



NEW IRON (II) COMPLEX WITH 4'-(1H-BENZIMIDAZOL-2-YL)-2, 2':6', 2''-TERPYRIDINE: SYNTHESIS, SPECTRAL AND INVESTIGATION OF ANTIOXIDANT, ANTICANCER AND BIOLOGICAL PROPERTIES

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ABSTRACT

This article describes the synthesis of $[\text{Fe}(\text{bitpy})_2](\text{ClO}_4)_2$ where bitpy is 4'-(1H-benzimidazol-2-yl)-2,2':6',2''-terpyridine and its characterization by using elemental analysis, UV-visible, Mass and IR spectroscopic techniques. The antioxidant activity of the complex was evaluated by using 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging method. In this work, we have observed that the newly synthesized bitpy ligand was found to be much more effective free radical scavengers than the Complex 1. The ligand and metal complex exhibited anticancer activity against human breast cancer (MCF-7) cell lines. Microbial assay of the above ligand and complex against two gram positive (*S.aureus*, *B.subtilis*), two gram negative (*K.aerogenes*, *E.coli*) and two fungi (*A.niger*, *C.albicans*) showed that ligand exhibited potential activity than the complex.

KEYWORDS: bitpy, anticancer, Fe(II) complex, DPPH.

INTRODUCTION

For the last 30 years increased research attention has been focused on the synthesis, spectroscopic characterization, electrochemistry and biological properties of polypyridyl based metal ion complexes. The terpyridine ligands are more rigid than the bidentate ligands such as bipyridine and phenanthroline. The physicochemical and biological properties of the terpyridine metal complexes can be tuned favourably by putting a suitable substituent on the 4' position of the terpyridine. A few researchers have investigated with benzimidazolyl terpyridine metal complexes^[1-3]. In view of this an attempt has been made to synthesize and characterize the ligand L1 and its metal ion complex with Fe^{II} so as to evaluate their electrochemical properties and biological activities such as, antioxidants, anticancer and antimicrobial activities.

Physical Parameters

C, H and N were estimated by using Elemental Vario EL III CHNS/O elemental analyser. The FT-IR and UV-vis spectra of bitpy(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 bitpy and its metal complex was recorded in Finnigan LCQ 6000 advantage max ion trap mass spectrometer equipped with an electron spray source. The magnetic moments were 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, iron(II) perchlorate hexahydrate are procured from Sigma Aldrich, USA and used as received. Other materials like sodium hydroxide, ammonium acetate and solvents like methanol, acetonitrile, ethonal, diethyl ether, and glacial acetic acid were of reagent grade.

Synthesis and Characterization of 4'-(1H-benzimidazol-2-yl)-2,2':6',2''-terpyridine L1.

4'-(1H-benzimidazol-2-yl)-2,2':6',2''-terpyridine (L1) was prepared^[4,5,29&30] by 2-Acetylpyridine and 1H-

benzimidazole-2-carbaldehyde were combined using a mortar and pestle. The grinding was continued until the formation of an orange red powder. The powder was added to a suspension of ammonium acetate in glacial acetic acid and heated to reflux for 3 hours. The crude product was precipitated out by the addition of water. The product was filtered, washed with water and cold ethanol and it was column chromatographed using 1:1 methanol dichloromethane system to get white powder.

Synthesis of Iron (II) Perchlorato Complex with bitpy Complex 1:

To a hot solution of hydrated $\text{Fe}(\text{ClO}_4)_2$ in methanol, bitpy dissolved in methanol was added slowly, the reaction mixture was stirred and refluxed for four hours. The deep violet solid that separated out upon slow evaporation of the solvent was filtered, washed with diethyl ether and dried.

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.^[6-10] 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 $\mu\text{g}/\text{mL}$ 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.

