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Electronic Supporting Information

Design and Synthesis of Dual Probes for Detection of Metal Ions by LALDI MS and Fluorescence: Application in Zn(II) Imaging in Cells

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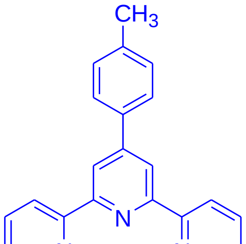
EXPERIMENTAL SECTION

1. General Experimental Procedure and Instrumentation:

All the starting materials used in this study were purchased from Sigma-Aldrich , Spectrochem, Alfa Aesar and were used without further purification. For thin layer chromatography (TLC), silica aluminum foils with fluorescent indicator 254 nm were used and compounds were visualized by irradiation with UV light. All NMR spectra were recorded using Bruker 400 MHz and 600 MHz. NMR shifts are reported as delta (δ) units in parts per million (ppm) and coupling constants (*J*) are reported in Hertz (Hz). Solvents such as hexane, EtOAc, DMF, CCl_4, toluene and THF were dried prior to use using standard protocols. The chemical shift of residual solvent signal was used as standard. UV and Fluorescence spectra were recorded using JASCO 700 spectrometer and HITACHI FL 7000 fluorometer respectively. All mass spectra were recorded on ultraFlextrene MALDI Time-of-Flight Mass Spectrophotometer from Bruker in positive ion mode. The instrument was calibrated for the mass range 50-2500 Da using a standard calibration kit (Bruker). The kit contains Bradykinin, Angiotensin II, Angiotensin I, Substance_P, Bombesin, Renin_substrate, ACTH_clip (1-17), ACTH_clip (18-39), Somatostatin. By using this kit whole mass range was calibrated in positive ion mode. UV Laser: Smartbeam II (N_2, NdYag), 355 nm wavelength, Laser rep rate 2000 Hz, Reflector mode.

2. Synthetic procedure of required compounds

2.1 Synthesis of 4'-(p-Tolyl)-[2,2':6', 2''] terpyridine (1):

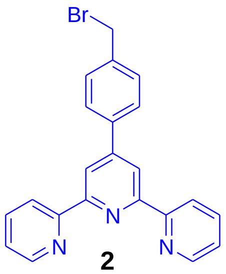


1

2-Acetylpyridine (1.3 g, 10.8 mmol) was added into a solution of 4-methylbenzaldehyde (0.65 g, 5.4 mmol) in MeOH (120 mL). NaOH pellets (0.22g, 5.4 mmol) and aq. NH_3 (60 mL, 25%) were then added to the solution. The solution was refluxed for 12 h. The mixture was cold to ambient temperature, and then the off-white solid was collected by filtration and washed with ice-cold water (10 mL) to obtain **1**. It was further purified by recrystallization from ethanol. **State:** White crystalline solid. **Spectral Data:** **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.72 (d, *J* = 4.8 Hz, 4H), 8.65 (d, *J* = 8.0 Hz, 2H), 7.88 – 7.80 (m, 4H), 7.37 –

7.26 (m, 4H), 2.42 (s, 3H).**^{13}C NMR** (100 MHz, $CDCl_3$) δ 156.6, 156.1, 150.4, 149.4, 139.3, 137.1, 135.7, 129.9, 127.4, 124.0, 121.6, 118.9, 21.5.

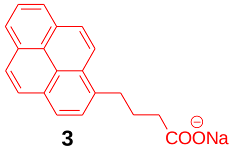
2.2 Synthesis of 4'-(4-Bromomethylphenyl)-[2, 2':6',2''] terpyridine (2):



