peak. The fragmentation of ligand may represent the $C_6H_5N_3$ gives at base peak m/z 119 (100%) value and remaining fragmentations are in agreement with the molecular formula $C_5H_5N_3$ gives m/z 107(10%),$C_5H_5N_2$ gives m/z 91(45%). **Figure 4**.

Electronic Spectra of the Ligand and Its Complexes

Electronic spectra were used to assign the stereochemistry of a metal complex. The UV-Vis spectra of the ligand and the complexes were recorded in DMSO in the range of 200–800nm at room temperature. The electronic spectra of the ligand showed two strong bands at 243nm (38461 cm^{-1}) and at 288nm (37004 cm^{-1}). The band at 260nm (37072 cm^{-1}) is assigned to $\pi\longrightarrow\pi*$ transition., the band observed at 243nm (36064 cm^{-1}) is attributed to $n\longrightarrow\pi*$ transitions of the C=N chromophores.

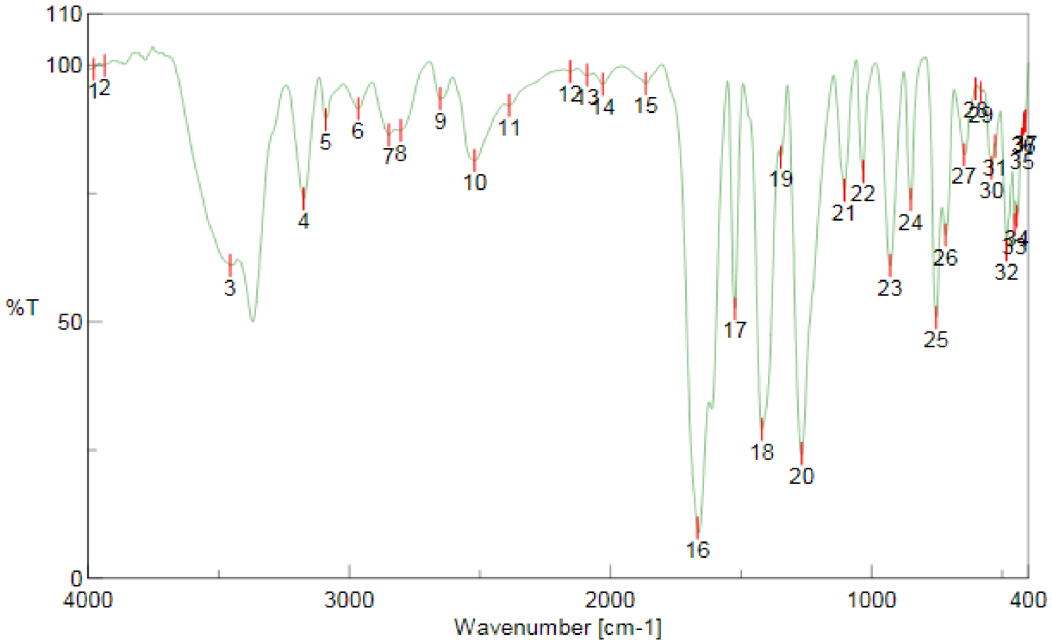


Figure 1: FTIR-Spectra.

