Multi Metal Ion Recognizing Unsymmetrical tetradentate Schiff bases Associated with Antifungal activity

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ABSTRACT

New unsymmetrical Schiff bases containing azomethine moiety with simple aromatic section in one side and ferrocene fragment attached imine on the other side have been synthesized. Advent of metal-to-ligand charge transfer band for the coordination of Cu²⁺ ions with receptors and appropriate changes in UV-Visible spectra for other metal ion combination with the sensor is reported. Observed extravagant Δ𝐸p values suggest quasi-reversible process. The ΔIpa amount calculated from the anodic current Ipa value noticed for receptor solution and different metal ion added sensor solution discloses the concentration of metal ions required for effective sensing. The synthesized ligands were subjected to antimicrobial activity against four bacterial and two fungal stains and the zone of inhibition (in mm) was calculated. Further molecular docking study was carried out and the binding energy (Kcal.mol⁻¹) for the synthesized ligand (R₁ and R₂) with the selected protein was intended.

Keywords: Azomethine, Cation sensors, Binding attitude, Molecular docking, MLCT Band, Unsymmetrical Schiff base.

INTRODUCTION

Researchers are interested to develop selective chemosensors to identify the trace amount of transition metal ions involved in many biological processes. Not only have that, the influence of metal ions having redox behavior in the environment also captivated the attraction of scientist to synthesize sensor compounds capable of identifying different metal ions¹. Qualities like non-destructive nature, easiness in synthesis, selectiveness towards target entities, quick retention time and capability to diagnose biological samples² project chemosensors to unique place rather than the other instrumental method of analysis³.

Preparation of sensors proficient in identifying cations in solution and converting the action of recognition into documentable signal are of flourishing field with enormous amount of published
works. Interlocking ability of the synthesized compounds with hazardous heavy metal cations and anions hinge on the π electrons cloud available on C=N group which in turn is influenced by heterocyclic aromatic part with nitrogen and inductive effect of substituent groups. Metal-to-Ligand charge-transfer and intramolecular charge transfer may emerge for the union of sensor and targeted ions.

Copper ions control the biological activity of cytochrome c oxidase, superoxide dismutase and tyrosinase enzymes. Despite, higher insertion modify the functions of enzymes and lead to antagonistic actions like, lethargy, hike in blood pressure, nausea and Alzheimer’s diseases. Solubility of Hg²⁺ in water makes it to penetrate through membrane of the cell leading to malfunction of brain, letdown of kidney functions, infirmity of nervous system and Minamata disease. Consumption of chocolate, milk and cookies made of milk, canned food and hydrogenated oils induces Ni²⁺ ion in biological systems. Development of cancer in respiratory organs, pneumonia, asthma and malfunctioning of nervous system has been reported for the excess intake of Ni²⁺ ions.

Consumption of Cd²⁺ ions toxicity prompts productivity, hepatic and cardiovascular dysfunctions. Harmfulness of Pb²⁺ ion exposure include procreative malady in human, neurological dysfunctions and loss of strength at bone joints. Health hazards persuaded by manganese toxicity include damage to immune system, central nervous system, kidney function and exocrine & endocrine function of pancreas.

Here in we report the synthesis of new unsymmetrical Schiff bases \( \text{N'}-(\text{E})-2\text{-nitrobenzylidene})-2\text{-((E)-2-(ferrocenylidene)} \) hydrazine-1-carbothiohydrazide and \( \text{N'}-(\text{E})-2\text{-hydroxy-5-nitrobenzyliden-2-(E)-2-(ferrocenylidene)} \) hydrazine-1-carbothiohydrazide containing aromatic part at one end and ferrocene compound at the other end of the main frame structure. Spectroscopic and redox studies exposes that synthesized receptor possess sensing aptitude towards metal ions like, Hg²⁺, Mn²⁺, Pb²⁺, Cd²⁺, Ni²⁺ and Cu²⁺.

