Synthesis, Characterization and Evaluation of Biological Activities of 4'-(Ferrocen-2-yl)-2, 2':6', 2''-terpyridine and its Metal Complex

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ABSTRACT

In the present investigation, 4'-(Ferrocen-2-yl)-2, 2':6', 2''-terpyridine ligand and its metal complex have been synthesized and spectroscopically characterized. It can be concluded from the results the ligand act as bidentate chelating agent, coordinates with transition metal ion to give octahedral environment. The ligand and its metal complex exhibited anticancer activity against breast cancer (MCF-7) cell lines. Using disk diffusion methods the ligand and its complex was evaluated invitro as antimicrobial agents against representative strains of two gram- positive ((*S.aureus, B.subtilis*) and two gram- negative (*K.aerogenes* , *E.coli*) and as an antifungal agents against *A.niger* , *C.albicans*. All the bacteria and fungus studied were screened against some antibiotics to compare with our chemical zone diameters. The antioxidant activity of the ligand and its metal complex was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. The results indicate that the ligand and its metal complex possess biological properties.

Key words: transition metal, complex, spectra, MTT assay, DPPH

INTRODUCTION

Presently there is a growing interest in the coordination chemistry of structurally modified bio – ligands. Transition metal complexes with pontential biological activity are the focus of extensive investigations. More than forty years after its serendipitous discovery [1] in 1951, ferrocene still enjoys a great deal of interest from scientists in many areas of research. Moreover ferrocene conjugates are stable in biological media, liphophilic and have unique redox property [2-3]. The molecule ferrocene is non-toxic but the ferrocenium ion produced is found to be toxic in various cancer cell lines [4]. Ferrocifen, a hormone independent chemotherapeutic agent containing ferrocene moiety is found to be more effective than tamoxifen which is a hormone dependent anticancer drug. Both ferocifen and tamoxifen are used in the treatment of breast cancer [5-6]. Medicinal application of ferrocenyl conjugates is currently an active area of research with many reports showing their activities as antitumour, antimalarial and antifungal agents [7]. Cis – platin the first discovered anticancer drug has toxic side effects such as nephrotoxicity. But by indroducing ferrocenyl moiety in the metal

complexes, the antitumor activity may be increased. Infact, ferrocenyl moiety containing metal chelates are multinuclear molecules possessing features of both organo-metallic and coordination chemistry [8]. Hence a study of metal complex and biological activities of terpyridine system substituted with ferrocenyl moiety at 4' – position has been attempted.

PHYSICAL PARAMETERS

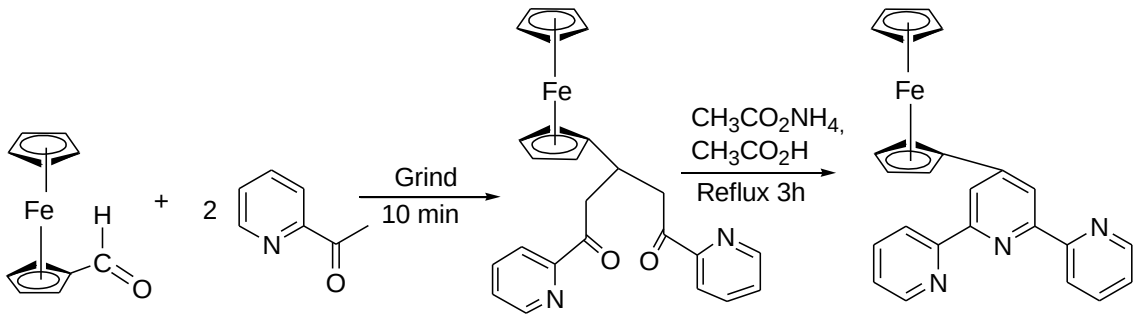
C, H and N were estimated by using Elemental Vario EL III CHNS/O elemental analyzer. The FT-IR and UV-vis spectra of fctpy(L1) and its metal complex was recorded as KBR pellets in the range of 400-4000 cm^{-1} region using a Shimadzu FT-IR 8000 spectrophotometer and Perkin Elmer lambda 35 Uv-vis spectrophotometer in the region of 200-800 nm respectively. The ESI-MS spectra of the fctpy and its metal complex was recorded in Finnigan LCQ 6000 advantage max ion trap mass spectrometer equipped with an electron spray source. The magnetic moment was carried out at room temperature by using a Gouy magnetic balance. Molar conductance was measured by DMF solutions at room temperature using a digital conductivity bridge, systronics direct reading conductivity meter 304 with a dip type conductivity cell. Cyclic voltammetric studies of the complex was carried out by using three electrode systems in a single compartment comprising of glassy-carbon working electrode and potentials were referenced to standard calomel electrode.

