## Accepted Manuscript

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Authors: P. Madhu, P. Sivakumar



PII:	S1010-6030(18)31306-6
DOI:	https://doi.org/10.1016/j.jphotochem.2018.11.033
Reference:	IPC 11605

To appear in: Journal of Photochemistry and Photobiology A: Chemistry

Received date:7 September 2018Revised date:23 November 2018Accepted date:23 November 2018

Please cite this article as: Madhu P, Sivakumar P, A novel pyridinepyrazole based selective ïurn-offfluorescent chemosensor for Fe(III) ions, *Journal of Photochemistry and amp; Photobiology, A: Chemistry* (2018), https://doi.org/10.1016/j.jphotochem.2018.11.033

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# A novel pyridine-pyrazole based selective "turn-off" fluorescent chemosensor for Fe(III) ions

P. Madhu<sup>a,b,\*</sup> and P. Sivakumar<sup>c</sup>

<sup>a</sup>Research and Development Centre, Bharathiar University, Coimbatore-641 046, TamilNadu, India

<sup>b</sup>Department of Chemistry, Thiruvalluvar Government Arts College, Rasipuram-637 401, TamilNadu, India

<sup>c</sup>Department of Chemistry, Arignar Anna Government Arts College, Namakkal-637 002, TamilNadu, India

\*Corresponding Author E-mail: madhup.chem@gmail.com

Tel: +91 9944502310

### Graphical abstract



#### Highlights

- The new pyridine-pyrazole based chemosensor was designed, synthesized and characterized for the detection of  $Fe^{3+}$  ions.
- The **PPPC** exhibits high selectivity and sensitivity toward Fe<sup>3+</sup> ions over other metal ions.
- Fluorescence quenching mechanism is due to the binding interaction of the  $Fe^{3+}$  ion with **PPPC**.
- $Fe^{3+}$  ion inhibits the ICT process by forming a complex with **PPPC**.

#### Abstract

A novel pyridine-pyrazole based "turn-off" fluorescent chemosensor namely, 5-N-(pyridine-2-yl)-3-(pyridine-4-yl)-1H-pyrazole-5-carboxamide (**PPPC**) was designed, synthesized and well characterized by NMR, ESI-MS and FT-IR spectroscopic techniques. UV-Vis absorption and fluorescence spectroscopic studies show that **PPPC** exhibits high selectivity and sensitivity towards  $Fe^{3+}$  ion in DMSO/H<sub>2</sub>O solution (9:1, v/v) over other metal ions. The binding constant (K) of **PPPC** with  $Fe^{3+}$  was calculated to be 5.1 x 10<sup>-2</sup> M and 6.1 x 10<sup>-2</sup> M from Benesi-Hildebrand plot using UV-vis and fluorescence spectrophotometer respectively. The detection limit of **PPPC** for  $Fe^{3+}$  was further determined as 57 nM and 88 nM by UV-vis and fluorescence titrations. Moreover, the binding mechanism of  $Fe^{3+}$  with **PPPC** was confirmed by DFT study.

**Keywords:** Chemosensor; Fluorescent sensor, Iron ion; Pyridine-pyrazole derivative; DFT studies.

#### 1. Introduction

The design and synthesis of chemosensors with high selectivity and sensitivity for heavy and transition metal ions have considerable research attention over the years because of its important applications in biological, industrial and environmental processes [1-3].

Among various metal ions, iron ion (Fe<sup>3+</sup>) is an essential element within the human body which plays an important role in the growth and development of living systems. Also, iron provides the oxygen carrying capacity of hemoglobin and acts as a cofactor in many enzymatic reactions *viz*. metabolism, electron transfer and nucleic acids (DNA and RNA) synthesis [4]. Presence of trace amount in the human body can cause an imbalance in the iron concentration and can have a dangerous effect on human health. The deficiency of Fe<sup>3+</sup> causes various biological disorders in living systems such as low blood pressure, anemia [5] and decreased immunity. Excess of Fe<sup>3+</sup> concentration may also equally harmful or even fatal [6], which could cause damage the lipids, proteins and nucleic acids. Recently, researchers revealed that Fe<sup>3+</sup> could involve some neurodegenerative diseases such as Parkinson's and Alzheimer's disease [7-11]. Thus, the development of new methods to detect iron has emerged and tremendous attention in the field of research. Nowadays some of the instrumental techniques such as atomic