Reported literature discloses that the biological and chemical activities of Schiff bases depend upon the sp² hybridized nitrogen donor atom of the azomethine group. The proteins present in microorganisms find suitability to form hydrogen bond with the active site of azomethine group containing high electro negative nitrogen atom, which in turn is responsible for the anticancer, antibacterial and antifungal activities. Prokaryotic nature of bacteria ( unicellular organism without nucleus, cell wall & organelles and survives on the host entities) helps to develop enormous amount of antibacterial commixture whereas eukaryotic nature of fungi ( multicellular organism with nucleus, cell wall & organelles and endures independently) prevent the formulation of antifungal agents. The newly synthesized sensor by us exhibit better antifungal activity rather than antibacterial activities.

**EXPERIMENTAL**

**Materials**

Chemicals [AR grade] such as carbon disulfide, 2-hydroxy-5-nitrobenzaldehyde, ferrocene carboxaldehyde, hydrazinehydrate, 2-nitrobenzaldehyde and silica gel were purchased from E.Merck industry. They were used without further purification. Analytical grade NiCl₂, MnCl₂, CuCl₂, HgCl₂, Pb(OAc)₂ and Cd(OAc)₂ used in the electronic spectral and CV studies were procured from Sigma–Aldrich. Acetonitrile [HPLC grade] obtained from E-Merck and absolute ethanol [spectral grade] acquired from Commercial Alcohols, Canada was used for spectral studies. Tetrabutylammoniumperchlorate [99+\%] secured from Chemical Center, Mumbai was used as such without purification.

**Instruments**

Bruker Daltonics esquire 3000 spectrometer was used to record mass spectra. BRUKER AVANCE spectrometer [500 MHz] engaging \( \text{C}_2\text{D}_2\text{OD} \) solvent was adopted to document proton NMR spectra. Perkin-Elmer 337 spectrometer was engaged to register FTIR spectra in the range of 400-4000 cm⁻¹ using KBr pellets. SHIMADZU MODEL UV-1800 240V spectrophotometer was affianced to observe UV–Visible spectral studies between 200 and 800 nm. CHI electrochemical
analyzer 1200B model was employed to draw cyclic voltammograms using platinum as counter electrode, Ag/AgCl as reference electrode and glassy carbon as working electrode. The C, H and N contents were analyzed with Herarus C-H-N rapid analyzer.

Synthesis of N’-((E)-2-Nitrobenzylidene)-2-((E)-2-(ferrocenyldiene)hydrazine-1-carbothiohydrazide [R1]

Hydrazinehydrate and carbon disulphide in 3:1 molar ratio was refluxed for ten hours at 80°C along with 0.15 mole of 2-chloroethanol as catalyst to prepare the precursor compound thiocarboxyhydrazide. To a clear solution [0.01 mole/25 mL] of purified thiocarboxyhydrazide in ethanol, a mixture of 2-nitrobenzaldehyde (0.01mol) and ferrocene carboxaldehyde (0.01 mol) in 180 mL ethanol was added. After half an hour stirring, the reaction mixture was refluxed for 6-7 hours. Thin layer chromatographic technique was used to check the progress of the reaction at various time intervals. Filtration was carried out after cooling and the filtrate was concentrated to get reddish yellow colored N’-((E)-2-Nitrobenzylidene)-2-((E)-2-(ferrocenyldiene)hydrazine-1-carbothiohydrazide. Column having silica gel as stationary phase was used for the purification of crude sample. Ethanol was used as eluent. Color: Dark reddish orange. Yield: 0.536 g (91%), m.p. 69°C.

Synthesis of N’-((E)-2-hydroxy-5-nitrobenzylidene-2-((E)-2-(ferrocenyldiene)hydrazine-1-carbothiohydrazide [R2]

Solution containing 0.01 mole of thiocarboxyhydrazide in 25 mL of ethanol was added with stirring to another solution having 0.01 mole of 2-hydroxy-5-nitrobenzaldehyde and 0.01 mole of ferrocene carboxaldehyde in 180 mL ethanol. The reaction mixture was stirred for another half an hour and then refluxed for 6-7 hours. The progress of the reaction at various time intervals was checked using thin layer chromatographic technique. The reaction mixture was filtered after cooling. Greenish yellow color solid was obtained after concentrating the filtrate. The product was further purified by column chromatography using silica gel as stationary phase and ethanol as eluent. Yield: 0.5442 g, (91%), Color: reddish yellow, m.p. 70°C.
the theoretical values. R1 (Found: C, 50.61; H, 3.73; N, 15.51; Fe, 12.18; Calc. for C_{19}H_{17}N_{5}O_{2}SFe: C, 50.66; H, 3.77; N, 15.55; Fe, 12.20 %). R2 (Found: C, 50.59; H, 3.70; N, 15.47; Fe, 12.16; Calc. for C_{19}H_{17}N_{5}O_{3}SFe: C, 50.66; H, 3.77; N, 15.55; Fe, 12.20 %).