MATERIALS AND METHODS

2-acetyl pyridine, hydrated metal (II) perchlorate and ferrocen-2-carbaldehyde are procured from Sigma Aldrich, USA and used as received. Other materials like sodium hydroxide, ammonium acetate and solvents like methanol, acetonitrile, ethanol, diethyl ether, and glacial acetic acid were of reagent grade.

SYNTHESIS OF 4'-(FERROCEN-2-YL)-2,2':6',2''-TERPYRIDINE -L1

4'-(Ferrocen-2-yl)- 2, 2': 6', 2''-terpyridine (fctpy) was prepared[9] (Scheme 1) by 2-Acetylpyridine and ferrocen-2-carbaldehyde were interacted by intimately grinding them in a mortar and pestle. The grinding was continued for 10 minutes for the formation of an orange red powder. The product was suspended in a mixture of ammonium acetate and glacial acetic acid and refluxed for 2h. The crude product of L1 was precipitated by adding water. The product was filtered, washed with water and then cold ethanol and dried. It was column chromatographed using silica gel and 1:1 methanol- dichloromethane system.

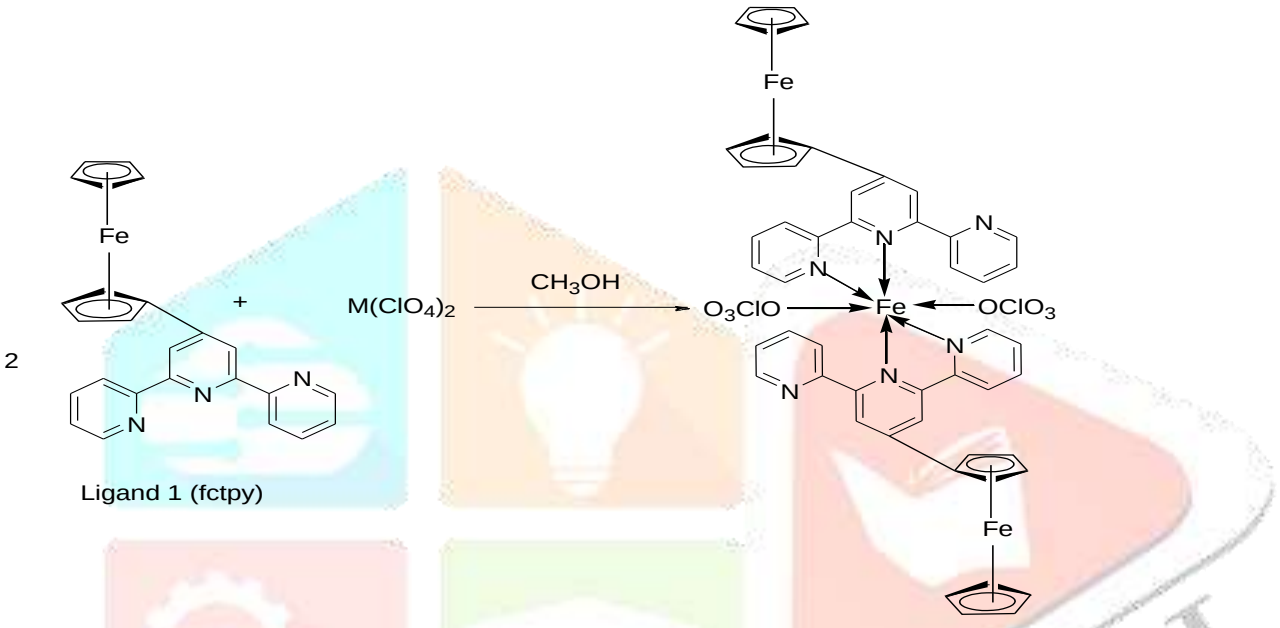


fctpy (L1)