absorption spectroscopy (AAS), inductively coupled plasma atomic emission spectrometry (ICP-AES), inductively coupled plasma mass spectrometry (ICP-MS) and electrochemical methods [12] have been used for the detection of iron ions, but these methods require highly expensive instrument and professional operators. Compared with the traditional analytical methods, colorimetric chemosensors are an excellent method because they have low cost, good resolution, high sensitivity and selectivity and easy monitoring of the target ions. Although enormous excellent fluorescent chemosensors were developed and reported to selective sensing ability for various transition metal ions such as Hg<sup>2+</sup> [13-24], Cu<sup>2+</sup> [25-29], Zn<sup>2+</sup> [30-33] and Ag<sup>2+</sup> [34-38], there are very few fluorescent probes are reported for Fe<sup>3+</sup> ions. Thus there is an immediate requirement to develop some highly selective fluorescent chemosensor for Fe<sup>3+</sup> to satisfy the biological and environmental needs.

A number of organic molecules containing a wide range of donor sites were reported for detection of heavy metals. Among them, pyrazole and its derivatives are one of the most important classes of a chelating ligand for the heavy metal atoms. The combination with pyridine moiety the pyrazole derivative strengthen in its complexing behavior and can act as a good candidate for complexation with a wide range of metals due to its more nitrogen donor sites. Thus, in this study, we designed and synthesized a novel pyridine-pyrazole based fluorescent chemosensor for  $Fe^{3+}$  ion (scheme 1), which showed a selective and sensitive recognition towards the  $Fe^{3+}$  ion over other metal ions. The sensing behaviour of  $Fe^{3+}$  mostly depends on the fluorescence quenching mechanism due to its high paramagnetic nature.

### 2. Experimental

#### 2.1. Materials and Instrumentation

All chemicals were purchased from Sigma-Aldrich/Merck and used as received without further purification. Solvents used were analytical grade and double distilled water was used throughout the experiment. The progress of all reactions was monitored by TLC using silica gel 60F254 and visualized under UV 254-366 nm and iodine.

<sup>1</sup>H NMR (300MHz) and <sup>13</sup>C NMR (75MHz) were recorded on Bruker NMR instrument in CDCl<sub>3</sub> and DMSO-d<sub>6</sub> as solvents and TMS as an internal standard. Electrospray ionization mass spectra (ESI-MS) were recorded on LCQ Fleet mass spectrometer (Thermo Fisher Instruments Ltd., US). Fluorescence measurements were recorded using fluorescence

spectrophotometer (Agilent Technologies, US). Shimadzu UV-1800 UV-vis spectrophotometer was used to record UV absorption spectra.

- 2.2. Synthesis and Characterization
- 2.2.1. Synthesis of ethyl 2,4-dioxo-4-(pyridin-4-yl)butanoate (1) [39]

To a sodium hydride (60 % in paraffin oil) (1.98 g, 0.049 mol), 10 mL of Dimethyl formamide was added dropwise at 5°C under nitrogen atmosphere. To this mixture, 4-acetylpyridine (3.0 g, 0.024 mmol) was added dropwise and stirred for 30 min at ambient temperature. Then diethyl oxalate (4.03 mL, 0.029 mol) was added slowly and the reaction mixture was again stirred for 3 h at room temperature. The completion of the reaction was monitored by TLC and quenched with crushed ice. Then the reaction mixture was extracted with ethyl acetate (1 x 100 mL), washed with water (100 mL) and brine solution (100mL). The organic layer was separated, dried over anhydrous sodium sulfate and evaporation of solvent affords the expected intermediate 1. (White solid, Yield 78%). <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.78 (d, *J* = 5.5 Hz, 2H), 7.85 (d, *J* = 5.5 Hz, 2H), 4.42 (q, *J* = 14.0, 7.1 Hz, 2H), 1.42 (t, *J* = 7.0 Hz, 3H). <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  192.89, 173.26, 165.10, 150.57, 137.70, 122.79, 61.75, 29.62, 14.15.

