Synthesis, Characterization and Antimicrobial Studies of a Chiral Compound and its Metal Complexes

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Abstract- A chiral Mannich base, [1-(piperidin-1-yl(thiophen-2-yl)methyl)] thiourea (TPPTU)was prepared by treating thiophene-2-carbaldehyde piperidine and thiourea . Using TPPTU as a ligand, metal complexes of Mn(II), Co(II), Ni(II), Cu(II) and Zn(II) were prepared. Both the compound and complexes were characterized by physical methods such as elemental analysis, melting point and TLC and spectral methods such as IR, UV –Visible, ^{1}H NMR, ^{13}C NMR and Mass spectral studies. For the complexes, molar conductivity, magnetic susceptibility and thermal studies were also been carried out. In vitro antimicrobial study was carried out for both the compound and complexes.

Index Terms- Mannich base complexes, Thiopene-2-aldehyde, Piperdine, Antimicrobial studies

I. INTRODUCTION

The compounds containing amide moieties such as urea, thiourea, nicotinic acid hydrazide, semi- and thiosemicarbazide and their derivatives have been investigated extensively due to their rapid coordinating tendencies [1- 7]. These ligands exhibit different tendency for coordination. It may take place through carbonyl oxygen in the case of urea and thionylsulphur in the case of thiourea. In both the cases possibility of coordination may through nitrogen atom of NH_2. Urea and Thiourea were highly exploited in the synthesis of Schiff bases because they easily undergo condensation reaction. During the recent years much work has been carried out in the metal complexes of Mannich bases. The study of Mannich reaction attracted the chemist owing to their wide range of pharmaceutical and industrial applications [8 - 12]. It has been reported that many Mannich compounds possess properties such as anti-bacterial, anti-fungal, anti-tumor, anti-convulsant, anti-inflammatory, anti-malarial, anti-biotic, anti-viral, anti-cancer, and anti-leishmanial. Mannich bases are also used as analgesic, cytotoxic, oxytocic, anti-psychotic, diuretic, centrally active muscle relaxant and tranquilizer. It exhibit complexation characteristic with many transition metal ions. Mannich base is a three component system obtained by reacting an aldehyde/ketone, a primary/secondary amine and a compound containing active hydrogen atom as substrate. Enormous research papers have been appeared in the literature for the synthesis of Mannich bases using formaldehyde. Although formaldehyde is usually used in the Mannich synthesis, the higher aldehydes such as succinaldehyde, benzaldehyde, acetaldehyde, anisaldehyde and their substituted products etc., are also reported. Mohamed kassim et al., have reported the synthesis and characterization of metal complexes of N-(1-piperidinobenzyl)acetamide and N-(1-morpholinenobenzyl)acetamide [M = Mn^{II}, Co^{II}, Ni^{II} and Cu^{II}]. In all the complexes, the ligand chelation occurs through its carbonyl oxygen and nitrogen atom of piperidine [13]. N-(1-piperidino(4-nitrobenzyl))acetamide and its metal complexes [M = Mn^{II}, Co^{II}, Ni^{II} and Cu^{II}] have been prepared and characterized. This study shows that the ligand forms complexes with metals through oxygen atom of carbonyl and nitrogen atom of piperidine [14]. N Raman et al., have synthesized and characterized metal complexes of Mannich bases [15,16]. Metal complexes of Mannich bases derived from urea as a substrate have been synthesized and characterized by [13, 17]. A Jameel et al., have synthesized and characterized Mannich bases using heteroaldehyde such as furan-2-carbaldehyde, thiopene-2-carbaldehyde and pyridine-2-carbaldehyde [5-6]. From the literature survey it is clearly revealed that for the synthesis of Mannich bases, aldehydes like formaldehyde, benzaldehyde, substituted benzaldehyde have been used along with secondary amines such as morpholine, piperidine and piperazine etc., and a compound containing an active hydrogen atom like alkyl ketones, phenols, carboxylic acid derivatives, heterocyclic compounds, alkynes and amides. This has been observed from the literature that a limited number of research works have been carried out in the synthesis of Mannich bases utilizing thiophene-2-carbaldehyde as a component. Now a day's tremendous investigation are going on for developing new drugs using heterocyclic derivatives. Heterocyclic compounds such as pyrrole, furan & thiophene derivatives show diverse pharmacological activities [18].Thiophene nucleus has been established as the potential entity in the largely growing chemical world of heterocyclic compounds possessing promising pharmacological characteristics. The similar compounds synthesized through different routes bear variable magnitudes of biological activities. The knowledge of various synthetic pathways and the diverse physicochemical parameters of such compounds draw the special attention of medicinal chemists to produce combinatorial library and carry out exhaustive efforts in the search of lead molecules. Based on the above mentioned importance of heterocyclic compounds, nitrogen and oxygen containing compounds and their metal complexes, the present study provides a synthesis, characterization and biological studies of a Mannich base having thiophene nucleus and its metal complexes.