Scheme 1. Synthesis of ferrocen-2-yl terpyridine (Ligand 1)

SYNTHESIS OF COMPLEX L1

Complex 1: Hot methanolic solution of hydrated $Fe(ClO_4)_2$ and the methanolic solution of fctpy were mixed and the reaction mixture was stirred for 2 hours. A violet coloured precipitate crystallized from the deep green solution on slow evaporation of solvent. The solid coordination complex was filtered, washed with ether and dried. The formation of metal (II) perchlorato complex with fctpy (L1) in methanolic medium is (Scheme 2) illustrated below.



Scheme 2. Synthesis of Metal Complex with fctpy

DPPH RADICAL SCAVENGING ASSAY

The free radical scavenging abilities of the terpyridyl ligand and its metal complex have been determined by their interaction with the stable free radical 2, 2'-diphenyl-1-picryl hydrazyl [10-14]. The reduction capability of DPPH induced by antioxidant has been determined by the decrease in its absorbance measured at 517 nm. The sample solution in DMSO (5, 10, 15 or 20 µL) was mixed with 1 mL of 1.5×10^{-5} M DPPH solution in ethanol so as to make the desired concentrations of 5-20 µg/mL and the mixture have been kept in dark for 30 minutes. The quantity of DPPH remaining in each mixed solution has been determined by measuring the absorbance of mixed solution at 517 nm using a spectrophotometer. The decrease in the absorbance of DPPH in the mixed solution indicates the free radical scavenging activity of the test drug. L-ascorbic acid has been used as standard and ethanol has been employed as control.

ANTICANCER ACTIVITY SCREENING- MTT ASSAY

3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) [15] is a yellow water soluble tetrazolium salt. A mitochondrial enzyme present in living cells namely succinate-dehydrogenase, cleaves the

tetrazolium ring, converting the MTT to an insoluble purple coloured formazan. Therefore, the amount of formazan produced is directly proportional to the number of viable cells present in the sample. After 48 h of incubation, 15 µL of MTT (5 mg/mL) in phosphate buffered saline (PBS) was added to each well and incubated at 37^0 C for 4h. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 µL of DMSO and then the absorbance was measured at 570 nm using a micro plate reader. Percentage cell growth inhibition was calculated using relative cell viability.

$$\textbf{\textit{Relative Cell Viability}}\ (\%) = \frac{\textbf{\textit{Absorbance of test drug}}}{\textbf{\textit{Absorbance of control}}}\ \textbf{\textit{x 100}}$$

$$\%\ \textbf{\textit{Cell Growth Inhibition}}\ =\ \textbf{100}\ -\ \textbf{\textit{Relative Cell Viability}}$$

ANTIMICROBIAL ASSAY

The *invitro* antimicrobial assay of the test drugs was tested for certain bacteria and fungi by using the Disc Diffusion Method and Mueller Hintonagar medium [16]. A loopful of strain in each was inoculated in 30 mL of the nutrient broth and incubated for 24 hours at 37°C to activate the strains. The dried surface of each agar-agar plate was swabbed with the respective suspension of the bacterial/fungal strain using a sterile cotton swab. The test drugs were dissolved in DMF to prepare stock solutions and concentrations of 100, 250 and 500 mg/mL were made by suitable dilutions. The sterilized filter paper discs were completely saturated with the test compounds and the impregnated dried discs were placed on the dried surface of inoculated agar plate. The agar plates inoculated with the bacterial organisms under test were incubated at 35°C to 37°C for 24 hours and the plates incubated with the fungi were incubated for 48 hours at the same temperature.