### 2.2.2. Synthesis of ethyl 3-(pyridin-4-yl)-1H-pyrazole-5-carboxylate (2) [39]

To acetic acid (10 mL) solution of intermediate 1 (2.0g, 9.04 mmol), hydrazine hydrate (2.26 mL, 45.2 mmol) was added dropwise over 5 min at 0°C. The reaction mixture was stirred for 15 h at room temperature and quenched with crushed ice, basified with sodium bicarbonate solution. Then the reaction mixture was extracted with ethyl acetate (150 mL) washed with water (150 mL) and brine solution (150 mL). The organic layer was separated, dried over anhydrous sodium sulfate and evaporated under reduced pressure yielded intermediate 2. (White solid. Yield 87 %). <sup>1</sup>H NMR (300 MHz, DMSO-D<sub>6</sub>)  $\delta$  14.36 (s, 1H), 8.64 (d, *J* = 5.7 Hz, 2H), 7.85 (d, *J* = 5.7 Hz, 2H), 7.51 (s, 1H), 4.36 (q, *J* = 14.0, 7.0 Hz, 2H), 1.34 (t, *J* = 7.1 Hz, 3H). <sup>13</sup>C NMR (75 MHz, DMSO-D<sub>6</sub>)  $\delta$  163.78, 150.81, 135.88, 127.73, 120.08, 107.35, 61.33, 14.68. ESI-LC/MS calculated m/z 217.09, found 218.08 (M+1)<sup>+</sup>. IR: 3124, 2978, 1725, 1608, 1571, 835, 765.

2.2.3. Synthesis of 4-(5-carboxy-1H-pyrazol-3-yl)pyridin-1-ium (3) [39]

Intermediate 2 (1.5 g, 6.9 mmol) was dissolved in 6N hydrochloric acid (7.5 mL) and heated to reflux for 3 h. The completion of the reaction was monitored by TLC. Then the reaction mixture was stirred with tetrahydrofuran (15 mL) then filtered and washed with tetrahydrofuran (15 mL). The filtered intermediate 3 was taken to the next step. (White solid, Yield 95%). <sup>1</sup>H NMR (300 MHz, DMSO-D<sub>6</sub>)  $\delta$  8.93 (bs, 2H), 8.47 (d, *J* = 5.3 Hz, 2H), 7.80 (s, 1H). <sup>13</sup>C NMR (75 MHz, DMSO-D<sub>6</sub>)  $\delta$  160.58, 148.04, 142.48, 122.51, 109.74. ESI-LC/MS calculated m/z 189.05, found 190.11 (M+1)<sup>+</sup>. IR: 3046, 2865, 1694, 1631, 1589, 830, 773.

