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RESEARCH ARTICLE

BIOLOGICAL ACTIVITY OF TRANSITION METAL COMPLEXES OF 4- FORMYLPYRIDINE THIOSEMICARBAZONE; EQUILIBRIUM STUDIES

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ABSTRACT

Metal complexes of 4-Formylpyridinethiosemicarbazone, H4FPT (L) with Zn(II), Cd(II) and Hg(II) were prepared and characterized by various spectro-analytical techniques such as elemental analyses, molar conductance, LC-MS, TGA, IR and ^{1}H-NMR. Elemental analyses and LC-MS studies reveal the composition as ML_2 for all the complexes. Equilibrium studies were carried out in 70% v/v DMF-water medium to calculate dissociation constant of H4FPT and the stability constants of complexes with Zn(II) and Cd(II) ions in solution. H4FPT acts as monobasic ligand with pK_a value of 11.60. Stability constants of 1:1 and 1:2 complexes of M(II)-H4FPT followed Irving-William order of stability constants. H4FPT and its complexes showed moderate to good activity against gram positive and gram negative bacterial strains.

INTRODUCTION

Transition metal complexes of thiosemicarbazones are of considerable interest in research field due to their pharmacological activity such as antitumor, antifungal, antibacterial, antiviral, anticancer and other properties (Nfor *et al*, 2011; Elsayed *et al*, 2010; Wiecek *et al*, 2010; Chandra *et al*, 2008). They coordinate to metal either in neutral form or anionic form and are bidentate or tridentate ligands. Medicinal importance of metal complexes of 2-formylpyridine thiosemicarbazone is extensively studied as compared to its 4-isomer. In recent years platinum and palladium complexes of 4-formylpyridinethiosemicarbazone were reported (Mendes *et al*, 1999). Owing to greater biological activity of metal complexes over ligands, it has been planned to synthesize transition metal complexes of 4-formylpyridine thiosemicarbazone. Hence knowledge of acid dissociation constant of the ligand and evaluation of stability constants of complexes is essential to plan synthesis of metal complexes. This communication reports synthesis of 4-Formylpyridine thiosemicarbazone (H4FPT, L) with a modified procedure. Synthesis, characterization and anti bacterial activity of novel binary metal complexes of H4FPT with IIB metal ions has been reported. Determination of proton dissociation constant of H4FPT and stability constant of its complexes with Zn(II), Cd(II) ions in solution has been carried out for the first time.

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EXPERIMENTAL

MATERIALS AND METHODS

All the chemicals used were of AR grade (Sigma-Aldrich). A digital Elico (L1-120) pH meter with a combined glass and calomel electrode was used for equilibrium studies. pH meter was calibrated using 4.0, 7.0 and 9.2 buffers. LC-MS of the ligand and its complexes were recorded on LCMS 2010A, Shimadzu spectrometer, elemental analysis was done on Thermo Finnigan/Eager 300 for EA 11120. Molar conductivity of the complexes ($1x10^{-3}$M) was measured using Digisun model 909 digital conductivity meter. Thermo gravimetric analyses of the complexes were carried on TG balance TA (Q/50) in the temperature range of 0 to 1000°C with a ramp of 20°C per min. IR spectra in KBr were recorded on Schimadzu (Prestige-21) FTIR spectrometer. ^{1}H-NMR (with D_2O exchange) and ^{13}C-NMR were recorded on Varian 400MHz NMR spectrometer and UV spectra in DMSO were recorded on Schimadzu UV 2450 spectrophotometer. Anti bacterial

activity was studied following disc diffusion method in sterile nutrient agar medium.

Synthesis of 4-Formylpyridinethiosemicarbazone (L)

H4FPT (Figure 1) was synthesized by stirring equimolar (0.01M) solutions of thiosemicarbazide in hot water and 4-Formylpyridine at room temperature for one hour. The progress of the reaction was monitored by TLC. Shiny cream coloured product formed was filtered, washed with water, dried and recrystallised from 1:1 ethanol and water.