RESULTS AND DISCUSSION

ELEMENTAL, MASS AND ELECTRICAL CONDUCTANCE

On the basis of elemental analysis data (Table 1), the complex have the general composition ML_2, where M = Fe, L= fctpy. The recorded mass spectra of the ligand, complex and the molecular ion peaks have been used to confirm the proposed formula. The mass spectra of ligand and complex peaks attributed to the molecular ions m/z at 417.2, 1089 (Fig. 1) respectively. Conductivity value for the complex was found to be 42.40 in DMF indicating that, non electrolytic nature [17].

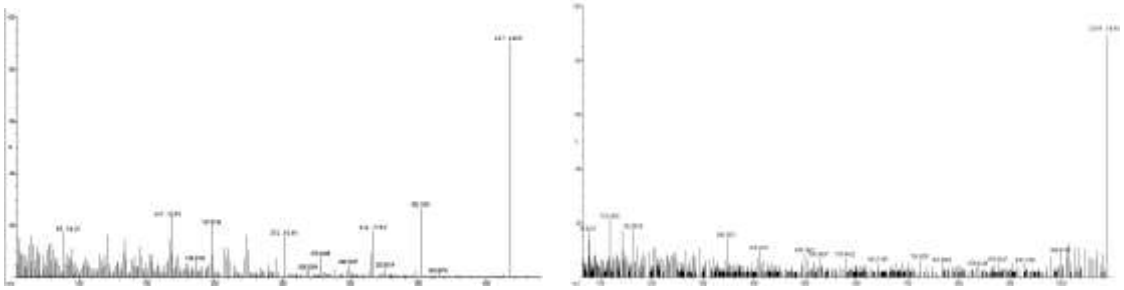


Fig 1.Mass Spectrum of L1 and Complex 1

Table 1

Elemental, Electrical Conductance and Mass Spectral Data for L1, Complex 1

Sl. No	Compound	% Found (Calculated)					λ_M (S cm^2mol^{-1})	Molecular Ion Peak (m/z)
		C	H	N	Fe	M		
1	fctpy(L1)	71.04 (71.96)	4.54 (4.56)	9.94 (10.08)	13.36 (13.40)	--	--	417.2
2	$[Fe(fctpy)_2(ClO_4)_2]$ Complex 1	55.04 (55.12)	3.46 (3.49)	7.62 (7.72)	15.36 (15.39)	--	42.40	1089

ELECTRONIC AND MAGNETIC SUSCEPTIBILITY MEASUREMENTS

The electronic absorption spectrum of the ferrocenyl terpyridine (fctpy) and its complex was recorded at 300 K in acetonitrile solutions. Electronic spectral data as well as the magnetic data of the fctpy, Fe complex are provided in Table 2. In the present study the ligand which is brown in colour shows electronic absorption at 42918 cm^{-1} and 38462 cm^{-1} which are ascribed to the π - π^* and n - π^* transitions. The absorption seen at 19084 cm^{-1} may be attributed to Fe (3d) –L(π) CT transition which may be responsible for the dark brown colour of the ligand. The complex 1 exhibits a deep violet clolour. The ligand field transition and Fe (3d) –L(π) CT transition are observed at 12590 and 19084 cm^{-1} [18-21] . The weak ligand field absorption in the red region may be assigned to the spin allowed transition of the central metal ion $^5T_{2g} \rightarrow {}^5E_g$. The strong absorption at 19084 cm^{-1} which is due to charge transfer transition produces a strong violet colour for the complex. The complex records an effective magmatic moment of 5.2 BM at room temperature [22]. This corresponds to the $t_{2g}^4\ e_g^2$ electronic configuration of the central metal ion . It should be noted here that the ferrocenyl iron is diamagnetic. Hence, based on experimental results, Complex 1 is assigned a high spin octahedral geometry.