2.2.4. Synthesis of N-(pyridin-2-yl)-3-(pyridin-4-yl)-1H-pyrazole-5-carboxamide (PPPC)

The intermediate 3 (1.0g, 5.29 mmol) was dissolved in Acetonitrile (20 mL) and then TBTU (2.03g, 6.33 mmol) and triethylamine (1.10 mL, 7.93 mmol) was added. The reaction mixture was stirred for 30 min at room temperature. To the reaction mixture, 2-aminopyridine (0.497 g, 5.29 mmol) was added at 0° C and again stirred for 24 h at room temperature. Then the reaction mixture was quenched with crushed ice. The precipitate obtained was filtered and dried in vacuum yielded the target compound **PPPC** and used for further studies. (White solid, Yield 92 %). <sup>1</sup>H NMR (300 MHz, DMSO-D<sub>6</sub>)  $\delta$  14.30 (s, 1H), 10.97 (s, 1H), 8.68 – 8.63 (m, 2H), 8.42 (bs, 1H), 8.23 (d, *J* = 7.3 Hz, 1H), 7.89 – 7.81 (m, 3H), 7.46 (s, 1H), 7.25 – 7.17 (m, 1H). <sup>13</sup>C NMR (75 MHz, DMSO-D<sub>6</sub>+CDCl<sub>3</sub>)  $\delta$  158.98, 148.43, 148.22, 146.16, 136.38, 118.01, 117.75, 112.10, 111.11, 104.61, 103.10. ESI-LC/MS calculated m/z 265.10, found 266.14 (M+1)<sup>+</sup>. IR: 3108, 2161, 1736, 1638, 1538, 830, 797.

### 2.3. Stock solution preparation

The stock solution of **PPPC** was prepared at a concentration of  $1 \times 10^{-3}$  M in DMSO/H<sub>2</sub>O (9:1, v/v). 20 µL of this stock solution was diluted to 2 mL with the respective solvent system to cover the final concentration as in 10 µM level. The stock solution of metal ions was prepared in  $1 \times 10^{-2}$  M concentration by adding the appropriate amount of metal cation using double distilled water. For selectivity experiment 10 equiv. of the metal ions were used and the stock solution was diluted (10 times) for the sensitivity experiments. For Job's plot analysis  $1 \times 10^{-5}$  M of **PPPC** and metal solutions were used. For the purpose of spectral measurements, detection solutions of **PPPC** and metal solutions were freshly prepared and used.

### 2.4. UV-vis and fluorescence spectral studies

Using UV-vis spectrophotometer and fluorescence spectrophotometer, the absorption and emission spectra were recorded respectively. 10  $\mu$ M solution of **PPPC** in DMSO/H<sub>2</sub>O solution (9:1, v/v) was prepared at room temperature and added with the metal ion solution to the quartz optical cell. After the addition of metal ions, spectral data were recorded. In the fluorescence measurements, the probe **PPPC** shows an admirable fluorescence band at 354 nm with the excitation wavelength 310 nm.

### 2.5. Calculation of binding constant

The binding constant (K) for the formation of complex, **PPPC-F**e<sup>3+</sup> has been determined by using both UV-vis and Fluorescence Spectrophotometer. First, the binding constant was determined by UV-vis Spectral titration. The concentration of **PPPC** was kept constant throughout the experiments and varying the concentration of the Fe<sup>3+</sup> ions. The constant value of **PPPC** with the corresponding Fe<sup>3+</sup> was determined by using Benesi-Hildebrand equation. Binding constant *K* was calculated by following the equation stated below.

$$1/(A-A_o) = 1/\{Ka(A_{max}-A_o) [Fe^{3+}]\} + 1/[A_{max}-A_o]$$

Here,  $A_o$  is the absorbance of the receptor, **PPPC** in the absence of guest  $Fe^{3+}$ , A is the absorbance recorded in the presence of added  $Fe^{3+}$  ions,  $A_{max}$  is absorbance in presence of added  $[Fe^{3+}]_{max}$  and K is the binding constant (M<sup>-1</sup>). The binding constant (K) could be determined from the slope of the straight line obtained in the plot of  $1/(A-A_o)$  against  $1/[Fe^{3+}]$ . Similarly, the binding constant value can also be determined by fluorescence spectrophotometer by using modified Benesi-Hildebrand equation stated below,

$$1/I - I_{min} = 1/I_{max} - I_{min} + (1/K[Fe^{3+}])(1/I_{max} - I_{min}).$$

Where,  $I_{min}$ , I, and  $I_{max}$  are the emission intensities of **PPPC**, at an intermediate  $Fe^{3+}$  concentration with **PPPC**, and at a concentration of complete saturation respectively. K is the binding constant and  $[Fe^{3+}]$  is the concentration of  $Fe^{3+}$  ions. From the plot of  $1/(I-I_{min})$  against  $[Fe^{3+}]^{-1}$  for **PPPC** with  $Fe^{3+}$  ion, the binding constant value (K) has been determined from the slope.