II. MATERIALS AND METHODS

2.1. Reactants

All the reactants and solvents used were of analytical grade and commercially available. All the solvents were dried before use by the literature methods and moisture was excluded from the glass apparatus using $CaCl_2$ drying tube. Urea, piperidine and thiophene-2-carbaldehyde were purchased from Merck Products and metal salts were used as received.

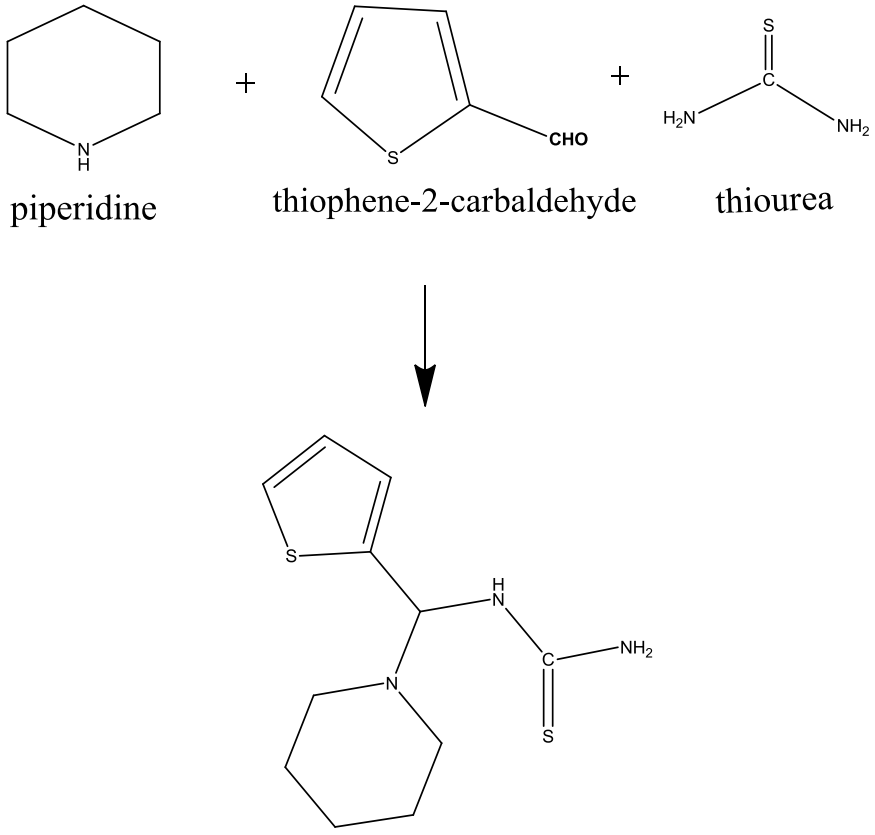
2.2. Measurements

The melting point of the compounds were determined in open capillary tubes and are uncorrected the purity of the compounds was checked by TLC using silica gel G coated glass plate with methanol and ethyl acetate 1:1 as irrigant and iodine vapour as visualizing agent. The IR spectra were recorded on Shimadzu FT- IR affinity 1 using KBr pellets. The absorption in the UV-Vis region was recorded by a Perkin Elmer Lambda 35 Spectrophotometer using DMSO as solvents. ^1H NMR and ^{13}C NMR spectra were recorded on 300 MHz Shimadzu spectrometer using DMSO-d^6 as solvent and TMS as an internal standard. Elemental analyses were carried out on a Perkin - Elmer series C, H, N & S analyzer 2000. Molar conductivity in DMF at room temperature was measured by Elico conductivity bridge type CM 82 T having conductivity cell with the cell constant 1.00 using 10^{-3} M solution of complexes. Magnetic measurements at room temperature were carried out by Gauoy's method.

2.3. Experimental

2.3.1 Synthesis of Mannich base

Thiophene-2-carbaldehyde, Piperidine and thiourea were taken in 1:1:1 ratio and reacted as shown in the Scheme – 1. 7.6 g (0.1 mole) of thiourea was taken in a round bottom flask and 10 mL of water was added. To this solution 9.8 mL (0.1 mole) of piperidine was added and stirred well for 15 min by keeping the reaction mixture on a magnetic stirrer. 9.2 mL (0.1 mole) of thiophene-2-carbaldehyde was added to the above mixture and stirring was continued under ice cold condition. The compound formed was filtered, washed and recrystallized using ethanol.