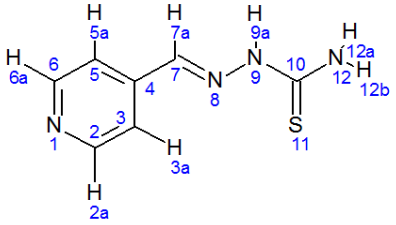

Figure 1. 4-Formylpyridinethiosemicarbazone

H4FPT: Shiny cream solid. Yield: 85%. m.p: 228-230°C. Analysis: Calculated (%) for $C_7H_8N_4S$: C, 46.6; H, 4.43; N, 31.06. Found (%): C, 46.82; H, 4.58; N, 30.91. APCI-MS(+): m/z 181 $[M+1]^+$, 121 $[C_6H_6N_3]^+$, 78 $[C_5H_4N]^+$, 61 $[CSNH_2]^+$. IR (KBr/cm^{-1}): 827, 991, 1294, 1598, 3151, 3261, 3419. ^{1}H NMR (400 MHz, DMSO-d_6, δ/ppm): 7.7 [2H, d, aromatic H (3a,5a)], 8.5 [2H, d, aromatic H (2a, 6a)], 7.9 [1H, s, H-C=N], 8.2 [1H, s, N-CS-NH, D_2O exchangeable], 8.4 [1H, s, N-CS-NH, D_2O exchangeable], 11.6 [1H, s, N-N-H, D_2O exchangeable]. ^{13}C NMR (DMSO-d_6, δ/ppm): 121.5 (C_3, C_5), 139.9 (C_4), 141.8 (C_2, C_6), 150.4 (C_7), 178.9 (C_{10}). UV-visible in DMSO, λ_{max}/nm: 331, 252.

Synthesis of metal complexes

To the hot methanolic solution of the ligand, aqueous metal salt MCl_2 [M= Cd(II), Hg(II)], $Zn(OAc)_2$ solutions in 1:2 (M:L) molar ratio were added. Mixtures were refluxed for 8 to 10hours. pH of the solutions were adjusted by addition of few drops of methanolic ammonium hydroxide solution. Solid complexes formed were filtered under hot condition, washed with hot methanol, water and with petroleum ether and finally dried in vacuum (Sireesha *et al*, 2006, 2016).

[Zn(H4FPT)$_2$(H$_2$O)$_2$](OAc)$_2$ (1)

Yellow solid. M.p: Dp > 300°C. Analysis: Calculated (%) for $C_{18}H_{26}ZnN_8S_2O_6$: C, 37.25; H, 4.48; N, 19.31. Found (%): C, 37.45; H, 4.62; N, 19.51. APCI-MS: m/z 581 $[M+1]^+$. IR (KBr/cm^{-1}): 825, 1024, 1290, 1614, 3180, 3286, 3420. ^{1}H NMR (400MHz, DMSO-d_6, δ/ppm): 7.7 [2H, d, aromatic H (3a,5a)], 8.5 [2H, d, aromatic H (2a, 6a)], 7.97 [1H, s, H-C=N], 8.2 [1H, s, N-CS-NH, D_2O exchangeable], 8.4 [1H, s, N-CS-NH, D_2O exchangeable], 11.6 [1H, s, N-N-H, D_2O exchangeable]. Conductivity in DMSO (Λ_M):183S cm^{2}M ^{1}.

[Cd(4FPT)$_2$(H$_2$O)$_2$] (2)

Yellow solid. M.p: Dp > 300°C. Analysis: Calculated (%) for $C_{14}H_{18}Cd\,N_8S_2O_2$: C, 33.14; H, 3.55; N, 22.09. Found (%): C, 33.71; H, 3.31; N, 22.56. APCI-MS: m/z 507 $[M+1]^+$. IR (KBr/cm^{-1}): 821, 1012, 1282, 1575, 1612, 3309 and 3437. ^{1}H NMR (400MHz, DMSO-d_6, δ/ppm): 7.68 [2H, d, aromatic H (3a,5a) br], 8.5 [2H, d, aromatic H (2a, 6a) br], 8.0 [1H, s, H-C=N], 8.5[1H, s, N-CS-NH, D_2O exchangeable], 7.68 [1H, s, N-CS-NH, D_2O exchangeable].

[Hg(4FPT)$_2$(H$_2$O)$_2$] (3)

Grey solid. M.p: Dp > 300°C. Analysis: Calculated (%) for $C_{14}H_{18}Hg\,N_8S_2O_2$: C, 28.23; H, 3.02; N, 18.82. Found (%): C, 28.85; H, 2.93; N, 19.12. APCI-MS: m/z 596 $[M+1]^+$. IR (KBr/cm^{-1}): 810, 995, 1597, 1604, 3242 and 3417. ^{1}H NMR (400MHz, DMSO-d_6, δ/ppm): 7.7 [2H, d, aromatic H (3a,5a) br], 8.5 [2H, d, aromatic H (2a, 6a) br], 7.9 [1H, s, H-C=N], 8.5[1H, s, N-CS-NH, D_2O exchangeable], 8.3 [1H, s, N-CS-NH, D_2O exchangeable].