Invitro Anticancer Activity Screening- MTT Assay^[11]

3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) 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^o 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.

$$\text{Relative Cell Viability (\%)} = \frac{\text{Absorbance of test drug}}{\text{Absorbance of control}} \times 100.$$

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

Antimicrobial Assay

The *in vitro* antimicrobial assay of the test drugs were tested for certain bacteria and fungi by using the Disc Diffusion Method and Mueller Hintonagar medium.^[12] A loopful of strain in each was inoculated in 30 mL of the nutrient broth and incubated for 24 hours at 37^oC 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^oC to 37^oC for 24 hours and the plates incubated with the fungi were incubated for 48 hours at the same temperature.

RESULTS AND DISCUSSION

Elemental Analyses, Mass Spectra and Molar Conductance

Elemental analyses are consistent with a 1:2 metal to ligand ratio for the complex.

Ligand 1 shows its molecular ion peak at $m/z = 349.0$ (Fig.1). The molecular ion peaks for Fe complex appeared at 952.85 (Fig.1). The molecular ion peak indicates the presence of the two perchlorate groups inside the coordination sphere as well as the neutral character of the coordination complex. The molar conductance value of the complex was $40.3 \text{ S cm}^2 \text{ mol}^{-1}$ in DMF solutions showing that complex are non electrolytic nature^[13] due to the coordination of perchlorate anions to the metal centre. The mass and elemental analysis data are confirm that the structure of the L1 and its metal complex.

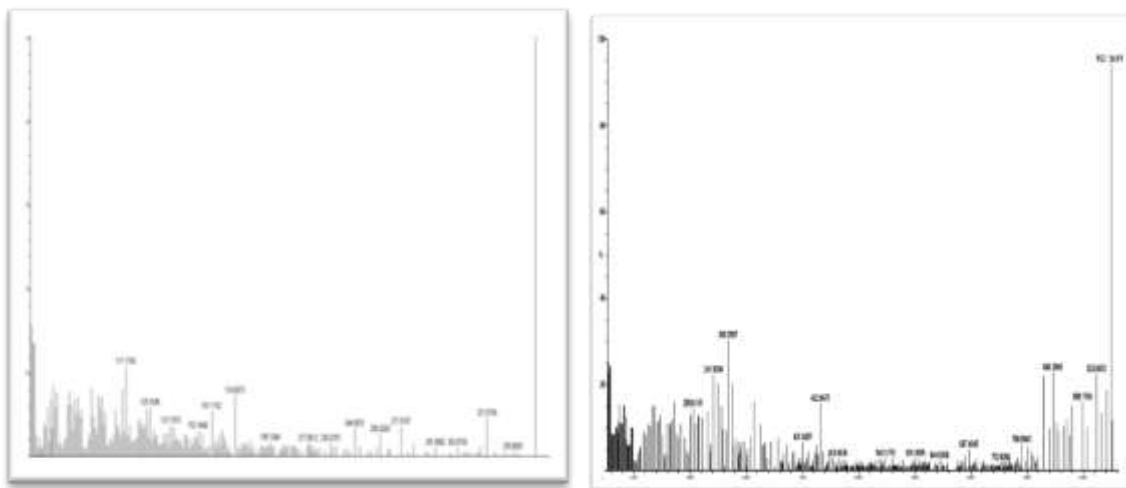


Fig 1: Mass spectra of L1 and its complex1.

Electronic Spectral and Magnetic Studies on bitpy (L1) and its Metal Complex

The L1 consist of three bands at 40486, 35336 and 31949 cm^{-1} . These bands are assigned to $\pi - \pi^*$ and $n - \pi^*$ transition (Fig.2). Generally compounds containing hetero atoms such as N, O, S etc., have nonbonding electrons (n) in addition to σ and π electrons. Thus the terpyridine derivative shows both $\pi - \pi^*$ and $n - \pi^*$

transitions in its UV spectrum. Complex 1 showed two bands at 12760 (single d-d transition) and 17150 cm^{-1} . These bands are assigned to ${}^5T_{2g} \rightarrow {}^5E_g$ and charge transfer band (Fig.2).^[14-17] The magnetic moment of the Complex 1-3 was found to be 5.1-2.98 BM.^[17] These results are typically characteristic for an octahedral configuration for the metal complex.