#### 3. Results and discussion

### 3.1. Synthesis and structural characterization PPPC



**Reagents and conditions:** (i) NaH, Diethyloxalate, DMF, 1h, RT; (ii) NH<sub>2</sub>NH<sub>2</sub>.H<sub>2</sub>O, ethanol, 24h, RT; (iii) 6N HCl, 6h, reflux; (iv) TBTU, 2-aminopyridine, TEA, 3h, 0 °C-RT

Scheme 1: Synthetic route of PPPC

As illustrated in scheme 1, the targeted probe **PPPC** was synthesized in a multi step process. Intermediate 1 was prepared from 4-acetylpyridine and diethyl oxalate in the presence of NaH. Treatment of intermediate 1 with hydrazine hydrate promotes cyclisation and gives the intermediate 2. The resulting intermediate 2 was hydrolyzed using HCl gives intermediate 3. Finally, the intermediate **3** reacts with 2-aminopyridine gives the probe **PPPC** via a condensation reaction. All the compounds were satisfactorily characterised using <sup>1</sup>H NMR, <sup>13</sup>C NMR, FT-IR and Mass spectral measurements. The characterization of PPPC can be well explained by <sup>1</sup>H NMR spectral studies. The singlets at 14.30 ppm and 10.97 ppm clearly indicates the presence of pyrazole NH and carboxamide NH respectively. The doublets for 2 protons and singlet for 1 proton at 8.65 ppm, 8.63 ppm appears for pyridyl protons (2 protons from the 4-substituted ring and 1 proton from the 2-substituted ring). The singlet appeared at 7.19 ppm indicates the presence of pyrazole CH. Similarly, the <sup>13</sup>C NMR spectrum also confirms the product formation. The appearance of a peak at 158.97 ppm indicates the presence of amide carbonyl carbon. The 3 peaks appeared around 148 ppm indicates the presence of pyridyl carbons (2 carbon from the 4substituted ring and 1 carbon from the 2-substituted ring). The peak appeared around 103.1 ppm indicates the presence of pyrazolyl carbon. In addition, the PPPC was characterized by using the ESI-Mass spectroscopic technique. The Mass spectrum clearly showed positive mode signal at

266.14. The FT-IR spectrum also gives some additional information about the probe PPPC. The band appeared around 1638 cm<sup>-1</sup> indicates the presence of carboxamide unit. Similarly, the band appeared around 1578 cm<sup>-1</sup> indicates the presence of C=N unit.

3.2. Selectivity of **PPPC** to Fe<sup>3+</sup> using UV-vis and fluorescence spectra

The selectivity of **PPPC** in DMSO/H<sub>2</sub>O solution (9:1, v/v) was investigated with various metal ions such as Mg<sup>2+</sup>, Ca<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup>, Fe<sup>3+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>, Ba<sup>2+</sup> and Hg<sup>2+</sup>. Initially, the synthesized probe **PPPC** (10  $\mu$ M) in DMSO/H<sub>2</sub>O solution (9:1, v/v) shows a single band at 255 nm. On the addition of 10 equiv. of various metal ions, no significant changes occurred except iron ions. As shown in Fig. 1, the addition of Fe<sup>3+</sup> ion a new absorption band was observed at 340 nm and Fe<sup>2+</sup> also produced some small interaction with the probe **PPPC**, but it is negligible compared to Fe<sup>3+</sup> ions.