On mass spectral analysis, the advent of molecular peak (ESI) m/z at 434 and 450 respectively for the compounds N’-((E)-2-Nitrobenzylidene)-2-((E)-2-(ferrocenylidene) hydrazine-1-carbothiohydrazide & N’-((E)-2-hydroxy-5-nitrobenzylidene-2-((E)-2-(ferrocenylidenedihydrazine-1-carbothiohydrazide confirm the formation of expected receptors.

FTIR Spectral analysis

In the FTIR spectrum of compound R1 (Fig. 1), the peak observed around 500 cm\(^{-1}\) and 830 cm\(^{-1}\) are assigned to ferrocene cyclopentadienyl ring tilt stretching vibration and C-H out of plane bend vibrations respectively\(^{19}\). The peak positioned between 900 cm\(^{-1}\) to 1080 cm\(^{-1}\) are allocated to the C-C-C-H bending vibration in the penta cyclic ring. The peak at 1104 cm\(^{-1}\) is allotted for breathing ring deformation\(^{20}\) vibration. The peaks appeared at 1340 cm\(^{-1}\), 1519 cm\(^{-1}\) and 1567 cm\(^{-1}\) are assigned for C=S group stretching vibration, C-C stretching vibration of pentacyclic ring and NO\(_2\) group vibration respectively. The appearance of \(-C=\text{N}\) stretching vibration peak at 1650 cm\(^{-1}\) confirms the formation of Schiff base and is lower than the vibration frequency of \(-C=\text{O}\) group of the \(-\text{CHO}\) group present in ferrocene (1678 cm\(^{-1}\))\(^{19}\). The absorption peak emerged at 2059 cm\(^{-1}\) has been earmarked for aromatic stretching vibration. The peaks observed between 3200 - 3400 cm\(^{-1}\) is attributed to stretching vibration of secondary amine and water of hydration. Compound R2 also give the above mentioned peaks and the stretching vibrational modes of phenolic -OH appears along with secondary amine and water of hydration peaks in 3200 – 3400 cm\(^{-1}\) region itself\(^{21}\).

NMR Spectral analysis

The proton NMR spectrum of R1 in C\(_2\)D\(_5\)OD solvent (Fig. 2) contains relevant peaks and are assigned accordingly \(\delta\), (ppm) 8.4(s, 2H, NCH), 8.3 (s, 1H, aromatic), 8.1(s, 1H, aromatic), 7.0 (s, 1H,Ar), 4.4(m, 4H, (cp (subst.)), 4.2 (m, 2H, (cp unsubst.)), 3.9(s, 5H, cp unsubst), 1.14(s,2H, NH), along with a prominent singlet at \(\delta\) 5.0(s, phenolic-OH) appear in the spectrum.

RESULTS AND DISCUSSION

Investigation of sensing nature of receptors

Exploration of the ability of receptors to imprisonment with the various metal ions was carried out by titration method while recording the UV-Visible spectra. Twenty μL aliquots of metal solutions (10\(^{-2}\) M) were added to 2.5 mL of receptor solution (10\(^{-5}\) M) taken in the quartz cell. Since, chloride salts of copper, mercury and nickel are soluble in acetonitrile, solution of the receptor in acetonitrile was used for the above three metal salt solutions. Alcoholic solution of receptors was used for the chloride salts of manganese and acetate salts of lead & cadmium as these salts are soluble in acetonitrile. Solution of the receptor in acetonitrile was used for the above three metal salt solutions. Alcoholic solution of receptors was used for the chloride salts of manganese and acetate salts of lead & cadmium as these salts are soluble in ethanol. In acetonitrile R1 shows two shoulders around 259 nm and 313 nm (Fig. 3a). Alcoholic solution of R1 displays three peaks near 205 nm, 243 nm and 313 nm (Fig. 3b). Aromatic ring \(\pi-\pi^*\) transitions are assigned for above observation\(^{22}\).
Effective coordination of Cu\textsuperscript{2+} ions with receptor is exposed by the development of new prominent peaks around 305 nm, 350 nm and 460 nm (Fig. 4a). The 460 nm peak (Fig. 4b) has been assigned\textsuperscript{23} for MLCT band which has developed after the coordination of Cu\textsuperscript{2+} ions with receptor. Development of additional peaks near 305 nm and 350 nm (Fig. 4c) at the expenses of the shoulder peaks of receptor also ascertain the sensing capacity of R1.