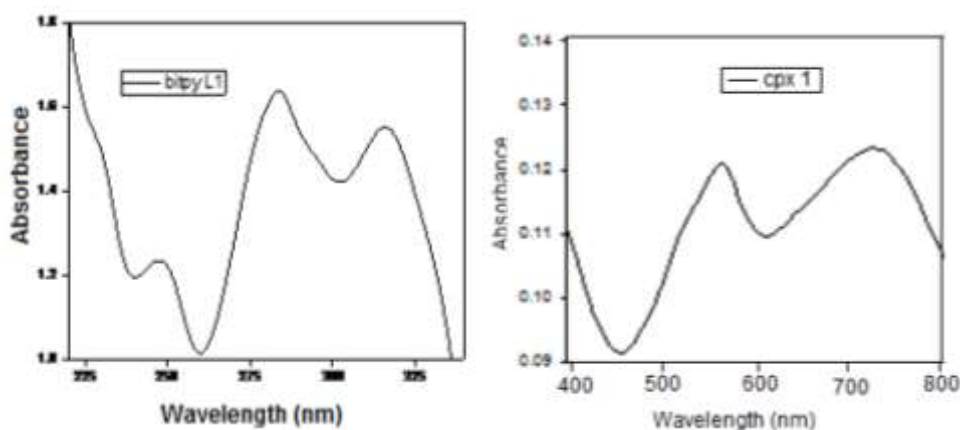


Fig 2: UV - visible Spectrum of bitpy (L1) and complex 1.

IR Spectroscopy

The IR spectra of bitpy(L) and its metal complex (Fig 3) exhibit several diagnostic vibrational frequencies of respective functional groups. IR spectrum of L1 displays bands at 3399, 3057-2922, 1523-1431, 624 and 434 cm^{-1} assigned to $\nu(\text{NH})$, $\nu(\text{CH})$, pyridine ring skeletal vibrations ($\nu\text{C}=\text{C}$ and $\nu\text{C}=\text{N}$), in-plane and out-of-plane deformations respectively. The Complex 1 $\nu\text{C}=\text{C}$ and $\nu\text{C}=\text{N}$ bands have shifted to higher position (1518-1449 cm^{-1}) due to the coordination of the pyridyl nitrogen atoms to the metalion.^[18] The pyridine ring in-plane deformation and out-of-plane deformation confirming the coordination of two nitrogen atoms at 1' and 1' positions.^[19-21] The new band at 496 cm^{-1} was also

being observed in the complex and was not found in the free ligand. These bands attributed to M-N and M-O bonds in complex 1^[22] respectively. Based on these data, Complex 1 proposed to have octahedral structure shown in the diagram.