Equilibrium Studies

The Zn(II) and Cd(II) metal ion solutions were prepared in double distilled water using corresponding AR grade metal nitrates and were standardized by known methods (Vogel 1997). pH-metric titrations were carried in 70% v/v DMF-water medium at 303K and ionic strength 0.1M KNO_3. Proton-ligand dissociation constant and formation constants of binary chelates have been determined by Irving Rossotti titration technique (Sireesha *et.al*, 2006; Irving *et.al*, 1954).

Biological Activity

Biological activity of all the complexes was tested by disc diffusion method (Chandra *et al*, 2009). 0.10mL of test bacteria [gram positive-Staphylococcus aureus, Bacillus subtilis and gram negative-Escherichia coli, Klebsiella pneumonia] was spread over the surface of nutrient agar. Sterile discs of 5mm diameter dipped in DMSO solutions of test samples are placed at equidistance. The potency of all the samples tested was 1000µg/disc. Capacity of the disc is 5µL of the sample. DMSO was taken as control, which has no antibacterial activity. Gentamycin was used as standard. Zone of inhibition was recorded after incubation for 24 hrs at 37°C. All these tests were made in triplicate and are averaged.

RESULTS AND DISCUSSION

Characterization of metal complexes

All the complexes synthesized are coloured, microcrystalline and stable. Complexes are soluble in DMSO and DMF. Elemental analyses data and mass spectral information revealed the formation of 1:2 (M:L) complexes. Molar conductivity measurements were recorded in 1x10^{-3}M DMSO solutions at room temperature. High molar conductance value (183Scm^{2}M ^{1}) for complex 1 suggests its electrolytic nature (Sakthilatha *et al*, 2013).

Liquid chromatograms

All the metal complexes have single peak with retention time in a range of 0.550 to 0.656min indicating their purity.

Mass spectra

Analysis of APCI (+) MS of complex 1 identified $[M+1]^+$ ion peak at m/z 581. 1:2(M:L) can be established from the peak at

m/z 427 and a small peak at m/z 361 indicate presence of two ligand moieties. APCI (+) MS of **2** showed $[M+1]^+$ peak at m/z 507, peak at m/z 472 is due to 1:2 (M:L) and a small peak at m/z 359 indicate presence of two deprotonated ligand moieties. APCI (+) MS of **3** showed $[M+1]^+$ peak at m/z 596, 1:2 metal to ligand peak at m/z 561 and a small peak at m/z 359 indicate presence of two deprotonated ligand moieties.

Thermogravimetric analysis

Thermogram of complex 1 showed three steps decomposition. Gradual weight loss from 100 to 300°C (7.4%) is due to decomposition of acetate ions and coordinated water. 65.73% of residue at 791°C revealed incomplete decomposition of the complex indicating its thermal stability. Thermogram of 2 indicated loss of coordinated water at 235 to 290°C (6%) and the decomposition steps at 290 to 315°C (18%), 315 to 800°C (36%) and 800 to 940°C (32.5%) suggested gradual decomposition of the complex followed by decomposition of metal. Thermogram of 3 revealed decomposition in three steps. Weight loss between 175 to 200°C (6%) is due to loss of coordinated water, gradual decomposition up to 550°C (81%) and sudden weight loss till 940°C (8%) indicate decomposition of both ligand and metal moieties.

IR spectra

A broad trough in the range of υ3100 to υ3600cm^{-1} reveals the presence of coordinated water molecules in all the complexes, which is also supported by thermo gravimetric analyses. The υ(C=N) in H4FPT at 1598cm^{-1} shifted to lower frequency (1575-1597cm^{-1}) in complexes 2 and 3 suggesting the coordination of azomethine nitrogen (West *et al*, 1999; Kanagaraj *et al*, 1993). However, an increase in frequency of complex 1 may be attributed to back bonding from metal to ligand. υ(N-CS-N) and υ(C=S) in the ligand shifted to lower frequency or found missing in the complexes suggesting the coordination of thiol or thiolate sulphur (Beraldo *et al*, 2001; de Lima *et al*, 1999). From the Far IR region of the spectra, there is evidence for the presence of υ(M-N), υ(M-S), υ(M-Cl) and υ(M-OH_2) bands in the complexes. Analysis of IR spectral data (table 1) shows that the ligand is bidentate with azomethine nitrogen and thione/thiolate sulphur as potential donor sites forming five membered chelates with the metal ions.