Scheme 1. Synthesis of 1-(piperidine-1-yl(thiophene-2-yl)methyl)thiourea

Preparation of Metal Complexes

The complexes were prepared by mixing the methanolic solution of the corresponding metal chlorides [Mn(II), Co(II),Ni(II), Cu(II) and Zn(II)] (0.1M) to the methanolic solution of the ligand in the mole ratio 1:2 respectively. The reaction mixture was refluxed on a water bath for 2 h. On cooling solid obtained was filtered, washed with methanol and chloroform mixture and dried over anhydrous $CaCl_2$ in desiccators. The analytical data of the ligand and complexes are presented in Table – 1

Table – 1 ANALYTICAL DATA OF TPPTU AND ITS COMPLEXES

Compounds / Colour	M P^{0}C / dec	Found (%) (Calculated)						λ_m Ω^{-1} cm^2 mol^{-1}	μ_{eff} BM
		C	H	N	S	Cl	M		
TPPTU (Colourless)	130	51.70 (51.73)	6.69 (6.71)	16.42 (16.45)	25.09 (25.11)	---	----	----	----
Mn(TPPTU)$_2$ $(H_2O)_2]Cl_2$ (Greenish yellow)	420-440	39.20 (39.28)	5.58 (5.69)	12.38 (12.49)	18.99 (19.07)	10.48 (10.54)	8.02 (8.17)	128.3	5.86
[Co(TPPTU)$_2$ $(H_2O)_2]Cl_2$ (Greenish brown)	510-530	39.12 (39.05)	5.58 (5.66)	12.40 (12.42)	18.89 (18.95)	10.40 (10.48)	8.68 (8.71)	112.4	4.54
[Ni(TPPTU)$_2$ $(H_2O)_2]Cl_2$ (Green)	450-480	38.98 (39.06)	5.60 (5.66)	12.37 (12.42)	18.92 (18.96)	10.39 (10.48)	8.60 (8.68)	132.2	3.82
[Cu(TPPTU)$_2$ $(H_2O)_2]Cl_2$ (Dark green)	415-425	38.70 (38.78)	5.59 (5.62)	12.28 (12.34)	18.80 (18.83)	10.39 (10.41)	9.30 (9.33)	118.6	1.75
[Zn(TPPTU)$_2$ $(H_2O)_2]Cl_2$ (colourless)	520-535	38.60 (38.68)	5.59 (5.61)	12.28 (12.30)	18.70 (18.78)	10.29 (10.38)	9.50 (9.57)	128.6	-----