2

A mixture of **1** (500 mg, 1.55 mmol), N-bromosuccinimide (NBS, 0.33 g, 1.85 mmol), and R,R'-azoisobutyroniltrile (AIBN, 20 mg, 0.12 mmol) in dry CCl_4 (5 mL) was refluxed for 2h. The warm reaction mixture was filtered to remove the succinimide, and the solvent was evaporated. The crude product was purified by recrystallization from ethanol (EtOH) to give compound **2** (560 mg, 90%).**State:** Pale yellow crystalline solid. **Spectral Data: ^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.71 (d, *J* = 3.8 Hz, 4H), 8.64 (d, *J* = 7.9 Hz, 2H), 7.85 (t, *J* = 8.0 Hz, 4H), 7.51 (d, *J* = 8.0 Hz, 2H), 7.39 – 7.27 (m, 2H), 4.54 (s, 2H).**^{13}C NMR** (100 MHz, $CDCl_3$) δ 156.1, 155.9, 149.4, 149.1, 138.6, 138.5, 136.9, 129.6, 127.7, 123.8, 121.3, 118.7, 32.3.

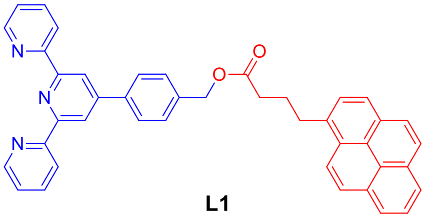
2.3 Preparation of sodium salt of pyrene-1-butyric acid (3):



3

Pyrene-1-butyric acid (50 mg, 0.17 mmol) was added to dry THF (3 mL) under N_2 atmosphere. To this, $NaHCO_3$ (15mg, 0.18 mmol) and 1-2 drops of water were added. The mixture was stirred at room temperature for overnight and THF was removed from the resulting reaction mixture under reduced pressure. The yellow crystalline solid of **3** was obtained in quantitative amount and it was lyophilised to remove any trace of water.

2.4 Synthesis of Ligand 1(L1):

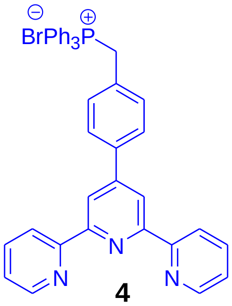


L1

Compound 2 (28 mg, 0.07mmol) was taken in dry DMF (4 mL) under N_2 atmosphere and compound 3 (22 mg, 0.07mmol) was added to it. The reaction mixture was stirred at room temperature for 8h. The organic layer was extracted by ethyl acetate and dried over anhydrous Na_2SO_4. Excess solvent was removed under reduced pressure to afford yellow residue. It was further purified by recrystallization from hexane-ethyl acetate (4:1) to obtain **L1** (35mg, 81%).**State:** Yellow amorphous solid. **Spectral Data: ^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.73 (d, *J* = 5.1 Hz, 4H), 8.67 (d, *J* = 7.9 Hz, 2H), 8.27 (d, *J* = 9.2

Hz, 2H), 8.15 (dd, *J* = 7.6, 4.1 Hz, 2H), 8.12 – 8.06 (m,2H), 8.02 (d, *J* = 2.8 Hz, 2H), 7.96 (t, *J* = 7.5 Hz, 2H), 7.89 (dd, *J* = 8.8, 1.9 Hz, 1H), 7.88 – 7.83 (m, 5H), 7.49 (d, *J* = 8.1 Hz, 2H), 7.36 (ddd, *J* = 7.4, 4.7, 1.2 Hz, 2H), 5.20 (s, 2H), 3.41 (dd, *J* = 8.7, 6.9 Hz, 2H), 2.55 (t, *J* = 7.2 Hz, 2H), 2.32 – 2.20 (m, 2H). **^{13}C NMR** (150 MHz, $CDCl_3$) δ 173.2, 156.1, 155.9, 149.6, 149.1, 149.1, 138.3, 136.9, 136.9, 136.8, 135.5, 131.3, 130.8, 129.9, 128.7, 128.7, 127.5, 127.5, 127.4, 127.3, 127.3, 127.3, 127.2, 126.6, 125.7, 125.0, 124.9, 124.8, 124.7, 124.7, 124.7, 123.8, 123.2, 123.2, 121.4, 118.8, 65.8, 33.8, 32.7, 26.7

