A Fluorescence Study of Pyrazole Derivative
2-(1,5-diphenyl-4,5-dihydro-1H-pyrazole-3-yl)pyridine Upon
Addition of La$^{3+}$ and Eu$^{3+}$ Ions

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Abstract. Synthesis of pyrazole derivative ligand 2-(1,5-diphenyl-4,5-dihydro-1H-pyrazole-3-yl)
pyridine (L) has been successfully carried out by the Claisen-Schmidt condensation followed by
the excess addition of phenyl hydrazine in ethanol solution. reddish yellow solid with a 28.85% yield
then characterized using FTIR, UV-Visible, and H$^1$NMR spectroscopy. UV-Visible spectroscopy
was conducted to determine the complex formation between lanthanide ions with ligands.
Fluorescent experiment with spectrofluorometer has conducted in compound emission intensity
change upon addition of La$^{3+}$ and the Eu$^{3+}$. UV-Visible result show complex formation in molar ratio
Eu$^{3+}$ : L (1:2) which occurred at a absorption wavelength of 366 nm, while the complex with the ratio
La$^{3+}$ : L (1:3) exhibited at 342 nm in acetonitrile. Fluorescence analysis showed ligands have two
excitation λ at 257 nm 365 nm with a noticeable molar absorptivity at concentration 2x10$^{-5}$ M. The
addition of La$^{3+}$ ions lead to the fluorescence enhancement effect on the λ$\text{em}$ at 355 nm. While the
addition of the Eu$^{3+}$ demonstrates the effect of fluorescence quenching at the λ$\text{em}$ 340 nm.

Introduction

Since the discovery of lanthanide metals in the late 18th Century by Axel Arrhenius, the need for
metal utilization is increasing as well as its application in the modern technological industries such as
computer, nuclear energy, and advanced material. Europium is surprisingly known as precursor
element to create the strong field magnetic material [1] and Lanthanum had been widely used as high
refractive-index glasses, hydrogen storage, electrode, and refinery catalyst [2]. Beside the highly
usage of metals in advanced material, lanthanides had the other potential including in medicine and
environmental field in particular luminescence-based sensor as they embrace the distinctive chemical
characteristic known as forbidden f-f orbital transition. As the global need of rare metal oxide had
reached almost 124,000 tons and the economic value varied approximately 3 billion US$ in 2013 [3],
it is considerably required for the lanthanide to be effectively detected regarding their low abundance.
Luminescence and fluorescence are the effective analyzing methods as they are qualified with the
high sensitivity, high selectivity, and inexpensive installations [4]. Organic compound or ligand
which contain conjugated aromatic system could perform as efficient chromophore group in
absorbing and emitting UV light. If ligand excited state lied in the higher energy level, the energy
could be transferred to metal excited state before turn to lower ground state by emitting radiation in
certain intensity and wavelength [5]. Several organic compounds which are able to conduct as
lanthanides fluorescence sensor are terpyridine, diazostilbene, and phenantroline [6].

In this study, pyrazole-derived ligand 2-(1,5-difienil-4,5-dihidro-1-H-pirazol-3-yl)piridin (Fig.1.)
has been synthesized to determine its fluorescence characteristic in addition of lanthanide metal La$^{3+}$
and Eu$^{3+}$. Pyrazole-derived ligand is studied because its good photophysical properties and synthesis
simplicity as the previous similar research that using methyl-substituted pyrazole ligand shows
favorable fluorescence when reacting with Cd$^{2+}$ dan Zn$^{2+}$[7]. The aim of using aromatic heterocyclic
ligand is to provide much chromophore which can effectively absorb wide range of photons with the
result in interesting enhancement of quenching effect upon the addition of lanthanide ions in solution.
Therefore, the resulting emission will have distinct spectral changes which specifically different in certain metals.

![Reaction pathway of pyrazoline compound.](image)

**Materials and Method**

**Reagents**

Chemicals including 2-acetylpyridine, phenylhydrazine, lanthanide oxides were purchased from Sigma-Aldrich whereas benzaldehyde, ethanol, and the other organic reagents from Merck. Spectroscopy was performed with CHROMASOLV® gradient grade acetonitrile for HPLC, ≥99.9% from Sigma-Aldrich. The lanthanide complexes used in this study was prepared by addition of nitric acid into lanthanide oxide to afford nitrate salts of La(NO₃)₃ and Eu(NO₃)₃.