Table 2

Magnetic and Electronic Spectral Data for Fe ^{II} Complex with fctpy (L1)

Sl. No	Compound	Colour	μ_{eff} (BM)	Absorption Maxima (cm^{-1})	Transition Assignments
1	Fctpy(L1)	dark brown	-	42918 38462 19084	π - π^* and n - π^* Fe (3d) –L(π) CT
2	$[Fe(fctpy)_2(ClO_4)_2]$ Complex 1	Violet	5.2	12590 19084	$^5T_{2g} \rightarrow {}^5E_g$ Fe (3d) – L (π) CT

IR SPECTRA AND MODE OF BONDING

The ir spectrum of the ligand and its metal complex was carried out in the range of 4000-200 cm^{-1}. In the IR spectrum of Complex 1(Fig. 2), the ring skeletal vibrations (Table 3) which originate from the heterocyclic rings appear at 1550 – 1452 cm^{-1} where as in L1 spectrum (Fig. 2) they are located at (Table 3)1562 – 1462 cm^{-1}. Thus from the appreciable positive shifting of the ring skeletal vibrations [23] . The pyridine ring nitrogen coordination is suggested. The in-plane pyridine ring deformation located at 644 cm^{-1} in the ligand spectrum has shifted to a higher value of 694 cm^{-1} in the complex indicating the pyridyl nitrogen binding. Also the out-of- plane ring deformation of L1 found at 459 cm^{-1} has experienced a positive shift to 476 cm $^{-1}$due to coordination of two N atoms at 1 and 1' positions [24-25]. The IR spectrum of Complex 1 shows vibrational absorptions at 1115 and 1084 cm^{-1} which are assigned to the absorption of the perchlorate groups bound to the metal ion in a unidentate fashion. The absorption band found at 476 cm^{-1} may be due to the mixing of ν_{MN} and ν_{MO} vibrations in the complex spectrum [26].

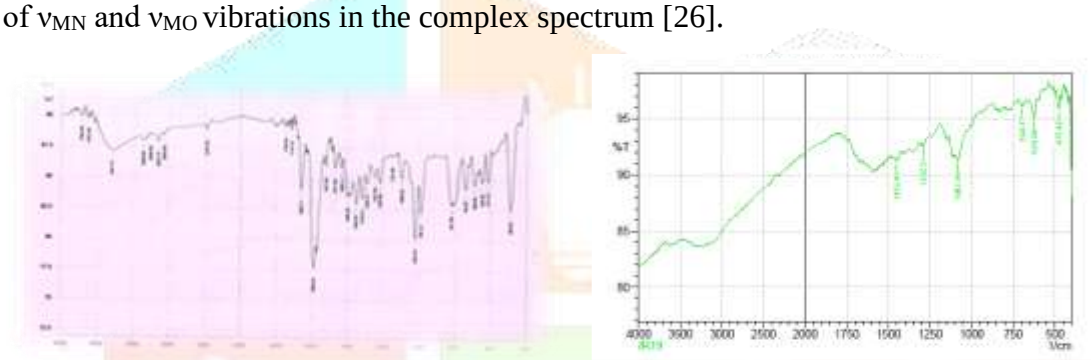


Fig. 2 FT-IR Spectrum of Ligand 1 and complex 1

Table 3

Selected IR Absorption Frequencies (cm^{-1}) for fctpy (L1) and its Complex

Sl. No	Compound	ν_{CH}	$\nu_{C=N}$ & $\nu_{C=C}$ of terpyridine	In plane pyridine ring Deformation	Out-of-plane pyridine ring deformation	ν_{ClO}	ν_{MO} & ν_{MN}
1.	fctpy(L1)	3132 - 2855	1562 - 1462	644	459	-	-
2.	$[Fe(fctpy)_2(ClO_4)_2]$ Complex 1	3100	1550 - 1452	694	476	1115 1084	476

ELECTROCHEMISTRY

The cyclic voltammograms of this complex (Fig.3) have been measured at 50, 100 and 200 mVs^{-1} scan rates. The redox parameters of this complex [27-28] are listed in Table 4. The E_{pa} indicates the energy required for oxidation , while the E_{pc} indicates the energy needed for reduction. The ΔE_p values indicates an irreversible one electron redox couple of Fe ^{II}/ Fe ^{I}. The peak current ratios (I_{pa}/I_{pc}) are less than unity suggesting that the electron transfer is followed by a chemical reaction. In other words EC mechanism is

followed. These positive values of $E_{1/2}$ reveal that this complex cannot undergo an easy reduction. As the σ-donating ability of the fctpy ligand would tend to stabilize the Fe^{II} in the chelate complex reduction of Fe^{II} becomes difficult.