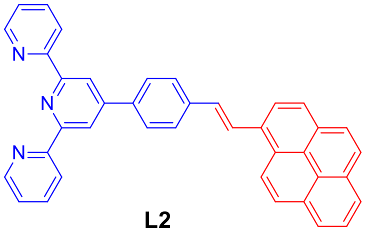
2.5 Synthesis of Wittig Salt (4):



4

A mixture of triphenyl phosphine (PPh_3) (0.21g, 0.8mmol) and compound 2 (0.23g, 0.57mmol) were dissolved in minimum amount of dry toluene and was refluxed for 16 h to produce a white solid which was removed by suction filtration. Then ethanol (1 mL) was added to it (for creating azeotrope) and excess solvent was removed under reduced pressure to afford yellow crystalline solid of **4** in quantitative amount.

2.6 Synthesis of Ligand 2 (L2):



L2

n-Butyllithium in hexane (0.23 mL, 0.48 mmol) was slowly added to a solution of the previously prepared Wittig salt (320mg, 0.48 mmol) in dry THF (4 mL) at 0°C under nitrogen atmosphere. The mixture was stirred for 20 min and the solution of pyrene aldehyde (37 mg, 0.16 mmol) in dry THF (5 mL) was injected under the same conditions. After the addition, the mixture was warmed to room temperature and stirred for 6h under nitrogen atmosphere. The mixture was quenched by a large amount of ice/water and extracted with ethyl acetate. The combined extracts were washed with water, dried with anhydrous Na_2SO_4 and concentrated under reduced pressure. Then the residue obtained was washed with hexane repeatedly to desired product **L2** (65 mg, 76%).**State:** Brown amorphous solid. **Spectral data:** **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.78 (d, *J* = 19.5 Hz, 4H), 8.70 (d, *J* = 8.2 Hz, 2H), 8.55 (d, *J* = 9.2 Hz, 1H), 8.37 (s, 1H), 8.30 (d, *J* = 16.2 Hz, 1H), 8.20 (d, *J* = 8.6 Hz, 4H), 8.07 (s, 2H), 8.01 (d, *J* = 7.5 Hz, 3H), 7.90 (t, *J* = 7.7 Hz, 2H), 7.82 (d,

J = 8.0 Hz, 2H), 7.48 – 7.32 (m, 3H).**^{13}C NMR** (100 MHz, $CDCl_3$) δ 156.1, 155.8, 149.5, 149.0, 138.4, 137.4, 136.8, 131.9, 131.6, 131.4, 130.9, 130.9, 130.8, 128.4, 128.3, 127.6, 127.3, 127.2, 127.1, 126.4, 125.9, 125.2, 125.0, 125.0, 124.9, 124.8, 123.7, 123.6, 122.9, 121.3, 118.4.