Fig. 1: Selectivity spectrum of the PPPC with 10 equiv. of the metal ions in UV-vis spectrum

To acquire further strong evidence of the selectivity of **PPPC** for metal ions, the fluorescence spectra were recorded between **PPPC** and various metal ions (Mg<sup>2+</sup>, Ca<sup>2+</sup>, Mn<sup>2+</sup>, Fe<sup>2+</sup>, Fe<sup>3+</sup>, Co<sup>2+</sup>, Ni<sup>2+</sup>, Cu<sup>2+</sup>, Zn<sup>2+</sup>, Cd<sup>2+</sup>, Ba<sup>2+</sup> and Hg<sup>2+</sup>) under the same conditions. Actually, the probe **PPPC** in DMSO/H<sub>2</sub>O solution (9:1, v/v) shows an excellent fluorescence band at 354 nm in fluorescence spectrometer with the excitation wavelength of 310 nm. Upon addition of Fe<sup>3+</sup> ions, the fluorescence intensity was quenched significantly (Fig. 2). The quantum yield ( $\Phi$ ) of

**PPPC** and **PPPC**-Fe<sup>3+</sup> were found to be 0.044 and 0.010 respectively. The dramatic changes in fluorescence can be attributed to the complex formed between the chemosensor **PPPC** and Fe<sup>3+</sup>. This "turn-off" fluorescence mechanism could be imputed to the paramagnetic quenching behaviour of Fe<sup>3+</sup>. Further to confirm the binding mode of **PPPC** with Fe<sup>3+</sup>, the phenyl analogue (**PA**) of **PPPC** (replacing 2-pyridyl moiety by phenyl) was synthesized and characterized (Fig. S14 and S15) and the sensing effect in emission spectroscopy was studied. In the presence of Fe<sup>3+</sup> the fluorescent of the **PA** was quenched (Fig. S16), but not like the present probe, **PPPC**. The quenching efficiency of the Fe<sup>3+</sup> was very less compare to **PPPC**. This is due to the **PA** have two binding sites and **PPPC** has three sites along with 2-pyridyl moiety which interact strongly with Fe<sup>3+</sup> than **PA**. Thus, the nitrogen atom in 2-pyridyl moiety plays a crucial role in the sensing system. Therefore the **PPPC** has better selectivity towards Fe<sup>3+</sup> ions and could be used as a selective chemosensor for Fe<sup>3+</sup> ions.



Fig. 2: Selectivity spectrum of PPPC with 10 equiv. of the metal ions in fluorescence spectroscopy

### 3.3. Sensitivity of **PPPC** to $Fe^{3+}$ using UV-vis and fluorescence spectra

The sensing behaviour of **PPPC** toward  $Fe^{3+}$  was examined by the absorption spectral titration of **PPPC** with  $Fe^{3+}$  in DMSO/H<sub>2</sub>O solution (9:1, v/v). Upon gradual addition of  $Fe^{3+}$  ions (0-22 µM) to the probe **PPPC**, good linearity was observed with the detection limit of 57

nM (Fig. 3 & 4). The binding constant for **PPPC** with  $Fe^{3+}$  was determined to be 5.1 x  $10^2 M^{-1}$  using Benesi-Hildebrand plot (Fig. 5) [40,41]. The complex stoichiometry of **PPPC** with  $Fe^{3+}$  also examined using Job's plot [42-44]. Fig. 6 confirmed the 1:1 stoichiometry between **PPPC** and  $Fe^{3+}$  where the absorbance at 340 nm got maximum when the mole fraction of  $Fe^{3+}$  was 0.5.



**Fig. 3:** Sensitivity spectrum of **PPPC** with increasing concentration Fe<sup>3+</sup> ions in UV-vis spectrum



Fig. 4: Linear plot derived from sensitivity titrations in UV-vis spectroscopy



**Fig. 5:** B-H plot analysis of Fe<sup>3+</sup> with **PPPC** from UV-vis. spectroscopy



Fig. 6: Job's plot analysis of the **PPPC** with  $Fe^{3+}$  ions (10<sup>-5</sup> M) in UV-vis spectroscopy.