Antibacterial Activity

To study the antimicrobial activity, nutrient agar was used as a medium. This was prepared by dissolving 5 g of yeast extract, 10 g meat extract, 5 g of peptone, 5 g of NaCl and 20 g agar in 100 mL of distilled water in a clean conical flask and the pH was maintained at 7. The solution was boiled to dissolve the medium completely and sterilized by autoclaving at 7 kg pressure (121^{0}C) for 15 minutes, after sterilization 20 mL media poured into the sterilized petri plates. These petri plates were kept at room temperature for some time, after a few minutes, the medium gets solidifies in plate. Then this was inoculated for 12 h. after the incubation, which was inoculated with microorganisms, using simple swabs. All these manipulation were carried out with atmospheric air under aseptic condition.

Antifungal activity

The potato dextrose agar PDA was used as a medium for antifungal activity, the nutrient agar was prepared by dissolving 20 g of potato extract, 20 g of agar and 20 g of dextrose in one liter of distilled water in a clean conical flask. The solution was boiled to dissolve the media completely and sterilized by autoclaving with 7 kg pressure (121^{0}C) for 30 minutes, after the strelization 20 mL media was poured in to the sterilized petri plates. These plates were kept at room temperature for some time. After a few minutes, the medium get solidifies in plate. DMSO (0.5 mL) was used as solvent and clotrimazole (10 mg\disk) as control, in a typical procedure, a well made on the agar medium was inoculate with microorganism and it was filled with test solution using a micro pipette (50 mL) and the plate was incubated at 35^{0}C for 72 hr. During this period the test solution diffuses and affects the growth of the inoculated microorganism. A zone was developed on the plate and the inhibition zone was measured by measuring the diameter of inhibited zone in mm, and the values are presented in the Table – 2.

Table – 2 ANTIMICROBIAL DATA OF TPPTU AND ITS COMPLEXES

Compounds	DMSO Extract added and Zone of inhibition 100 µl (mm/ml)		
	Staphylococcus aureus	*E.coli*	*A.niger*
TPPTU	10	10	10
$[Mn(TPPTU)_2(H_2O)_2]Cl_2$	25	20	10
$[Co(TPPTU)_2(H_2O)_2]Cl_2$	15	15	12
$[Ni(TPPTU)_2(H_2O)_2]Cl_2$	10	25	14
$[Cu(TPPTU)_2(H_2O)_2]Cl_2$	30	22	11
$[Zn(TPPTU)_2(H_2O)_2]Cl_2$	18	16	11
DMSO	-	-	-
STANDARD	30	25	10

III. RESULTS AND DISCUSSION

3.1 [1-(piperidin-1-yl(thiophen-2-yl)methyl)] thiourea (TPPTU) (a)

IR (KBr, cm^{-1}) 3444 (-NH Symm.str), 2950(CH Aromtic.str), 1645(N-H bending), 1150(C=S), 1103(C-N-C). **^{1}H** NMR **(500 MHz, $CDCl_3$ δ ppm)** 10.07 (s, 2H, NH_2), 7.79-7.20 (m, 3H, Ar-H), 5.45 (s, 1H, methine) 3.89 (s,1H, NH-(CO), 2.80-2.30(m, 8H, **piperidine**). **^{13}C NMR (**100 MHz, $CDCl_3$ **δ ppm)**. 183(C =S), 144 142, 135 & 128 (Thiophene ring), 85 (C-H), 50, 47, 27, 26, & 24 (piperidine), MS(EI) m/z: (%) 255, (M^+ +1) 256.14, 240, 185, 66

Characterization of complexes

Physical and analytical data of the complexes are presented in Table - 1. Molar conductivity values indicate the electrolytic nature of all the complexes.