Successive addition of Hg\textsuperscript{2+} ions generate new peak around 237 nm (Fig. 5a) and Pb\textsuperscript{2+} ions cause overall blue shift for all the base peaks (Fig. 5b) of the receptor R1. Similarly consecutive addition of Ni\textsuperscript{2+} ions gives a shoulder around 269 nm (Fig. 5c). Increase in absorbance value is noticed in the overall wavelength region for cumulative addition of Mn\textsuperscript{2+} and for Cd\textsuperscript{2+} ions along with the disappearance of shoulder at 243 nm\textsuperscript{22}.

Aromatic ring $\pi-\pi^*$ transition of R2 appear as a shoulder around 298 nm in acetonitrile and as a prominent peak at 313 nm in ethanol (Figure. 6).

Spectral changes observed for the addition of Cu\textsuperscript{2+}ions to R2 also generate peaks near 303 nm, 354 nm and 460 nm (Fig. 7a,b,c), which confirms that R2 is efficiently sensing the Cu\textsuperscript{2+} ions.

Discerning ability of R2 towards Hg\textsuperscript{2+}, Ni\textsuperscript{2+} and Pb\textsuperscript{2+} ions is exposed by the formation of new peak at 230 nm for Hg\textsuperscript{2+} (Fig. 8a), blue shift of 298 nm shoulder to 278 nm for Ni\textsuperscript{2+} (Fig. 8b) and conversion of 313 nm peak to a broad shoulder at the same wavelength (Figure 8c).
Fig. 5. Spectral changes noticed for R1 with the addition of a) Hg²⁺ ions b) Pb²⁺ ions c) Ni²⁺ ions

Fig. 6. UV-Visible spectrum of R2 in a) acetonitrile b) ethanol

Fig. 7. Change in the absorbance spectrum of R2 with Cu²⁺ ions a) overall changes b) formation of MLCT band c) generation of new peaks at 303 nm and 354 nm
Interaction studies with cyclic voltammetry

Responses to the applied potential were documented in cyclic voltammetry to establish the sensing priority order. Increasing $\Delta E_p$, $I_{pa}$ & $I_{pc}$ values (Table 1) noticed in the voltammograms recorded with different scan rate (20, 50 & 100 mV/sec) for metal free R1 (Fig. 9) and over blewed $\Delta E_p$ values (99-140 mV other than the expected 59 mV) emphasized the quasi-reversible one-electron redox process.

![Fig. 9. Cyclic voltammograms of R1 (1X10^-3 M) with different scan rate in a) acetonitrile b) ethanol](image)

The detected positive potential shift for oxidation peak and negative potential shift for reduction peak in the voltammograms logged in the CV titration (to 10 mL of 10^-3 molar R1 solution 20 μL of 10^-3 molar metal solution were added up to 7eq) under equimolar (10^-3 R1/10^-3 M2+) and multimolar (10^-3 R1/10^-1 M2+) concentration reveals that the synthesized receptors is capable of sensing deferent metal ions. Fig. 10 chronicled for the addition of Cd^{2+} ions is presented here as a reference.

![Table 1: Electrochemical parameters for R1](image)
The changes noticed in the I\textsubscript{pa} values (Table 2) for the addition of different metal ions with 10^{-3}M concentration (Fig. 11) and experiential magnified ΔE\textsubscript{p} amount (111-138 mV) discloses the different binding ability of metal cation and also the effect of electrostatic repulsion operated between the oxidized ferrocene moiety and bonded metal cations. Accessing the differences (ΔI\textsubscript{pa} %) between the I\textsubscript{pa} values noticed for the Fe\textsuperscript{II}/Fe\textsuperscript{III} oxidation wave of receptor solution and different metal ions added receptor solutions, uncover the coordination order of R1 as Hg\textsuperscript{2+} > Pb\textsuperscript{2+} > Ni\textsuperscript{2+} > Mn\textsuperscript{2+} > Cd\textsuperscript{2+} > Cu\textsuperscript{2+}.