Table 1. IR Spectral data (KBr, cm^{-1}) of H4FPT and complexes

Compound	$\upsilon_{C=N}$	υ_{N-H}	υ_{N-N}	N-CS-N	$\upsilon_{C=S}$
L	1598	3151	991	1294	827
Complex 1	1614	3180	1024	1290	825
Complex 2	1612,1575	----	1012	1282	821
Complex 3	1604-1597	----	995	----	810

^{1}H NMR spectra

In ^{1}H-NMR spectra of complexes 2 and 3, peak at δ11.66ppm that belongs to hydrazine proton in the spectrum of ligand is absent. The characteristic signal of thiol proton at 4ppm is also absent revealing the coordination of thiolate sulphur to metal ions. NH_2 peaks of the ligand had shifted downfield in all the complexes due to electron withdrawal of sulphur (Elsayed *et al*, 2010). The corresponding peaks are observed to merge with α and β protons of pyridine ring in 2 and with α-H of the pyridine ring in 3, which is evidenced by the higher integration of the band and also supported by D_2O exchange studies (Bhargavi *et al*, 2002). From the analysis of results of equilibrium studies and various spectro analytical data of complexes, the tentative structures proposed are given in Figure 2.

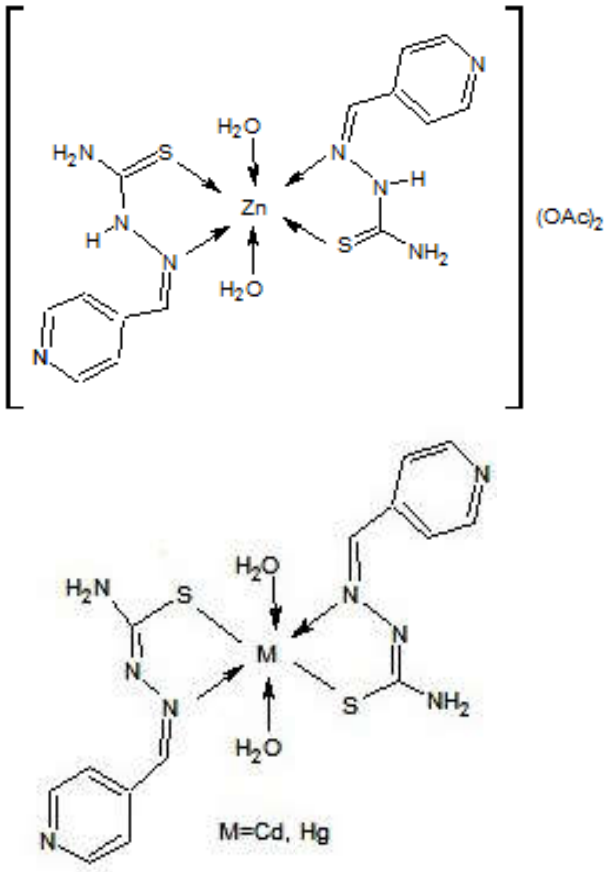

Figure 2. Proposed structures of complexes

Equilibrium studies

To understand the chelation ability of H4FPT, potentiometric titration of the following sets of solutions (50 mL) were carried out by Irving Rossotti titration technique. (i) HNO_3 ($4.0x10^{-3}$M) (ii) HNO_3 ($4.0x10^{-3}$M) + H4FPT ($1.0x10^{-3}$M) and (iii) HNO_3 ($4.0x10^{-3}$M) + H4FPT ($1.0x10^{-3}$M) + M(II) ion ($2.0x10^{-4}$M) against 0.1M NaOH solution in 70% v/v DMF-Water medium at 303K and 0.1M KNO_3 ionic strength. The titration curves are presented in Figure 3. Dissociation constant (pK_a) was computed from the linear plots of log [(1- n_A)/ n_A] vs pH. From the results, it is evident that the ligand has one dissociable proton, with pK_a value 11.60 corresponding to hydrazine proton. Ligand can undergo thione-thiol tautomerism and the dissociation of proton occurs through thiol-1 form (Figure 4). This indicates monobasic nature of the ligand. Values of n varies from 0.4 to1.9 indicate formation of 1:1 and 1:2 complexes in solution. The stability constants of binary complexes were calculated from linear plots of log [(1-n)/ n] Vs pL and log [(2- n)/(n -1)] Vs pL (figures 5 and 6) and they were also calculated by using BEST computational programming. The values obtained for logK_1, log K_2 and log β_2 are presented in Table 2.