IR spectra

The IR spectral data of the ligand and its metal complexes are presented in Table – 3, and the spectra are shown in fig 1. The IR spectrum of ligand was compared with the IR spectra of the complexes to indentify the coordination sites of the ligand. The IR spectrum of ligand showed a characteristic sharp band at 3412 cm^{-1} and 1645 cm^{-1} can be attributed to the ν (NH) stretching and bending ν (C=N) (amide II) observed, In the spectra of the complexes ν (C=S) mode of the free ligand is not absorbed indicating the enolisation of C=S followed by deprotonation. The ν (C=N) mode of the ligand appeared at 1645 cm^{-1} in the spectrum of the ligand has been found shifted to lower wave numbers in the spectra of the complexes indicate the involvement of nitrogen atom of the azomethane in binding with the metal ion. The band appeared at 1150 cm^{-1} due to ν(C-S-C) of thiophene moiety of the ligand has been shifted to lower frequency by 20 – 30 cm^{-1} in the spectrum of each complex corroborating the coordination of sulphur atom of thiophene with the metal ion. The stretching ν(NH) of the ligand is not much altered in the spectra of the complexes indicate the non participation of nitrogen atom of NH_2 .Hence it is concluded that the compound TPPTU act as a neutral bidentate ligand. Further all the complexes exhibits bands around 680 – 640 and 573 – 528 cm^{-1} which are assignable to ν (M – N) and ν (M – S) respectively.

Table – 3 IR - SPECTRAL DATA OF TPPTU AND ITS COMPLEXES

Compounds	*v (NH)*	*v(CH)*	*v(C=N)*	*v(C-S-C)*	*v(M-S)*	*v(M-N)*
TPPTU	3412	2950	1645	1150	-----	----
$[Mn(TPPTU)_2(H_2O)_2]Cl_2$	3409	2950	1630	1131	513	683
$[Co(TPPTU)_2(H_2O)_2]Cl_2$	3418	2846	1615	1135	543	686
$[Ni(TPPTU)_2(H_2O)_2]Cl_2$	3411	2914	1600	1136	583	642
$[Cu(TPPTU)_2(H_2O)_2]Cl_2$	3416	2904	1604	1137	528	642
$[Zn(TPPTU)_2(H_2O)_2]Cl_2$	3405	2811	1604	1134	552	652