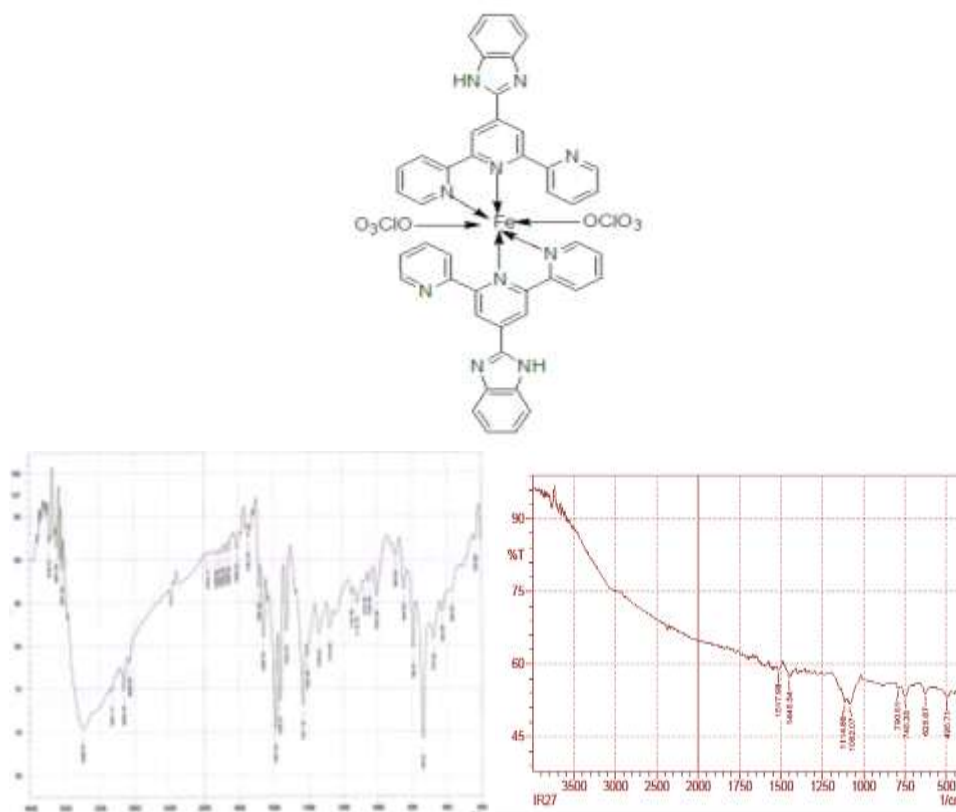


Fig 3: IR Spectrum of L1 and its Complex 1.

Cyclic Voltammetry

Electrochemical studies have been carried out on complex 1. The cyclic voltammograms have been recorded at three different scan rates viz. 50, 100 and 200 mVs^{-1} . Complex 1, the values (Table 1) of anodic peak potentials (E_{pa}) indicate the energy required for oxidation and the values of cathodic peak potential (E_{pc}) indicate

the energy required for reduction of the metal ion.^[23-24] ΔE_p values are higher than 200 mV, indicates an irreversible $\text{Fe}^{\text{II}}/\text{Fe}^{\text{I}}$ redox couple. The peak current ratios are less than unity suggesting that the electron transfer is followed by chemical reaction. i.e. EC mechanism is followed. The positive values of $E_{1/2}$ show that the complex 1 cannot undergo easy reduction (Fig 4).

Table 1: Electrochemical Parameters Measured for Iron(II) Complex with bitpy.

| Compound | Scan Rate (mVs^{-1}) | E_{pa}^1 (V) | E_{pa}^2 (V) | E_{pc} (V) | ΔE_p (mV) | $E_{1/2}$ (V) | $I_{pa}^1 \times 10^{-6}$ (A) | $I_{pa}^2 \times 10^{-6}$ (A) | $I_{pc} \times 10^{-6}$ (A) | I_{pa} / I_{pc} |
|---|---------------------------------|----------------|----------------|--------------|-------------------|---------------|-------------------------------|-------------------------------|-----------------------------|-------------------|
| [Fe(bitpy) ₂ (ClO ₄) ₂] Complex 1 | 50 | 1.1057 | 1.7543 | 1.0026 | 751.7 | 1.3784 | 2.5683 | 4.6797 | 8.0109 | 0.5841 |
| | 100 | 1.2087 | 1.8005 | 1.0151 | 785.4 | 1.4078 | 3.9672 | 7.0655 | 5.5737 | 1.2676 |
| | 200 | 1.2532 | 1.8449 | 1.0595 | 785.4 | 1.4522 | 5.4303 | 1.0225 | 1.0902 | 0.9379 |

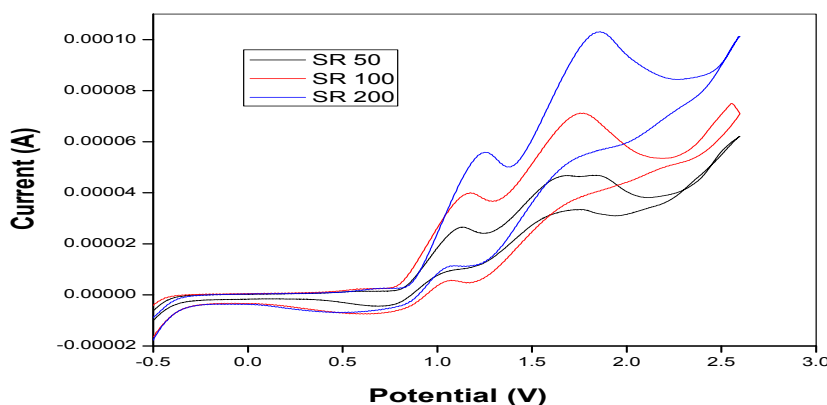


Fig 4: Cyclic Voltammograms of Complex 1 at 50, 100 and 200 mVs^{-1} .