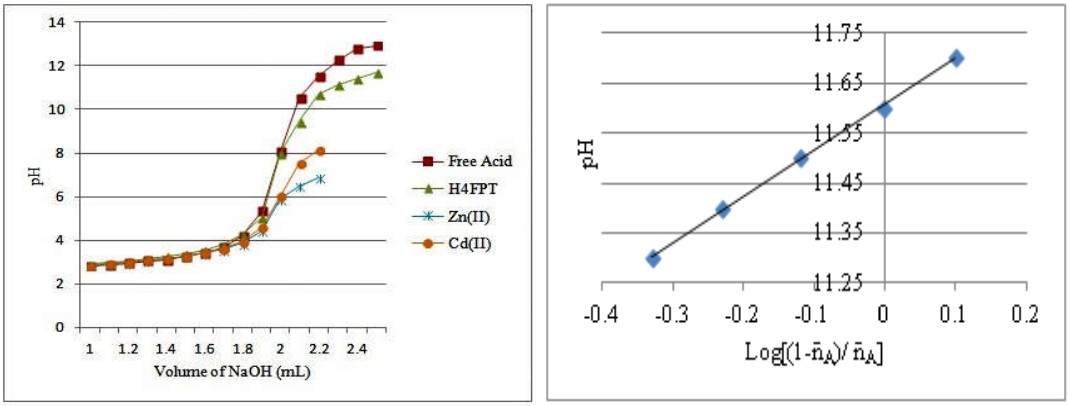

Figure 3. pH titration curves of H4FPT and Linear Plot of Log [(1- n $_A$)/ n $_A$] Vs pH of H4FPT

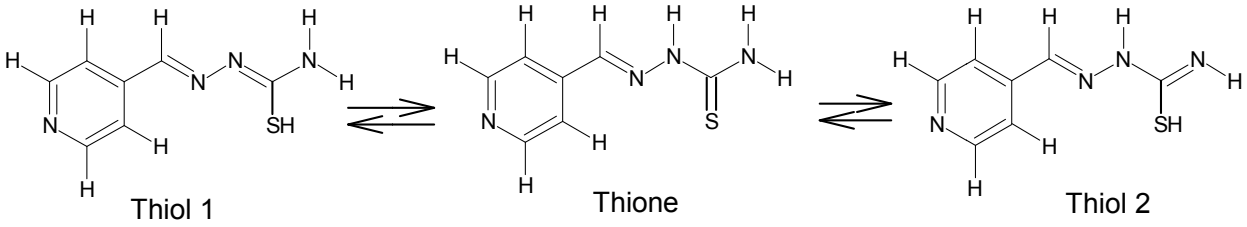

Figure 4. Thione and Thiol forms of the H4FPT

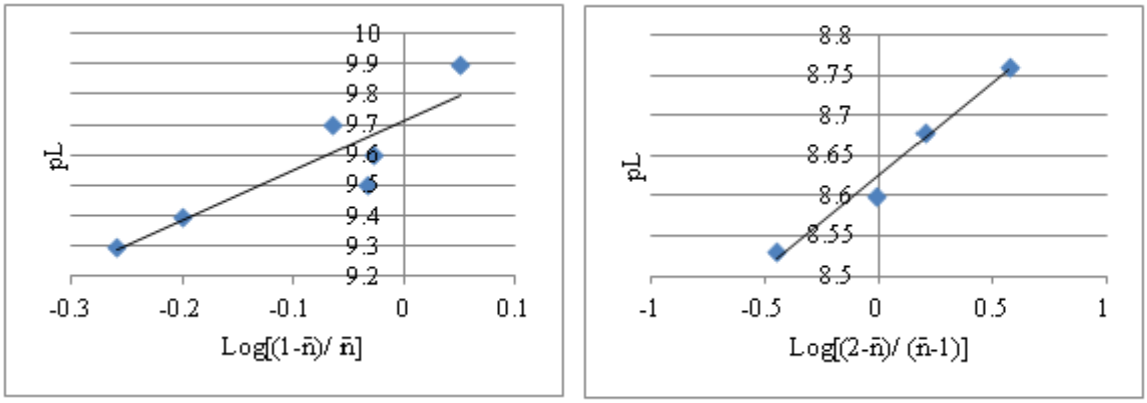

Figure 5. Linear Plots of Log [(1- n)/ n] Vs pL and Log [(2- n)/ n -1)] Vs pL of complex (2)