Table 2: Electrochemical data for equimolar titration (R1, 10^{-3} M/M\textsuperscript{2+}, 10^{-3} M) (Scan Rate 50 mV/ sec)

| Addition | E\textsubscript{pa} (V) | E\textsubscript{pc} (V) | ΔE\textsubscript{p} (V) | E\textsubscript{1/2} (V) | I\textsubscript{pa} x10^{-5} (μA) | I\textsubscript{pc} x10^{-6} (μA) |
|----------|------------------------|------------------------|------------------------|------------------------|-------------------------------|-------------------------------|
| Solvent - Acetonitrile |
| Receptor | 0.766 | 0.63 | 0.136 | 0.698 | -1.352 | 4.959 |
| Hg\textsuperscript{2+} | 0.754 | 0.622 | 0.132 | 0.688 | -7.458 | 1.752 |
| Cu\textsuperscript{2+} | 0.766 | 0.628 | 0.138 | 0.697 | -1.386 | 4.112 |
| Ni\textsuperscript{2+} | 0.762 | 0.619 | 0.142 | 0.691 | -1.139 | 3.217 |
| Solvent - Ethanol |
| Receptor | 0.756 | 0.644 | 0.111 | 0.7 | -7.303 | 2.627 |
| Cd\textsuperscript{2+} | 0.754 | 0.64 | 0.113 | 0.697 | -7.624 | 3.591 |
| Mn\textsuperscript{2+} | 0.745 | 0.624 | 0.121 | 0.685 | -6.361 | 2.985 |
| Pb\textsuperscript{2+} | 0.748 | 0.624 | 0.124 | 0.686 | -8.869 | 4.353 |

For multimolar concentration, witnessed (Table 3) binding power based on declining in ΔI\textsubscript{pa} (%) of oxidation tendency of metal ion coupled with R1 is Cu\textsuperscript{2+} (86.07)>Hg\textsuperscript{2+} (85.6)>Ni\textsuperscript{2+} (85.47)>Cd\textsuperscript{2+} (15.4)>Mn\textsuperscript{2+} (81.96)>Pb\textsuperscript{2+} (83.15). Comparison of sensing priority of R1 towards several metal ions at homo and hetero molecular concentrations divulge R1 is effective towards Cu, Hg, and Ni ions at higher concentration of metal salts and at lower concentration adept lead for Hg, Pb and Ni ions (Figure 12).
Table 3: Electrochemical data for multimolar titration (R1, 10^{-3} M/M^2+, 10^{-1} M) (Scan Rate-50 mV/sec)

| Addition | E_{pa} (V) | E_{pc} (V) | ΔE_p (V) | E_{1/2} (V) | I_{pa} \times 10^{-6} (μA) | I_{pc} \times 10^{-6} (μA) |
|----------|------------|------------|----------|------------|-----------------|-----------------|
| Solvent - Acetonitrile |
| Receptor | 0.766 | 0.63 | 0.136 | 0.698 | -1.352 | 4.959 |
| Hg^{2+} | 0.791 | 0.638 | 0.152 | 0.714 | -9.39 | 3.592 |
| Cu^{2+} | 0.801 | 0.642 | 0.159 | 0.722 | -9.709 | 2.47 |
| Ni^{2+} | 0.774 | 0.651 | 0.123 | 0.712 | -9.31 | 2.231 |
| Solvent - Ethanol |
| Receptor | 0.756 | 0.644 | 0.111 | 0.7 | -7.303 | 2.627 |
| Cd^{2+} | 0.764 | 0.628 | 0.136 | 0.696 | -8.63 | 3.869 |
| Mn^{2+} | 0.793 | 0.603 | 0.19 | 0.698 | -1.317 | 6.233 |
| Pb^{2+} | 0.781 | 0.607 | 0.173 | 0.694 | -1.23 | 4.393 |

Like R1, R2 also display same trend (Table 4) in the values of ΔE_p, I_{pa} and I_{pc} upon scanning with different scan rate.