Antioxidant activity screening

The bitpy and its metal complex were evaluated for DPPH radical scavenging activity.^[25, 26] The antioxidant activity of each test drug increases with increase in its

concentration. The determined results (Table 2) show that the bitpy (L1) exhibits the highest activity at 94.74% while the Fe^{II} complex displays the lowest activity at 84.21% at the concentration of 20 µg/mL.

Table 2: *In vitro* Antioxidant Activities of bitpy (L1) and its Metal Complex.

| Sl. No | Test Drug | Concentration (µg/mL) | Absorbance | % DPPH Scavenging Activity |
|--------|--|-----------------------|------------|----------------------------|
| 1. | bitpy(L1) | 05 | 0.19 | 50.00 |
| | | 10 | 0.10 | 73.68 |
| | | 15 | 0.05 | 86.84 |
| | | 20 | 0.02 | 94.74 |
| 2. | [Fe(bitpy) ₂ (ClO ₄) ₂] Complex 1 | 05 | 0.29 | 23.68 |
| | | 10 | 0.16 | 57.89 |
| | | 15 | 0.10 | 73.68 |
| | | 20 | 0.06 | 84.21 |
| 3. | Standard (Vitamin C) | 2.0 | 0.15 | 60.52 |
| | | 2.5 | 0.10 | 73.68 |
| | | 3.0 | 0.05 | 86.84 |
| | | 3.5 | 0.01 | 97.34 |
| 4. | Control (Ethanol) | - | 0.38 | - |

Antiproliferative Effect of L1 (bitpy) and its Metal Complex

The present study examined the efficacy of Ligand - 1 and its complex to inhibit the human breast cancer cell lines (MCF-7) at 24 h as determined by MTT assay. The cells were treated with five different concentrations of test drugs ranging from 0.25 to 100 µM. The free ligand as well as the complex suppressed the growth of breast cancer cell lines in a dose dependent manner. The maximum cell inhibitions of 68.21 and 61.39% at 100 µM concentration were determined for free Ligand (L1), and Complex-1 respectively and minimum inhibitions (3.48, 6.36) at 0.25µM concentration were observed for free Ligand (L1), Complex-1 respectively (Fig 5,6).

The IC₅₀ values for free ligand (L1), Fe^{II} complex treated MCF-7 cells were obtained at 51.16, 58.05 µM or at 17.85, 55.32 µg/mL (Table 3, 4) respectively. The test drugs having IC₅₀ values higher than 30 µg/mL are considered to be as inactive. Compounds with IC₅₀ equal to 5.0 µg/mL or below are considered to be highly active while those with IC₅₀ values greater than 5 - 10.0 µg/mL are associated with moderate activity.^[27-28] The benzimidazolyl terpyridine ligand (L1) measuring IC₅₀ value of 17.85 µg/mL may be considered to exert only weak antiproliferative effect and complex 1 inactive on the breast cancer cell lines.