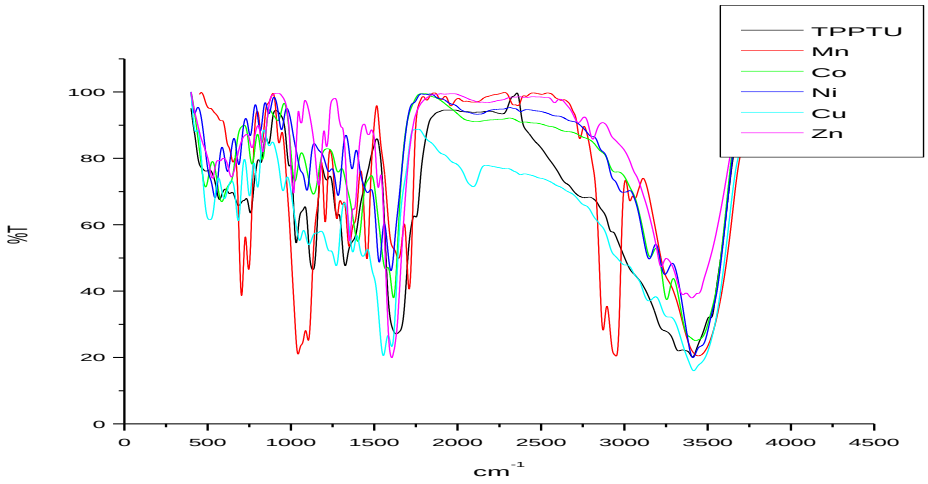


Fig : 1 IR spectra of ligand and complexes

UV – visible spectra

The electronic spectra of the ligand and its complexes were recorded in DMSO solution, the results are shown in Table - 5 and the spectra are shown in the fig 2.The electronic spectrum of Mn (II) complex gives two distinct transitions at 24271 cm^{-1} and 19271 cm^{-1} which are assignable to 6A1g→4Eg and 6A1g→4T1g transitions respectively, which are typical of Mn(II) complexes with an octahedral coordination geometry[19]. For Co (II) complex, two distinct bands appeared at 26,455 cm^{-1} and 15,510 cm^{-1} are assignable to $^4T_{1g} \rightarrow {}^4T_{1g}$ (P) and $^4T_{1g} \rightarrow {}^4A_{2g}$ transitions respectively. These absorptions favor octahedral geometry. The electronic spectrum of Ni (II) complex display two moderately intense bands with maxima centered in the region 12,432 – 12,875 cm^{-1} and 27,397 cm^{-1}. Which are assignable to $^3A_{2g} \rightarrow {}^3T_{1g}$ and $^3A_{2g} \rightarrow {}^3T_{1g}$ (P) transitions. These transitions suggesting octahedral geometry. The electronic spectrum of Cu (II) complex exhibit three bands in the region 11,450 – 16400 cm^{-1} , 18,250 cm^{-1} and 22,222 cm^{-1} corresponding to $^2B_{1g} \rightarrow {}^2A_{1g}$, $^2B_{1g} \rightarrow {}^2B_{2g}$ and $^2B_{1g} \rightarrow {}^2E_g$ respectively which are in good agreement with the distorted octahedral geometry. The Zn (II) complex is diamagnetic and the electronic spectrum of the complex is dominated only by ligand bands.

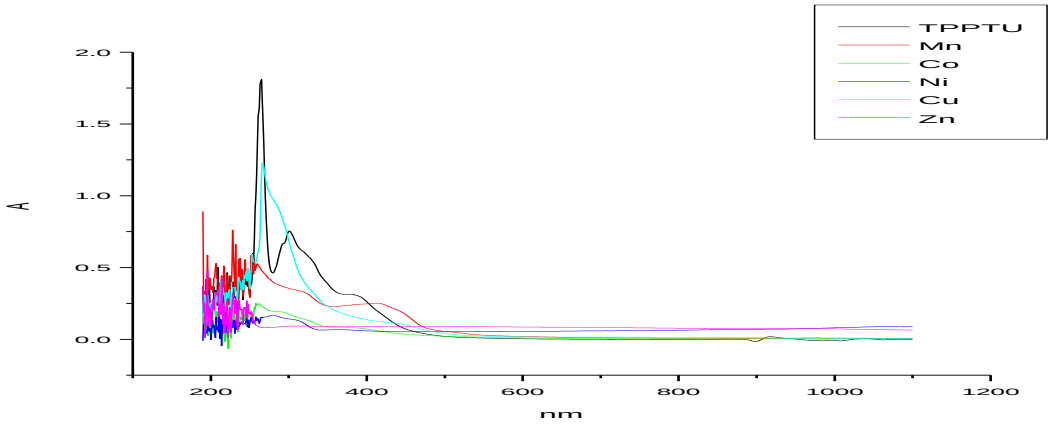


Fig: 2 UV spectra of ligand and complexes

^{1}H NMR Spectra

^{1}H NMR Spectra of the ligand and its zinc complex were recorded in $CDCl_3$ solution and the spectra are shown in fig 3. The ligand showed a singlet at δ 10.07 is assigned to NH_2 of thiourea moiety. A multiplet between δ 6.80 –7.76 is due to aromatic proton. A singlet appeared at δ 3.89 is assigned to methine proton. A multiplet at δ 2.80 – 1.60 is assigned to N – CH_2 and CH_2 protons of piperidine. A singlet appeared at δ 1.50 is attributed to –N = C – SH proton. In the spectra of the complex the multiplet δ 6.80 –7.76 is shifted to downfield confirms the participations of sulphur atom of thiophene. The signal due to SH proton of the ligand has been found shifted to downfield corroborating the involvement of nitrogen atom of azomethine. The signal appeared at δ 10.07 in the ligand is present in the complex also indicating the non involvement of nitrogen atom of the terminal NH_2 in complex formation.