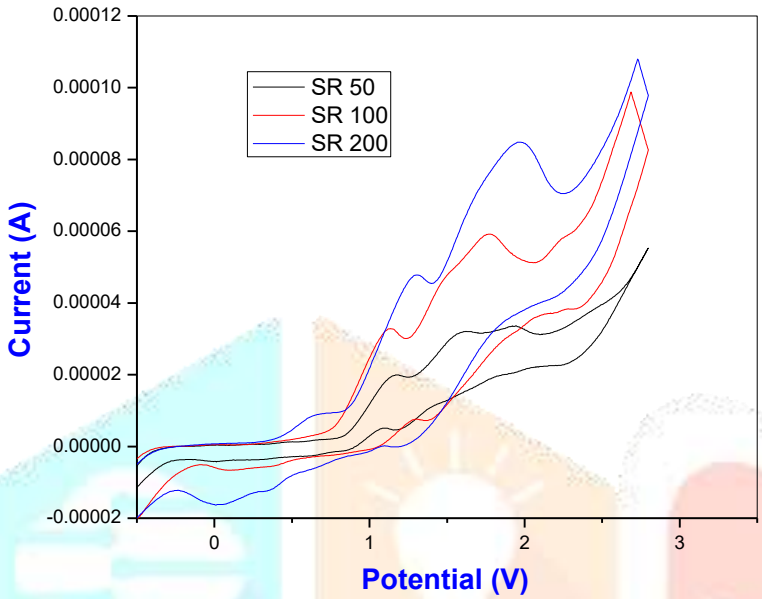


Fig 3 Cyclic Voltammograms of Complex 1 at 50, 100 and 200 mVs^{-1}

Table 4

Electrochemical Parameters Measured for Iron(II) Complex of fctpy(L1)

Compound	**Scan Rate (mVs^{-1})**	**E_{pa}^1 (V)**	**E_{pa}^2 (V)**	**E_{pc} (V)**	**ΔE_p (mV)**	**$E_{1/2}$ (V)**	**I_{pa}^1 X 10^{-6} (A)**	**I_{pa}^2X 10^{-6} (A)**	**I_{pc} × 10^{-6} (A)**	**I_{pa} / I_{pc}**
$[Fe(fctpy)_2(ClO_4)_2]$ Complex 1	50	1.223	1.88	1.152	728.6	1.516	--	5.934	6.819	0.870
	100	--	1.778	1.530	248	1.654	--	1.495	3.285	0.455
	200	--	1.342	1.082	259.9	1.212	--	1.412	3.442	0 .410

INVITRO EVALUATION OF ANTIOXIDANT ACTIVITY BY DPPH ASSAY

The fctpy and its metal complex were evaluated for DPPH radical scavenging activity [29-31]. The antioxidant activity of each test drug increases with increase in its concentration. The determined results (Table 5) show that the fctpy (L1) exhibits the highest activity at 94.74% while the Fe II complex displays the lowest activity at 86.84% at the concentration of 20 µg/mL.

Table 5

Antioxidant Activities of fctpy (L1) and its Metal Complex

Sl.No	Test Drug	Concentration (µg/mL)	Absorbance	% DPPH Scavenging Activity
1	fctpy(L1)	05	0.16	57.89
		10	0.09	76.32
		15	0.05	86.84
		20	0.02	94.74
2	$[Fe(fctpy)_2(ClO_4)_2]$ Complex 1	05	0.27	28.95
		10	0.18	52.63
		15	0.12	68.42
		20	0.05	86.84
3	Std (Vitamin C)	2.0	0.15	60.52
		2.5	0.10	73.68
		3.0	0.05	86.84
		3.5	0.01	97.37
4	Control (Ethanol)	-	0.38	-