Homo molar (10^{-3}, R2/10^{-3}, M^{2+}) and hetero molar (10^{-3}, R2/10^{-1}, M^{2+}) titration studies (Fig.13) exemplify similar sensing behavior to metal ions. Calculated ΔI_{pa} (%) values depict, fastening trend for R2 under same molar condition as Pb^{2+}(81.3)>Cu^{2+}(76.8)>Mn^{2+}(73.35)>Ni^{2+}(23.6)>Cd^{2+}(22.12)>Hg^{2+}(8.4) (Table 5) and for different molar it is Cd^{2+}(82.1)>Mn^{2+}(80.4)>Pb^{2+}(79)>Cu^{2+}(26.1)>Ni^{2+}(22.3)>Hg^{2+}(18.1) (Table 6). Above observation relate that R2 shows better recognition to Pb, Cu and Mn ions at lower concentration. Higher quantity of Cd^{2+} is requisite for finding (Figure 14).

**Antimicrobial Studies**

Disc diffusion method (Mueller Hinton Agar base) was adopted to discover antibacterial activity of R1 & R2 against *Streptococcus faecalis*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Escherichia coli* (Fig.15). Likewise, antifungal studies for fungi *Candida albicans* and *Aspergillus niger* was done using Sabouraud’s Dextrose agar as base (Fig.16). Table 7 highlights the zone of inhibition in mm perceived for the synthesized compounds R1 & R2 in antimicrobial analysis.

Table 4: Electrochemical parameters for R2

| Scan Rate-mV/sec | E_{pa} (V) | E_{pc} (V) | ΔE_p (V) | E_{1/2} (V) | I_{pa} \times 10^{-6} (μA) | I_{pc} \times 10^{-6} (μA) |
|------------------|------------|------------|----------|------------|-----------------|-----------------|
| Solvent - Acetonitrile |
| 20 | 0.787 | 0.683 | 0.103 | 0.735 | -0.746 | 2.822 |
| 50 | 0.795 | 0.685 | 0.109 | 0.74 | -1.137 | 4.846 |
| 100 | 0.797 | 0.683 | 0.113 | 0.74 | -1.628 | 7.525 |
| Solvent - Ethanol |
| 20 | 0.741 | 0.648 | 0.092 | 0.695 | -0.397 | 0.681 |
| 50 | 0.75 | 0.651 | 0.099 | 0.7 | -0.638 | 2.531 |
| 100 | 0.766 | 0.642 | 0.124 | 0.704 | -1.037 | 5.328 |
Table 5: CV data for homo molar titration ($R_2$, $10^{-3}$ M/M$^{2+}$, $10^{-3}$ M) (Scan Rate-50 mV/sec)

| Addition | $E_{pa}$ (V) | $E_{pc}$ (V) | $\Delta E_{pa}$ (V) | $I_{pa} \times 10^{-5}$ (μA) | $I_{pc} \times 10^{-6}$ (μA) |
|----------|--------------|--------------|---------------------|-----------------------------|-----------------------------|
| Acetonitrile - solvent |
| Receptor | 0.799        | 0.685        | 0.113               | 0.742                        | -1.143                      | 4.888                       |
| $Hg^{2+}$ | 0.785        | 0.675        | 0.109               | 0.73                         | -1.248                      | 5.426                       |
| $Cu^{2+}$ | 0.799        | 0.685        | 0.113               | 0.742                        | -1.152                      | 3.961                       |
| $Ni^{2+}$ | 0.803        | 0.681        | 0.121               | 0.742                        | -1.498                      | 4.849                       |
| Ethanol - solvent |
| Receptor | 0.75         | 0.651        | 0.099               | 0.7                           | -6.277                      | 2.593                       |
| $Cd^{2+}$ | 0.752        | 0.642        | 0.109               | 0.697                        | -8.057                      | 4.05                        |
| $Mn^{2+}$ | 0.781        | 0.613        | 0.167               | 0.697                        | -1.672                      | 8.89                        |
| $Pb^{2+}$ | 0.774        | 0.634        | 0.14                | 0.704                        | -1.172                      | 4.565                       |