**Synthesis Procedure of 2-(1,5-diphenyl-4,5-dihydro-1H-pyrazol-3-yl)pyridine.** Following the procedure that previously reported [7], 2-acetylpyridine (0.60 g, 5 mmol) and 5 mL of 10% of NaOH aqueous solution were added to 20 ml of anhydrous ethanol and stirred smoothly at around 60 seconds. Benzaldehyde (0.52 mL, 5 mmol) was then added and stirred again for 24 hours. After 24 hours, the solid product was filtered, dried, and washed by cooled ethanol to give the pale white crystals of chalcone (0.88 gram, 84.18% yield). The synthesized chalcone (0.418 g, 2 mmol) was then diluted in 10 ml of ethanol and stirred 10 minutes precisely in room temperature. Phenylhydrazine (0.432 g, 4 mmol) was then added to a rapidly stirred solution of chalcone and the mixture was refluxed to 80°C for 24 h. The solvent was filtrated and the crude orange solid was purified by column chromatography with silica gel using n-hexane : ethyl acetate (3:2) to afford the desired pyrazoline as an reddish orange solid (0.1731 g, 28.85% yield). IR (KBr, cm⁻¹): 3055, 2922, 2359, 1601, 1570, 750; ¹H-NMR (500 MHz, CDCl₃): δ 8.63-8.66 (m, 1H), 8.0 (m, 1H), 7.68-7.73 (td, 2H), 7.29-7.35 (m, 5H), 7.21-7.26 (m, 2H), 7.09-7.21 (m, 2H), 6.78-6.84 (m, 1H), 5.33 (q, 1H); 3.94 (dd, 1H); 3.64 (dd, 1H).

**Fluorescent Experiment**

The stock solutions of ligand (5.0×10⁻⁵ M) were prepared by dissolving 2.22 mg of solid in 150 ml of acetonitrile. The cationic stocks were all in similar solvent with concentration of 5.0×10⁻³ M for UV–Vis absorption and fluorescence spectra analysis. For metal ions fluorescence experiments, each time 2 mL solution of ligand filled in a quartz cell of 1 cm optical path length, and we increased concentrations of lanthanide ions by stepwise addition of different equivalents using a micro-pipets. After each addition of Ln³⁺ ion, the solution was shaked for 1 min. The volume of cationic stock solution added was less than 200 µL with the purpose of keeping the total volume of testing solution without obvious change. For all measurements of fluorescence spectra of compound, the excitation was at 257 nm.

**Result and Discussion.**

**Synthesis and Structural Characteristic**

The ligand was obtained by the reaction of pre-synthesized chalcone with phenylhydrazine in ethanol under reflux condition. The yield of ligand was 28.85% and its structure was identified by using FTIR, H¹-NMR, and UV-Visible Spectroscopy. The IR Spectrum of compound showed a narrow
bands at 3055 and 2922 cm\(^{-1}\) attributed to stretching of phenyl and C-H \(sp^3\) groups, respectively. As characteristic of C=\(N\) and C-N vibration were assigned at 1601 cm\(^{-1}\) and 1156 cm\(^{-1}\) whereas strong band at 1570 cm\(^{-1}\) referring to carbon-carbon double bonds. The noticeable feature was the disappearance of strong band at 1680 cm\(^{-1}\) that signified to reduction of carbonyl group of chalcone. The further structural identification was confirmed by \(^1\)H-NMR spectrum. The \(^1\)H-NMR spectrum of synthesized pyrazoline shows a doublet coupling at 3.64 and 3.94 ppm for two protons of C-H\(_2\) at pyrazoline ring. The downfield-shifted multiple signals at 5.33-5.56 ppm was assigned to single proton of pyrazole’s carbon that attached to phenyl. For the first phenyl ring that bonded to nitrogen atom of pyrazoline (N-N-Ph), multiples at 6.78-7.26 ppm were attributed to five protons of each phenyl carbons. The downfield shift of multiples at 7.29-7.35 were accounted for second phenyl ring (N-C-Ph) while the protons from the third phenyl (N=C-Ph) were signified by multiples at 7.70 and 8.65 ppm, and doublets at 7.68-7.73 ppm. The absorption maximum for the ligand \(\pi\rightarrow\pi^*\) transition is at 210 nm (log \(\varepsilon\) = 4.14 ) with two shoulders, one at 267 nm and the other at approximately 336 nm. The absorption is similar to that of a related methyl-substituted pyrazoline derivative (Ciupa et al, 2012)