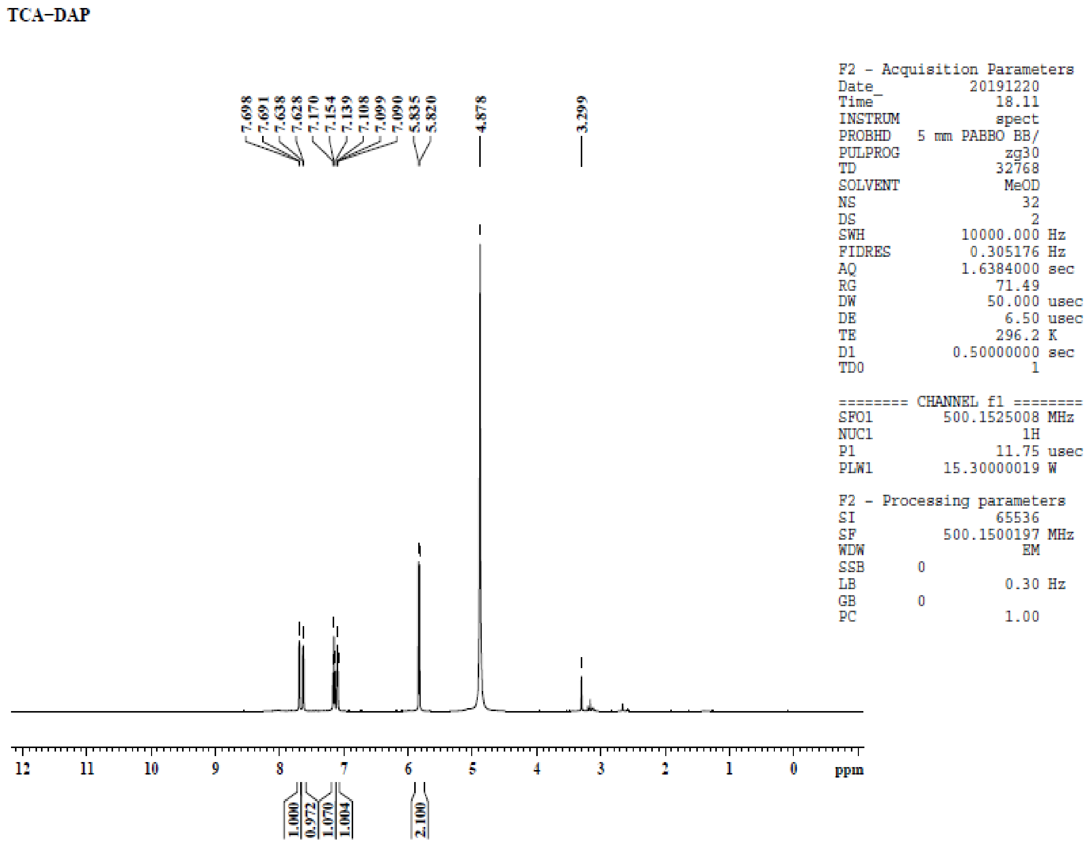


Figure 2: ^{1}H NMR spectrum.

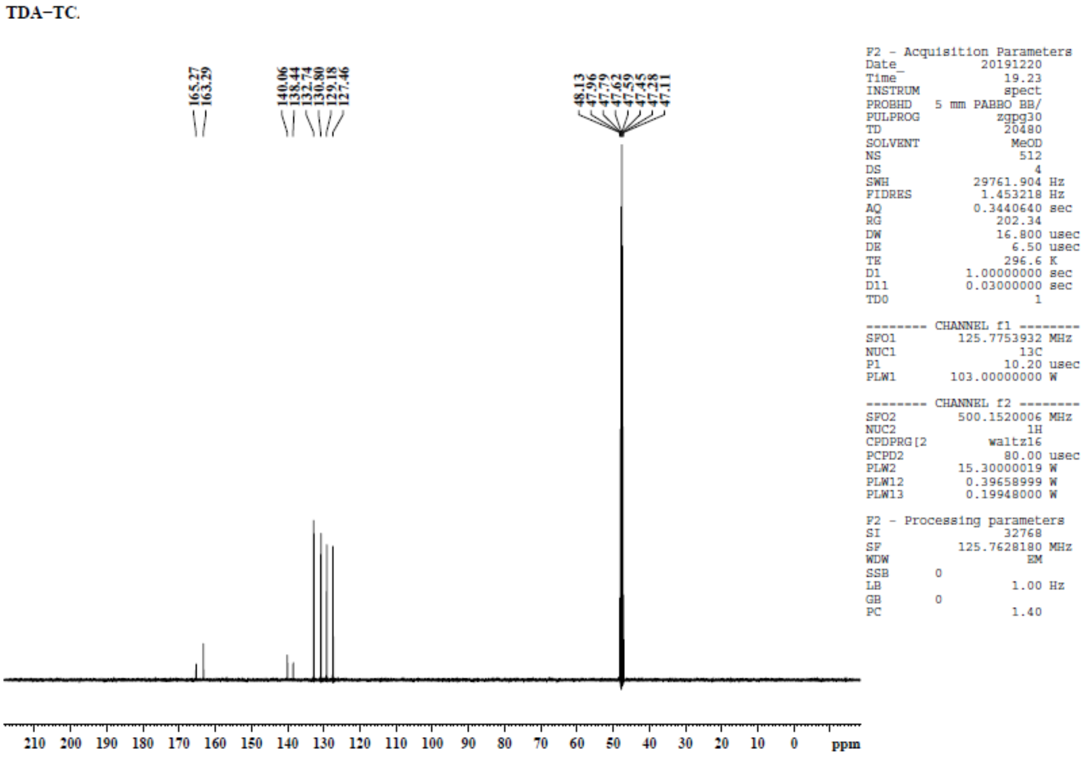


Figure 3: ^{13}C NMR Spectrum.