To promote further insight into the binding ability of **PPPC** with Fe<sup>3+</sup>, titration of Fe<sup>3+</sup> against **PPPC** in DMSO/H<sub>2</sub>O solution (9:1, v/v) was monitored using fluorescence spectra. In the absence of Fe<sup>3+</sup>, the free **PPPC** showed an excellent fluorescence band at 354 nm. Upon gradual addition of Fe<sup>3+</sup> ions (0-34  $\mu$ M) to **PPPC**, a good linearity was observed from 6 to 30  $\mu$ M with a strong fluorescence quenching. Furthermore, the lower detection limit of **PPPC** for Fe<sup>3+</sup> was calculated as 88 nM using the formula  $3\sigma$ /slope (Fig. 7 & 8), which is much lower than the maximum level of Fe<sup>3+</sup> (5.4  $\mu$ M) permitted by the US Environmental protection agency [45, 46] in drinking water. The association constant for **PPPC** with Fe<sup>3+</sup> was calculated to be 6.1 x  $10^2 \text{ M}^{-1}$  using a Benesi-Hildebrand plot (Fig. 9).



**Fig. 7:** Sensitivity spectrum of **PPPC** with increasing concentration Fe<sup>3+</sup> ions in fluorescence spectroscopy



Fig. 8: Linear plot derived from sensitivity titrations in fluorescence spectroscopy



Fig. 9: B-H plot analysis of Fe<sup>3+</sup> with **PPPC** from fluorescence spectroscopy

### 3.4. Competitive experiments of **PPPC** for $Fe^{3+}$ with other metal ions

The selectivity of **PPPC** for  $Fe^{3+}$  over other metal ions was further investigated by performing competitive experiments in DMSO/H<sub>2</sub>O solution (9:1, v/v). Chemosensor **PPPC** was mixed with 10 equiv. of  $Fe^{3+}$  ions in the presence of 15 equiv. of other metal ions ( $Fe^{2+}$ ,  $Zn^{2+}$ ,  $Cu^{2+}$ ,  $Ni^{2+}$ ,  $Co^{2+}$ ,  $Mn^{2+}$ ,  $Mg^{2+}$ ,  $Ba^{2+}$ ,  $Ca^{2+}$ ,  $Cd^{2+}$  and  $Hg^{2+}$ ) prepared separately. The resulting individual solution was analyzed by UV-vis and fluorescence spectrophotometers. As illustrated in Fig. 10 & 11, the probe **PPPC** shows remarkable sensing ability towards  $Fe^{3+}$  ions with a higher concentration of other metal ions, which reveals that the probe **PPPC** has higher affinity with  $Fe^{3+}$  than other metal ions.









3.5. Proposed mechanism

To determine the binding mechanism of **PPPC** with  $Fe^{3+}$ , the <sup>1</sup>H NMR titration was carried out both in the absence and presence of  $Fe^{3+}$  (Fig. 12). Gradual formation of **PPPC**-  $Fe^{3+}$  complex with the addition of  $Fe^{3+}$  to **PPPC** caused the N-H proton signal of pyrazole ring and N-

H proton signal of amide moiety was disappear gradually in <sup>1</sup>H NMR spectra, indicating that the pyrazole nitrogen atom and amide nitrogen atom are the binding sites for Fe<sup>3+</sup>. In addition, to assigning the sensing mechanism of **PPPC** with Fe<sup>3+</sup>, FT-IR spectral analyses were done for **PPPC** with and without Fe<sup>3+</sup> ions. IR spectrum of P**PPC** showed a strong band at 1578 cm<sup>-1</sup>, which is shifted in **PPPC**-Fe<sup>3+</sup> (Fig. S17) indicating that the N-atom in 2-pyridyl moiety is another binding site in the **PPPC**-Fe<sup>3+</sup> complex.

The plausible recognition of **PPPC** to  $Fe^{3+}$  was further supported by the results of mass spectral studies. ESI-Mass spectra gave a peak at m/z 320.02, which corresponds to **PPPC**+Fe<sup>3+</sup>+H<sup>+</sup> (Fig. S18). This clearly indicates the 1:1 binding stoichiometry between **PPPC** and Fe<sup>3+</sup>. Research for the <sup>1</sup>H NMR, FT-IR, and ESI-Mass spectra gave a remarkable evidence for the proposed binding mechanism.