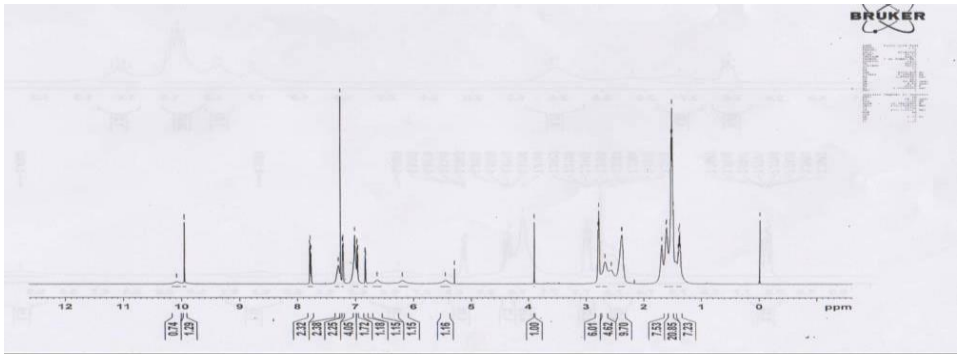


Fig : 3 ^{1}H NMR Spectrum of TPPTU.

^{3}C NMR spectra

^{13}C NMR spectrum of TPPTU is shown in the figure. The spectrum showed signals at 25.07, 25.31, 25.43, 49.87 and 50.38 are assigned to 2,3,4,1 and 1^{1} respectively. A signal appeared at 85.02 is due to methine carbon(C - 9). The signals appeared at 124.05, 125.62, 125.77 and 135.15 are assigned to C - 6, 7, 5 and 8 respectively. A signal appeared at 183.00 is assigned to azomethine carbon (C - 10).

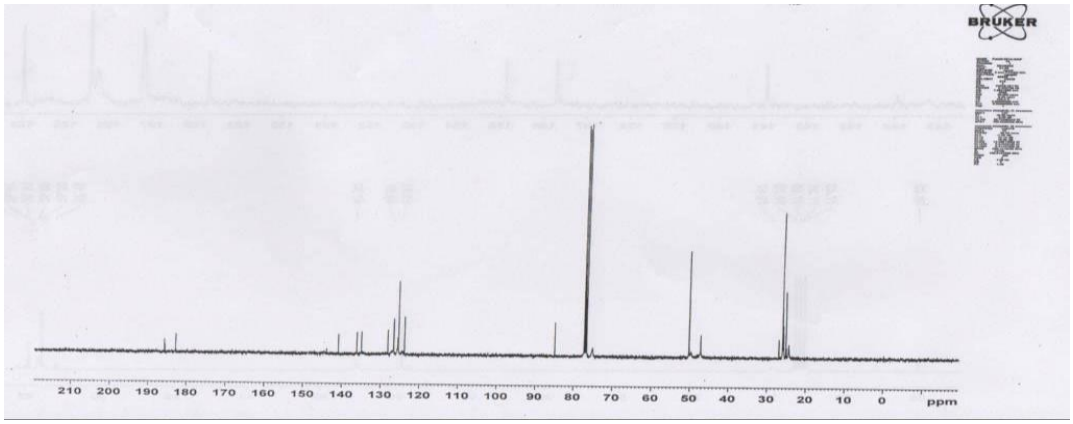


Fig – 4 ^{13}C NMR spectrum of TPPTU

Based on the above data, the following structure has been proposed for the metal complexes.