Table 6: CV data for hetero molar titration ($R_2$, $10^{-3}$ M/M$^{2+}$, $10^{-1}$ M) (Scan Rate-50 mV/sec)

| Addition | $E_{pa}$ (V) | $E_{pc}$ (V) | $\Delta E_{pa}$ (V) | $I_{pa} \times 10^{-5}$ (μA) | $I_{pc} \times 10^{-6}$ (μA) |
|----------|--------------|--------------|---------------------|-----------------------------|-----------------------------|
| Solvent - Acetonitrile |
| Receptor | 0.799        | 0.685        | 0.113               | 0.742                        | -1.143                      | 4.888                       |
| $Hg^{2+}$ | 0.795        | 0.659        | 0.136               | 0.727                        | -1.397                      | 5.451                       |
| $Cu^{2+}$ | 0.797        | 0.661        | 0.136               | 0.729                        | -1.548                      | 5.785                       |
| $Ni^{2+}$ | 0.777        | 0.659        | 0.117               | 0.718                        | -1.473                      | 5.653                       |
| Solvent - Ethanol |
| Receptor | 0.75         | 0.651        | 0.099               | 0.7                           | -6.277                      | 2.593                       |
| $Cd^{2+}$ | 0.777        | 0.6221       | 0.154               | 0.699                        | -1.117                      | 5.803                       |
| $Mn^{2+}$ | 0.756        | 0.624        | 0.132               | 0.69                         | -1.226                      | 5.649                       |
| $Pb^{2+}$ | 0.768        | 0.619        | 0.148               | 0.694                        | -1.306                      | 7.112                       |

Fig. 14. Comparison of binding ability of $R_2$ and metal ion concentration

Fig. 15. Zone of inhibition for a) Streptococcus faecalis, b) Staphylococcus aureus, c) Salmonella typhimurium and d) Escherichia coli

Fig. 16. Zone of inhibition for a) Candida albicans b) Aspergillus niger

Defense mechanism rendered by $R_1$ and $R_2$ to prevent the growth of fungus *Aspergillus niger* is nearly 150 to 160% higher than that of the value witnessed for the standard Ketoconazole, which is unusual. Fungus *Candida albicans* progress is also prevented up to 50% of the standard value. Above result discloses that the compound $R_1$ can be examined for antifungal agents formulation as there are only minimum numbers of antifungal agents available in the market\textsuperscript{15}. Retardant nature of $R_1$ & $R_2$ displayed for two Gram-positive and two Gram-negative bacteria are on par with standard Ciprofloxacin.
Table 7: In-vitro antimicrobial studies data

| S.No | Microorganisms          | Control | R1 | R2 | Ciprofloxacin/Ketoconazole |
|------|--------------------------|---------|----|----|----------------------------|
|      |                          | zone of inhibition in mm for bacteria | zone of inhibition in mm for fungi |
| 1    | Staphylococcus aureus    | -       | 10 | 8  | 25                         |
| 2    | Streptococcus faecalis   | -       | 6  | -  | 24                         |
| 3    | Escherichia coli         | -       | 8  | 7  | 12                         |
| 4    | Salmonella typhimurium    | -       | 8  | 6  | 27                         |
| 1    | Candida albicans         | -       | 10 | 10 | 25                         |
| 2    | Aspergillus niger        | -       | 12 | 13 | 8                          |

Molecular docking studies

The purpose of molecular docking is to determine the mode of interaction of the complex protein-ligand. Docking results arrived are presented in the Table 8. For selected fungi and bacteria, ligand R1 binding 3D and 2D views (Fig. 17) and ligand R2 binding 3D and 2D views (Fig. 18) are presented. The binding scores for both the compounds fall between -3.61 to -7.45 Kcal mol⁻¹. Compound R1 exhibited better binding affinity with the protein 6KVQ (-6.52 Kcal mol⁻¹) and R2 showed higher binding affinity with proteins 3K4Q and 7BU2 with -7.32 and -7.45 Kcal.mol⁻¹ respectively. Proteins 3K4Q & 6TZ6 are present in fungi, whereas protein 7BU2 is present in bacteria. Both the compounds R1 and R2 tested were involved in H-bond against active site residue 47 ILE, 50 PHE, 59 VAL, 60 ILE and 155 THR. Above results confirm that such type of ligand would represent a promising class for further development of a new class of antimicrobial agents which deserves further exploration.