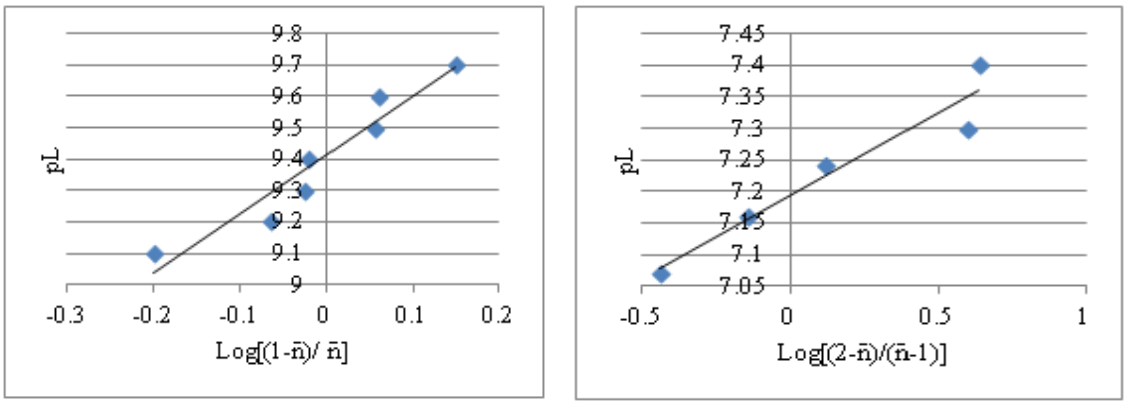

Figure 6. Linear Plots of Log [(1- n)/ n] Vs pL and Log [(2- n)/ n -1)] Vs pL of complex (3)

Table 2. Dissociation and stability constants* for M(II)-H4FPT in 70% v/v DMF-water medium and 0.1M ionic strength

Ligand/Metal	pK_a/ Log K_1	Log K_2	Logβ_2
H4FPT	11.60	----	----
Zn(II)	9.72	8.63	18.35
Cd(II)	9.41	7.18	16.59

*Units: K_1, K_2, β_1-mol^{-1}L; β_2-mol^{-2}L^2

Log β_2 values of M(II)-H4FPT follow the order Zn(II)>Cd(II) in accordance to Irving-William order of stability constants (Zewdu *et al*, 2015). The greater stability constant of Zn complex can be attributed to high charge to radius ratio of zinc as compared to cadmium. Equilibrium studies of H4FPT with Zn(II) and Cd(II) metal ions also support the formation of 1:2 (M:L) complexes.

Antibacterial studies

Activity of H4FPT and its complexes have been tested on both gram positive and gram negative bacteria (Table 3). The ligand and complex 2 are found to inhibit the growth of only gram positive bacteria under study. Complex 1 is active against gram negative bacteria. Complex 3 is active against both types It is observed that all the complexes exhibit either equal or more activity than ligand. This can be attributed to the chelating capacity of the metal to ligand. Metal atom partially shares its positive charge with the donor atoms of the ligand leading to delocalization of π electron cloud over the chelating ring. Due to this the lipophilic character of the metal gets enhanced and favours its permeability into bacterial cell membranes and inhibits the growth of the bacteria (Chandra *et. al*, 2009).

Table 3. Anti Bacterial Activity of ligand and complexes

Compound.	S.aureus	B.subtilis	E. coli	K.pneumoniae
L	6 mm	7mm	----	----
Complex 1	----	----	6mm	6mm
Complex 2	6mm	15mm	----	----
Complex 3	9mm	10mm	8mm	8mm

Conclusion

4-Formylpyridinethiosemicarbazone acts as bidentate ligand with azomethine nitrogen and thione/thiol sulphur as potential donor sites forming 1:2 mononuclear complexes with distorted octahedral geometry. Equilibrium studies reveal that H4FPT acts as monobasic ligand releasing a proton from hydrazine nitrogen and form stable 1:1 and 1:2 complexes in solution. H4FPT and its IIB metal complexes show moderate to good activity against bacteria under study.

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