Table 3: Percentage Cell Growth Inhibitions and IC₅₀ Values Determined for bitpy (L1).

| Concentration of Test Drug (µM) | Mean Absorbance | Relative Cell Viability | Percentage Cell Growth Inhibition | IC ₅₀ | R ² |
|---------------------------------|-----------------|-------------------------|-----------------------------------|-------------------------------|----------------|
| 0.25 | 0.387 | 96.52 | 3.48 | 51.16 µM or 17.85 µg/mL | 0.9834 |
| 2.50 | 0.374 | 93.2 | 6.80 | | |
| 25.00 | 0.296 | 73.86 | 26.14 | | |
| 50.00 | 0.190 | 47.39 | 52.61 | | |
| 100.00 | 0.127 | 31.79 | 68.21 | | |
| Control | 0.401 | - | - | | |

Table 4: Percentage Cell Growth Inhibitions and IC₅₀ Values Determined for [Fe(bitpy)₂(ClO₄)₂], Complex 1.

| Concentration of Test Drug (µM) | Mean Absorbance | Relative Cell Viability | Percentage Cell Growth Inhibition | IC ₅₀ | R ² |
|---------------------------------|-----------------|-------------------------|-----------------------------------|-------------------------------|----------------|
| 0.25 | 0.353 | 93.64 | 6.36 | 58.05 µM or 55.32 µg/mL | 0.9555 |
| 2.50 | 0.328 | 86.93 | 13.07 | | |
| 25.00 | 0.271 | 72.00 | 28.00 | | |
| 50.00 | 0.186 | 49.39 | 50.61 | | |
| 100.00 | 0.145 | 38.61 | 61.39 | | |
| Control | 0.377 | - | - | | |

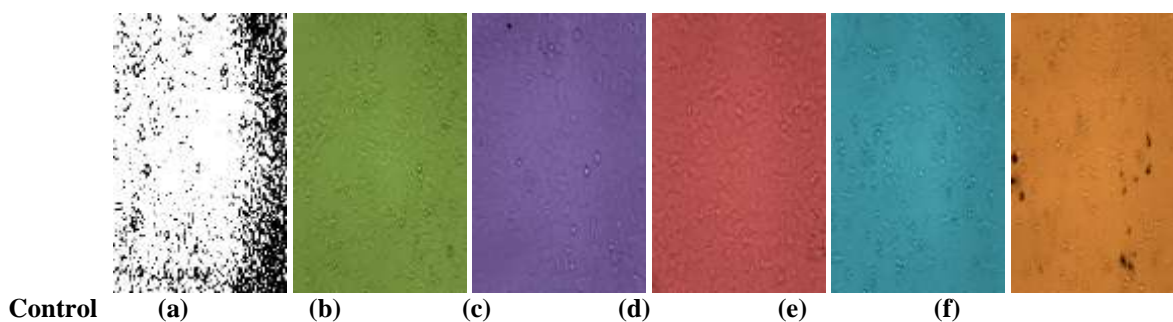


Fig 5: *In vitro* Cell Line MCF-7 Treated with Ligand 1.

- (a): Control showed the presence of human breast cancer cell line without any treatment
 (b): 0.25 μM concentration of L1 treated on MCF-7 cell line
 (c): 2.5 μM concentration of L1 treated on MCF-7 cell line
 (d): 25 μM concentration of L1 treated on MCF-7 cell line
 (e): 50 μM concentration of L1 treated on MCF-7 cell line
 (f): 100 μM concentration of L1 treated on MCF-7 cell line

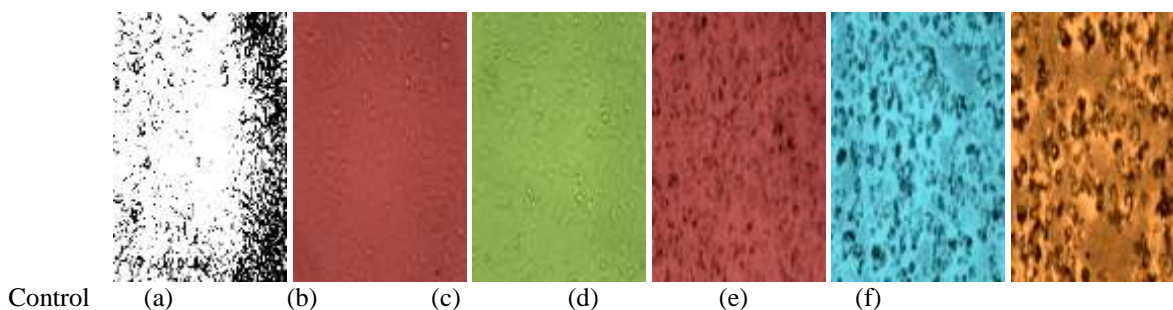


Fig 6: *In vitro* Cell Line MCF-7 Treated with Complex 1.