CYTOTOXIC EFFECTS OF FCTPY AND ITS METAL COMPLEX

The present study examines the efficacies of ligand -1 and its metal complex to inhibit the growth of the human breast cancer cell lines (MCF-7) at 24 h. The cells were treated with five different concentrations of test drugs ranging from 0.25 – 100 µM. The average absorbance, relative cell viability, percentage cell inhibition and IC_{50} values determined for each of the test drugs are provided in Table 6, 7. The nonlinear regression graphs constructed for the test drugs are reproduced in Fig 4. The IC_{50} values for the test drugs have been determined using the Graph Pad Prism software. The ligand (L1) as well as the complex 1 suppressed the growth of breast cancer cell lines in a dose dependent manner. The maximum cell inhibitions of 62.00 and 26.97 % at 100 µM concentration were determined for Complex-1 and L1 and minimum inhibitions of 0.08, 0.17 % at 0.25µM concentration were observed for ligand, Complex - 1 (Fig 4, 5). The IC_{50} values for ligand and complex treated MCF-7 cells were obtained at 280.1, 68.29 µM respectively. The corresponding IC_{50} values in µg/mL for L1 and its complex are 116.9, 74.40 µg/mL . The free ligand L1 and Complex 1 are considered to be inactive as they measure IC_{50} values higher than 30 µg/mL [32,33].

Table 6

Percentage Cell Inhibitions and IC_{50} Determined for fctpy (L1)

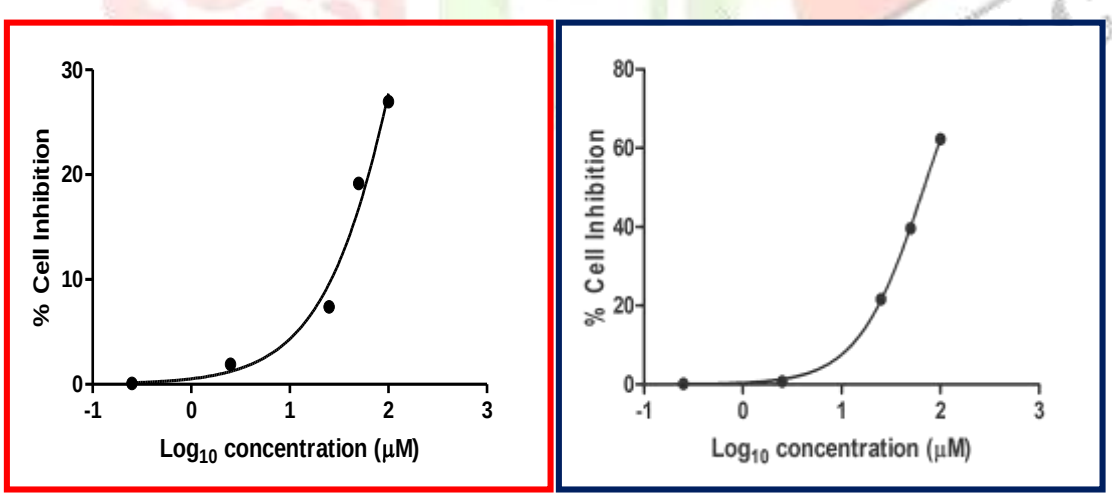
Concentration	Mean Absorbance	Relative Cell	Percentage Cell	IC_{50}	R^2

of Test Drug (µM)		Viability	Inhibition		
0.25	0.4013	99.917	0.083	280.1 µM or 116.9 µg/mL	0.9785
2.50	0.3940	98.092	1.908		
25.00	0.3720	92.615	7.385		
50.00	03246	80.830	19.170		
100.00	0.2933	73.030	26.970		
Control	0.4017	-	-		

Table 7

Percentage Cell Inhibitions and IC_{50} Determined for $[Fe(fctpy)_2(ClO_4)_2]$, Complex 1

Concentration of Test Drug (µM)	Mean Absorbance	Relative Cell Viability	Percentage Cell Inhibition	IC_{50}	R^2
0.25	0.3767	99.824	0.176	68.29 µM or 74.40 µg/mL	0.9998
2.50	0.3743	99.205	0.795		
25.00	0.2957	78.357	21.643		
50.00	0.2277	60.336	39.664		
100.00	0.1423	37.721	62.279		
Control	0.3773	-	-		



(a) **(b)**

Fig 4.Antiproliferative Nonlinear Regression Graphs for (a) L1, (b) Complex 1

Control (b) (c) (d) (e) (f)