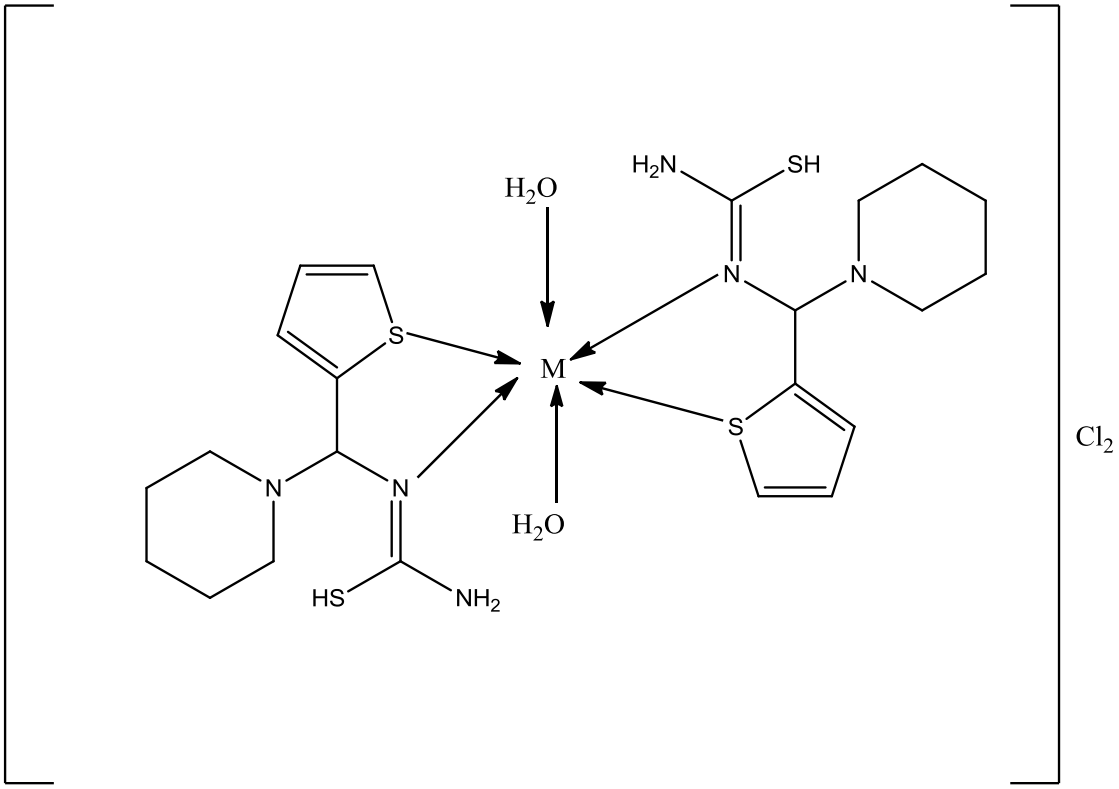


Fig: 5 General structure of the complexes

Antimicrobial studies

The ligand and complexes were screened for their invitro antibacterial and antifungal activities by disc diffusion method. The activities were done at 100 µg/mL concentration DMSO solvent using *S. aureus, E.coli and A.niger*. The standard drugs used are Gentamycin for bacteria and Amphotericin - P for fungi. The minimum inhibitory concentration (MIC) values are presented in the Table -2. The comparison of activity of the ligand and the complexes is given in the fig 1. From the table - 2 it has been observed that Cu (II) complex posses equivalent activity against *S.aureus* and moderate activity against *E.coli* when compared with their standards. Ni(II) complex posses equivalent activity against *E.coli* and highest activity against *A.niger*. In general complexes possess more activity than the free ligand, the is may be explained on the basis of Overtone's concept of cell permeability, the ligand membrane that surrounds the cell favours the passage of only lipid – soluble materials which control the antimicrobial activity. On chelation, the polarity of the metal ion reduces considerably. This is due to overlap of the ligand orbital and the partial sharing of the positive charge of the metal ion with donor groups. Further it increases the delocalization of π electrons over the whole chelate

ring and enhances the lipophilic character of the metal complexes. This increased lipophilicity leads to breakdown of permeability barrier and permits the complexes to penetrate in to the lipid membrane.

IV. Conclusion

In summary, five complexes have been prepared using Mannich base, [1-(piperidin-1-yl(thiophen-2-yl)methyl)] thiourea (TPPTU) and characterized by physical and spectral methods. The metal ions were coordinated through the sulphur atom of thiophene and the nitrogen of the azomethine group. The binding of ligand to metal ions was confirmed by the analytical data, as well as spectral and magnetic studies. For all the complexes octahedral geometry have been proposed based on the analytical and spectra data. Some metal complexes were found to possess higher antibacterial and antifungal activities and some were moderate activities than the ligand.

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