- (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

Antimicrobial activity

Bacterial species such as *S.aureus*, *B.subtilis*, *K.aerogenes* and *E.coli* and fungal species such as *A.niger* and *C.albicans* were used in the study. Ciprofloxacin, Nystatin was the standard antibiotics used in the antibacterial and fungal screening. The inhibition activities of the test compound increase with increase of concentrations of test compound (Table 5, 6). The activities of the L1 and its metal complex against

S.aureus : L1, Complex 1 shows better activity. The activities of test drugs against *B.subtilis* shows the order $\text{L1} > \text{Fe}^{\text{II}}$. In the case of *K.aerogenes* the activities decrease in the order: $\text{Fe} > \text{L1}$. When the test drugs are assayed against *E.Coli*, the order of activity is $\text{L1} \sim \text{Fe}$. The activities of test drugs against *A.niger* decrease in the order: $\text{bitpy} > \text{Fe}$. The sensitivities of test drugs to *C.albicans* are in the order: $\text{L1} > \text{Fe}$.

Table 5, 6: Antibacterial, Antifungal Activities of bitpy and its Metal Complex.

| Sl. No | Test Drug | Zone of Inhibition (mm) | | | | | | | | | | | |
|--------|--|-------------------------|----------|----------|--------------------|----------|----------|---------------------|----------|----------|----------------|----------|----------|
| | | <i>S. aureus</i> | | | <i>B. subtilis</i> | | | <i>K. aerogenes</i> | | | <i>E. coli</i> | | |
| | | 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 | bitpy(L1) | 17 | 20 | 24 | 12 | 16 | 18 | 8 | 8 | 10 | 15 | 16 | 20 |
| 2 | [Fe(bitpy) ₂ (ClO ₄) ₂] Complex 1 | 16 | 18 | 21 | 12 | 12 | 16 | 12 | 12 | 15 | 15 | 17 | 20 |
| 3 | Ciprofloxacin Standard | - | - | 35 | - | - | 40 | - | - | 30 | - | - | 38 |

| Sl. No | Test Drug | Zone of Inhibition (mm) | | | | | |
|--------|---|--------------------------|----------|----------|-------------------------|----------|----------|
| | | <i>Aspergillus niger</i> | | | <i>Candida albicans</i> | | |
| | | 100 mg/L | 250 mg/L | 500 mg/L | 100 mg/L | 250 mg/L | 500 mg/L |
| 1 | bitpy(L1) | 16 | 16 | 20 | 15 | 18 | 19 |
| 2 | [Fe(bitpy) ₂ (ClO ₄) ₂] Complex 1 | 12 | 15 | 16 | 12 | 14 | 17 |
| 3 | Nystatin Standard | - | - | 30 | - | - | 25 |

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

CONCLUSION

In this study, we have reported the synthesis of bitpy ligand and its Fe^{II} complex. The structural characterizations of the synthesized compounds were made by spectroscopic methods (FT-IR, Electronic, ESI-MS), elemental analysis, electrical conductance, magnetic studies and cyclic voltammetry. From the spectroscopic characterization, it is concluded that bitpy act as bidentate ligands, coordinating through its two 1, 1' positions to the metal. The biological activity screening showed that the complex selectively inhibited the bacterial (*S.aureus*, *B.subtilis*, *K.aerogenes* and *E.coli*) and fungal (*A.niger*, *C.albicans*) species, the ligand has more activity than iron complex. The antioxidant properties of ligand and complex have been tested using DPPH radical scavenging method in which ligand exhibited potential antioxidant capacity than that of iron complex. Ligand shows only weak antiproliferative effect on the breast cancer cell lines. However, further studies on the mechanisms of antioxidant are required.

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