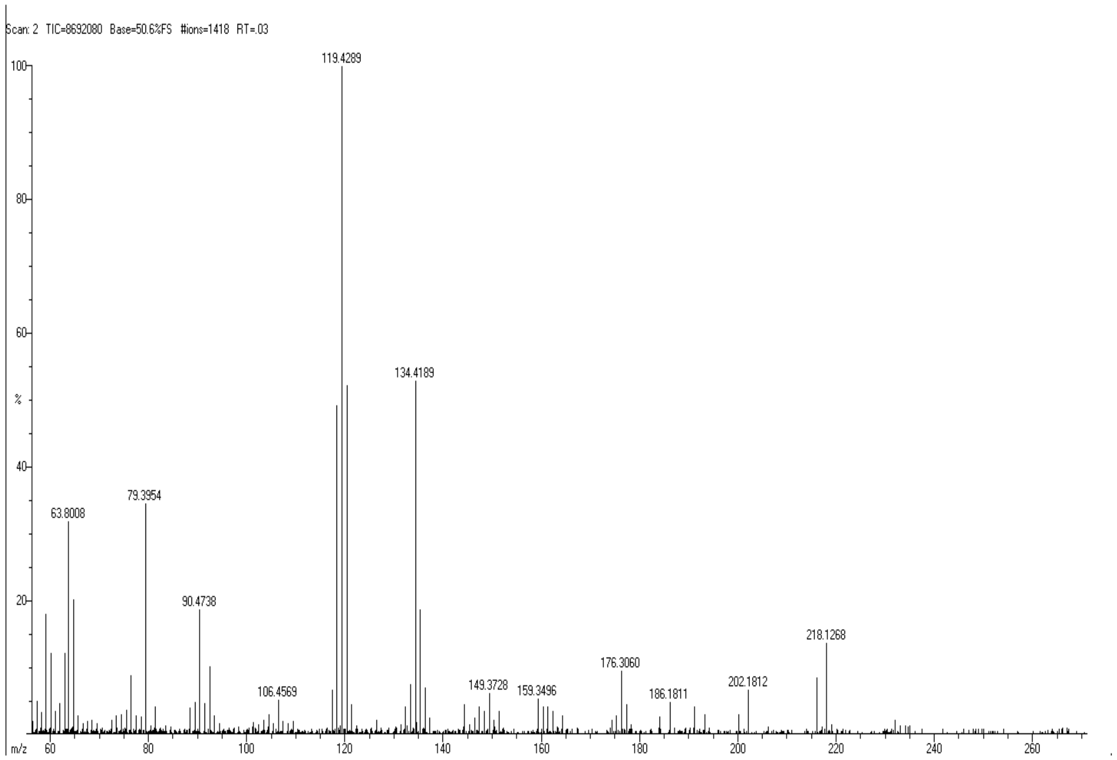


Figure-4- Mass spectrum

Antimicrobial Results

In vitro Antimicrobial Activity

The *In-vitro* antimicrobial activity of all newly synthesized compounds were screened against *E.coli*, *S.aureus*, *B.subtilis* and *S.typi* bacteria and *C.albicans*, *C.oxysporum*, and *A.niger* fungal strains by minimum inhibitory concentration (MIC) method. The MIC profile of the entire compound against bacteria and fungi are summarized in Table 4. A comparative study of the ligand and their metal complexes indicates that complexes exhibit higher antimicrobial activity than free ligand and this activity enhanced on coordination with metal ions. This enhancement in the activity may be rationalized on the basis that ligands mainly possess C=N bond. The enhanced activity of the complexes over the ligand can be explained on the basis of chelation theory.[27, 28] It is observed that in complex, the positive charge of the metal is moderately mutual with the donor atoms present in the ligand and there may be π-electron delocalization over the whole chelating. This increases the lipophilic character of the metal chelates and favors its permeation through the lipoid layer of the bacterial membranes and also other factors which increase the activity, namely solubility, conductivity, and bond length between the metal and the ligand.

Molar Conductivity of the Complexes

The conductivity measurements were carried out at room temperature by dissolving 20 mg of each complex in 30mL of DMSO. Specific conductance (K) of the, Hg(II) Pb(II), Zn(II), Co(II) and Cu(II) complexes was obtained as mention in analytical table, respectively. The molar conductance (ΛM) of the complexes was calculated using the relation ΛM 1000 K/C (where C is the molar concentration of the metal complex solution and K is specific conductance); the molar conductivity of the Cu (II), Co (II), complex and Pb (II), Zn(II)and Hg(II) complex was obtained as 9.8, 9.4 10.2 ,12.1and 5.5 Ω^{-1} cm^2mol^{-1}, respectively, which suggests these complexes have electrolytic nature in a 1 : 1 ratio, is was in line with the chloride test.[29,30]

Table 4: Minimum inhibitory concentration (MIC μg mL^{-1}) of Schiff base ligand and its metal complexes.

MIC Value in μ g mL^{-1}							
Compounds	**Bacteria**					**Fungi**	
	E.coli	**S.aureus**	**B.subtilis**	**S.typhi**	**C.albicans**	**C.oxysporum**	**A.niger**
Schiff base	50	76	50	100	75	51	50
Cu complex	13	25	25	50	25	13	13
Co complex	25	50	13	50	13	13	25
Pb complex	13	25	25	50	25	13	25
Zn complex	25	50	25	75	50	25	25
Hg complex	13	13	13	25	13	25	13
Gentamycin	13	13	13	13	-	-	-
Fluconazole	-	-	-	13	13	13	13

CONCLUSION

The newly synthesized Schiff base ligand 2,6-DiaminoPyridine and 2-Thiophenecarboxamide, behaves as bidentate N-S donor and the complexes of Hexa planar type [ML (Cl) $_6$]. The help of various physicochemical and spectroscopic methods such as IR, ^{1}H,^{13}C NMR, Mass spectra , the Hexa planar geometries of Cu(II), Co(II), Pb(II), Zn(II) and Hg(II) complexes have been proposed **scheme 2**. The newly synthesized metal complexes having good antimicrobial activity when compared to Schiff base ligand.

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