**Fig. 12.** <sup>1</sup>H NMR titration of **PPPC** in the presence of the varying amounts of Fe<sup>3+</sup> (in DMSO-d<sub>6</sub>)

### 3.6. Density functional theory (DFT) calculation

To get more evidence for the interaction between **PPPC** and  $Fe^{3+}$ , the energy-optimized structure of **PPPC** and its corresponding complex **PPPC-Fe<sup>3+</sup>** were obtained by density functional theory (DFT) calculations with the B3LYP/6.311++G(d,p) and LANL2DZ level of the Gaussian 09W program. As shown in Fig. 12, the probe **PPPC** shows more electron density on pyrazole part at HOMO. In LUMO, these electron densities were transferred to pyridine moiety. This observation clearly indicates that the probe involved intra-molecular charge transfer (ICT), which leads to fluorescence on in emission profile. In general,  $Fe^{3+}$  ions have best quenching behaviour due to its paramagnetic nature. Here, the Fe<sup>3+</sup> ion not only acts as a perfect quencher, but it also inhibits the ICT process by forming a complex with **PPPC**. This was clearly observed in Fig. 13. In the energy-optimized structure of **PPPC-Fe<sup>3+</sup>**, the electrons are highly localized on pyridine ring at HOMO, whereas in LUMO, the electron cloud transferred into the metal environment. In addition, the energy gap between HOMO and LUMO for PPPC (4.73 eV) is higher than in the **PPPC-Fe<sup>3+</sup>** complex (3.31 eV). On the other hand, the energy gap between HOMO and LUMO of **PPPC-Fe<sup>3+</sup>** complex has decreased to 1.42eV due to the formation of the **PPPC-Fe<sup>3+</sup>** complex. The results show that the probe **PPPC** was easily bind with  $Fe^{3+}$  ions and form stable **PPPC-**  $Fe^{3+}$  complex as shown in scheme 2.



Fig. 13: DFT analysis of PPPC and iron complex (left to right).



Scheme 2: Proposed sensing mechanism of PPPC with  $Fe^{3+}$  ion.

### 4. Conclusion

In conclusion, we have designed and synthesized a novel pyridine-pyrazole based fluorescence chemosensor **PPPC** by the multi-step process and satisfactorily characterized by different spectroscopic techniques. UV-vis and fluorescence spectral studies reveal that the chemosensor **PPPC** exhibits excellent selectivity and sensitivity toward  $Fe^{3+}$  ion over other metal ions. In the presence of  $Fe^{3+}$  ions, the fluorescence intensity of **PPPC** was selectively quenched due to the formation of the complex **PPPC**-Fe<sup>3+</sup>. Job's plot analysis supports the stoichiometry of the complex between **PPPC** and  $Fe^{3+}$  ion as 1:1. Furthermore, DFT investigation confirms the complexation behaviour of **PPPC** with  $Fe^{3+}$  from the HOMO-LUMO orbitals of **PPPC** and **PPPC**-Fe<sup>3+</sup> complex.

#### Acknowledgement

The authors grateful to Biochemie Innovations Lab, Tindivanam-604001 for consultancy service during this research work.

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### **Author Biographies**

**Mr.P. Madhu** is an Assistant Professor in the Department of Chemistry, Thiruvalluvar Government Arts College, Rasipuram, India. He is pursuing his Ph.D. degree in chemistry in Bharathiar University, India, under the guidance of Dr P. Sivakumar. His current research interest includes synthesis of Fluorescence Chemosensors and Biosensors.

**Dr P. Sivakumar** is an Assistant Professor in the Department of Chemistry, Arignar Anna Govt. Arts College, Namakkal, India. He received his Ph.D. degree in chemistry from Anna University, Chennai, India. His current research interest includes Nanomaterials, Photocatalysis and Fluorescence Chemosensors.