**Fluorescent Studies in Addition of Lanthanide Metals**

The present study shows that synthesized compound exhibits a fluorescent peak at 357 nm when excited by 257 nm wavelength photons in acetonitrile. The intensity of fluorescent peak is found to increase on interaction with \(La^{3+}\) in contrast with slight decrease in the intensity when observed on interaction with \(Eu^{3+}\). These observations indicate that ligand can be used to distinguish certain lanthanide metals based on fluorescent sensing. The luminescence of ligand in the presence of increasing amounts of \(La^{3+}\) is shown in Fig. 2. The integrated luminescence intensity increases slightly, the emission maximum shifts to the red and the spectrum becomes more structured with a new band appearing at 360 nm. The luminescence is fairly enhanced, as the intensity of emission reached 5.91% at \(La^{3+}\) concentration 1x10\(^{-8}\) M and then raised to 60% at 5x10\(^{-5}\) M. Plot of intensity as a function of metal ion concentration is also shown in Fig. 3. When \(La^{3+}\) ion was added, the intensity of fluorescent peak at 355 nm increased from 253 a.u. to 341 a.u. i.e., is approximately 1.35 times increase, till the final concentration of \(La^{3+}\) became 5x10\(^{-5}\) M. Thus, the fluorescent sensing can be regarded as off-on type which is affected by interaction between metals and the pyrazole compound. Furthermore, to determine the the number of \(La^{3+}\) binds to pyrazolines in the complex, Job’s method was employed by using the absorption changes at 327 nm as a function of molar fraction of \(La^{3+}\). A maximum absorption was observed when the molar fraction of \(La^{3+}\) reached approximately 0.23 (Fig. 4), indicating that \(La^{3+}\) ions form a 1:3 complex with the sensing compound.

![Fluorescence spectra](image_url)

Figure 2. Emission spectra of pyrazoline compound in acetonitrile following addition of different quantities of \(La^{3+}\) (left) and \(Eu^{3+}\) (right). [L] = 5x10\(^{-5}\) M (\(\lambda_{ex}\) = 257 nm).

Oppositely, addition of \(Eu^{3+}\) induces distinct emission spectral changes and, in addition, the luminescence is significantly quenched as shown in Fig. 2. As with the \(La^{3+}\) addition, a red shift is observed at 360 nm whereas in \(Eu^{3+}\) addition, an opposite small shift occurred to 340 nm in acetonitrile. The emission is slightly quenched, from nearly 250 a.u. at initial ligand without \(Eu^{3+}\)
presence to 175 a.u. when final amount of Eu$^{3+}$ was added. The plot of intensity in Fig 3. versus concentration clearly presents the quenching had reached 28% of decrease. Moreover, Job’s plot showed the complexation of ligand and Eu$^{3+}$ was occurred when the molar ratio is 1:2 at absorption wavelength 366 nm.

![Figure 3](image1.png)

**Figure 3.** Plot of the emission intensity as a function of La$^{3+}$ (left) ($\lambda_{\text{max}} = 355$ nm) and Eu$^{3+}$ (right) ($\lambda_{\text{max}} = 340$ nm) concentrations.

![Figure 4](image2.png)

**Figure 4.** Jobs Plot to determine the ratio between ligand and lanthanides in MeCN. Total concentration = $2.0 \times 10^{-4}$ M.

The fluorescence mechanism of either enhancement or quenching is not completely clear in both systems. The enhancement in emission intensity of compound on interaction with La$^{3+}$ ions may be explained on the basis of photo induced electron transfer system (PET) [8]. As La$^{3+}$ is known to be a strong electropositive metal, binding of the metal will lowers the energy or the HOMO of the receptor part of the compound and the PET process would stops and resulting in the raise in fluorescent intensity [9]. Besides, the electrostatic force of metal may also induce ionically to change the flexibility of ligand as Fig. 4 confirmed that the La$^{3+}$ bind three number of molecules and as consequence, it will affect the rigidity. The relative changes in chromophore structure will increase the number of excited electrons and could be responsible for fluorescent enhancement [10]. On the other side, the mechanism for fluorescence quenching may involves the increasing of efficiency for *intersystem crossing* in excited state of ligand as a result of spin-orbit coupling interactions of compound with the Eu$^{3+}$ metals following complexation [11]. The paramagnetic properties of Eu$^{3+}$ will change the electron orientation and turn the singlet excited states into triplet. Since the transition become forbidden, the rate of electron relaxation will become slower and resulting in extinguished fluorescence [12].

**Summary**

To summarize, a novel fluorophore of lanthanide metal sensing was obtained by joining chalcone with phenylhydrazine to form pyrazoline. The compound was used for the determination of La$^{3+}$ and
Eu$^{3+}$ ions with distinguish sensing type in acetonitrile. This ligand performed off-on type on interaction with La$^{3+}$ whereas the fluorescent quenching effect occurred when added with Eu$^{3+}$. The chromophore pyrazoline, having three heterocyclic aromatic systems, coordinates La$^{3+}$ more fixed than Eu$^{3+}$ as further Jobs plot showed 1:3 complex with first lanthanide while 1:2 molar ratio observed with the paramagnetic one. Eventhough, further X-Ray Diffraction analysis should be conducted to confirm the complex structure.

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**References**