Fig 4 Cytotoxic Effect of Ligand 1

(a): Control showed the presence of human breast cancer cell line without any treatment

(b): 0.25 µM concentration of ligand 1 treated on MCF-7 cell line

(c): 2.5 µM concentration of ligand 1 treated on MCF-7 cell line

(d): 25 µM concentration of ligand 1 treated on MCF-7 cell line

(e): 50 µM concentration of ligand 1 treated on MCF-7 cell line

(f): 100 µM concentration of ligand 1 treated on MCF-7 cell line

Control (b) (c) (d) (e) (f)

Fig 5. Cytotoxic Effect of Complex 1

(a): Control showed the presence of human breast cancer cell line without any treatment

(b): 0.25 µM concentration of complex 1 treated on MCF-7 cell line

(c): 2.5 µM concentration of complex 1 treated on MCF-7 cell line

(d): 25 µM concentration of complex 1 treated on MCF-7 cell line

(e): 50 µM concentration of complex 1 treated on MCF-7 cell line

(f): 100 µM concentration of complex 1 treated on MCF-7 cell line

INVITRO ANTIMICROBIAL ACTIVITIES OF (L1) AND ITS COMPLEX 1

The results of antibacterial and fungal sensitivities [33] of the test drugs (fctpy and its metal complex) against the bacterialand fungal strains are furnished in Table 8-9 and its Plates 1. The activities of the test compounds increase with increase in concentration. The test drugs are inactive to *E.coli*. The Fe^{II} complex is insensitive to *S.aureus*. In the case of assays against *K.aerogenes*, it is seen that fctpy, , Fe^{II} complex are

inactive to *K.aerogenes* . Interestingly, fctpy do not show any activity against both *A.niger* and *C.albicans*. Only $[Fe(fctpy)_2(ClO_4)_2]$ is found to be active against *A.niger*. The activity of the iron (II) complex is much less than that of the standard.

Bacillus subtilis *Klebsiella aerogenes* *Aspergillus niger*

Plate 1. Antibacterial and Antifungal Activities of Complex 1

Table 8

Antibacterial Activities of fctpy and its Metal Complex

Sl. No	**Test Drug**	**Zone of Inhibition (mm)**											
		S. aureus			***B. subtilis***			***K. aerogenes***			***E. coli***		
		100 mg/L	250 mg/L	500 mg/L	100 mg/L	250 mg/L	500 mg/L	100 mg/L	250 mg/L	500 mg/L	100 mg/L	250 mg/L	500 mg/L
1	fctpy(L1)	-	-	-	-	-	-	-	-	-	-	-	-
2	$[Fe(fctpy)_2(ClO_4)_2]$ Complex 1	-	-	-	10	15	15	8	12	15	-	-	-
6	Ciprofloxacin Standard	-	-	35	-	-	40	-	-	30	-	-	38

Table 9

Antifungal Activities of fctpy and its Metal Complex

Sl. No	**Test Drug**	**Zone of Inhibition (mm)**					
		Aspergillus niger			***Candida allbicans***		
		100 mg/L	250 mg/L	500 mg/L	100 mg/L	250 mg/L	500 mg/L
1	fctpy(L1)	-	-	-	-	-	-
2	$[Fe(fctpy)_2(ClO_4)_2]$	12	13	16	-	-	-

	Complex 1						
6	Nystatin Standard	-	-	30	-	-	25

Note: Zone size less than 15 mm - Least active; 16 – 20 mm - moderately active; Above 20 mm – highly active

CONCLUSION

Based on the above observations of the physico-chemical result, it is possible to determine the type of coordination of the ligand in its metal complex. These spectral data show that fctpy exist as bidentate ligand by bonding to the metal ion through the N atoms. The proposed structure of such complex was also shown in the Scheme 2. The free ligand L1 and Complex 1 are considered to be inactive as they measure IC_{50} values higher than 30 µg/mL . The antioxidant properties of ligand and complex have been tested using DPPH radical scavenging method in which ligand and its metal complex exhibited potential antioxidant activity. However, further studies on the mechanisms of antioxidant activity are required.

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