Table 8: Results obtained from molecular docking studies

| PDB  | R1 Free binding energy, kcal mol⁻¹ | R1 Hydrogen bonds with receptor amino acids | R1 Distance (Å) | R2 Hydrogen bonds with receptor amino acids | R2 Distance (Å) |
|------|-----------------------------------|-------------------------------------------|----------------|-------------------------------------------|----------------|
| 1PTF | -3.61                             | 30-TYR                                    | 3.93           | 23-VAL                                    | 3.44           |
|      |                                   | 50-PHE                                    | 3.69           | 24-GLN                                    | 3.95           |
|      |                                   | 59-VAL                                    | 3.88           | 28-LYS                                    | 3.80           |
|      |                                   | 60-ILE                                    | 3.98           | 47-ILE                                    | 3.66           |
|      |                                   | 114-PHE                                   | 3.23           |                                           |                |
|      |                                   | 45-LYS                                    | 3.04           | 27-GLN                                    | 3.91           |
| 3K4Q | -4.18                             | 47-ILE                                    | 2.15           | 277-LYS                                   | 3.55           |
|      |                                   |                                           |                | 278-LYS                                   | 3.37           |
| 4YXB | -5.21                             | 22-VAL                                    | 3.90           | 26-ALA                                    | 3.67           |
|      |                                   | 155-THR                                   | 3.19           | 28-ILE                                    | 3.83           |
|      |                                   | 157-GLU                                   | 3.56           | 29-PRO                                    | 3.78           |
|      |                                   | 190-LEU                                   | 3.70           | 46-ILE                                    | 3.27           |
|      |                                   | 225-LEU                                   | 3.30           | 51-ARG                                    | 3.23           |
|      |                                   | 153-LYS                                   | 2.27           | 188-ASN                                   | 3.32           |
| 6KVQ | -6.52                             | 155-THR                                   | 3.05           | 189-VAL                                   | 3.50           |
|      |                                   | 192-ASN                                   | 3.27           |                                           |                |
|      |                                   | 198-GLY                                   | 3.76           |                                           |                |
|      |                                   | 103-ASP                                   | 2.95           | 50-PHE                                    | 3.84           |
| 6TZ6 | -5.23                             | 142-ARG                                   | 2.73           | 59-VAL                                    | 3.74           |
|      |                                   | 142-ARG                                   | 2.79           | 60-ILE                                    | 3.65           |
|      |                                   |                                           |                | 97-TYR                                    | 3.72           |
| 7BU2 | -5.48                             | 50-PHE                                    | 3.84           | 42-HIS                                    | 3.64           |
|      |                                   | 59-VAL                                    | 3.74           | 180-LEU                                   | 3.85           |
|      |                                   | 60-ILE                                    | 3.65           | 240-ASN                                   | 3.31           |
|      |                                   |                                           |                | 332-ARG                                   | 3.75           |
CONCLUSION

Synthesis of unsymmetrical Schiff base compounds having ferrocencarboxaldehyde azomethine at one end and imine with aromatic aldehyde at the other end has not been reported so far. Our team have overawed the hurdles faced by the scientist by synthesizing Schiff bases \(N'-(E)-2\text{-nitrobenzylidene}-2-(E)-2\text{-}(\text{ferrocenylidene})\text{hydrazine-1-carbothiohydrazide}\) and \(N'-(E)-2\text{-hydroxy-5-nitrobenzylidene-2-(E)-2-}(\text{ferrocenylidene})\text{hydrazine-1-carbothiohydrazide}\). Spectral analysis by FTIR, 'HNMR and Mass spectrum authenticate the formation of desired sensors. Multi-metal ions sensing competency of newly prepared materials have been uncovered in UV-Visible spectral studies. Results elucidated from electrochemical studies are harmonized with the data of electronic spectral studies. Assessment of difference in anodic current perceived \((\Delta I_{pa})\) throwback the relation between the concentration of metal ions and receptors for appropriate binding.
Exaggerated antifungal activities identified in in-vitro studies and high free binding energy values observed in molecular docking studies for fungus *Aspergillus niger*, provoke the rhythm of pharmaceutical research to be under taken.

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Conflicts of interest

“There are no conflicts to declare”.

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