2.8 NMR Spectra of synthesized compounds

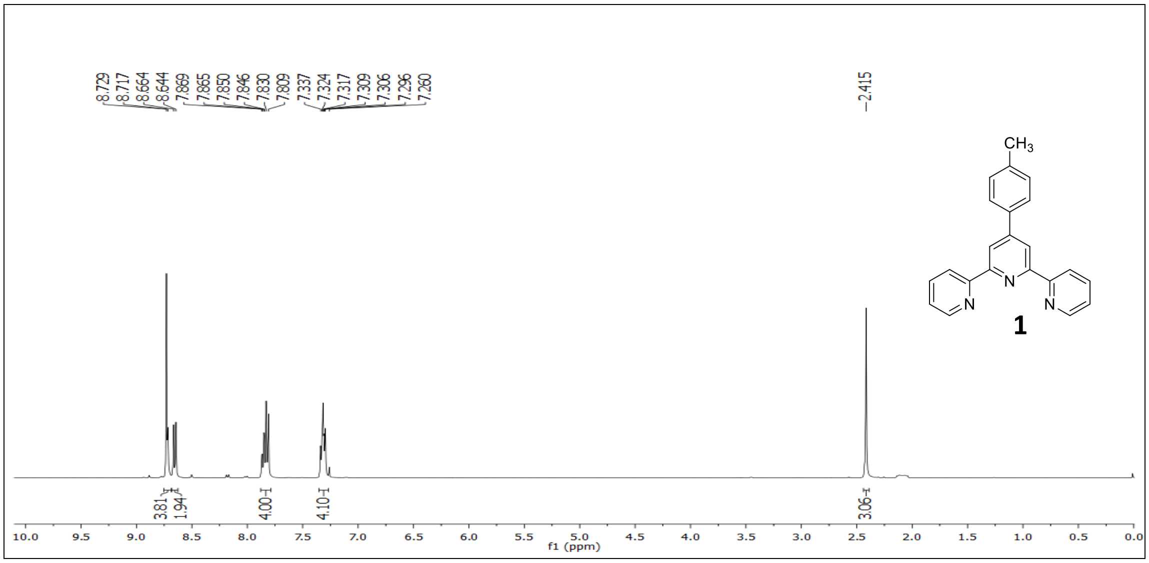


Fig. 1: ^{1}H NMR ($CDCl_3$, 400 MHz) of **1**

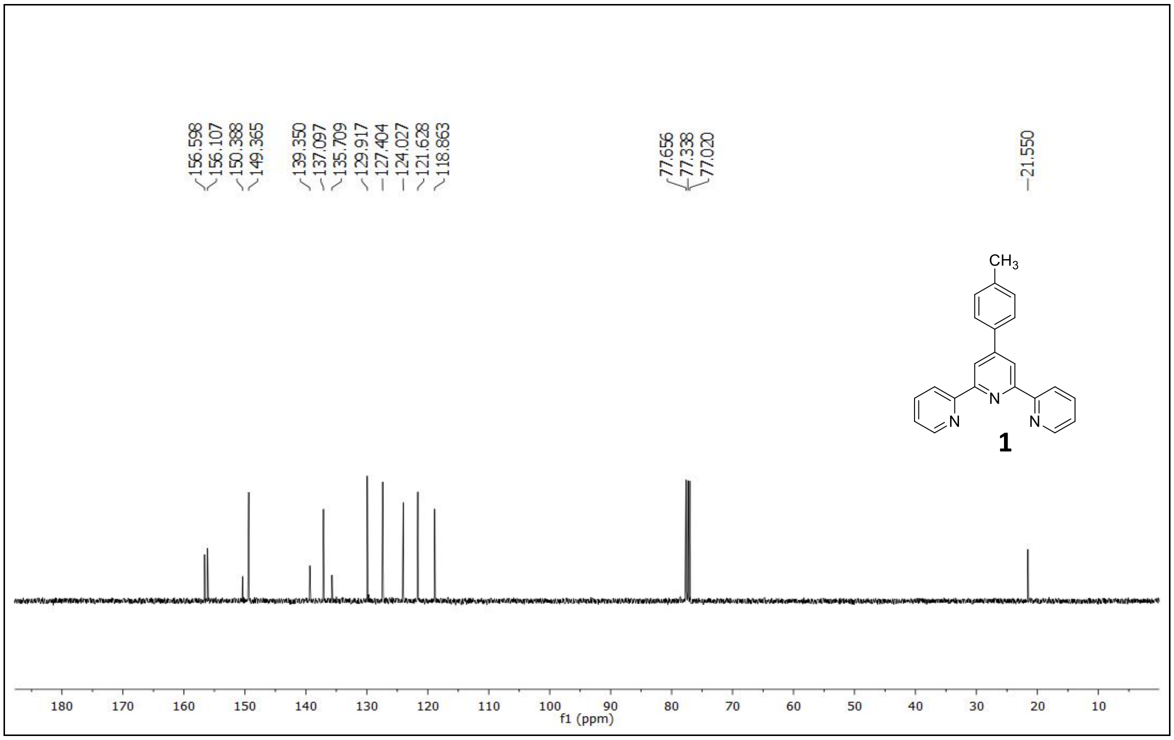


Fig. 2: ^{13}C NMR ($CDCl_3$, 100 MHz) of **1**

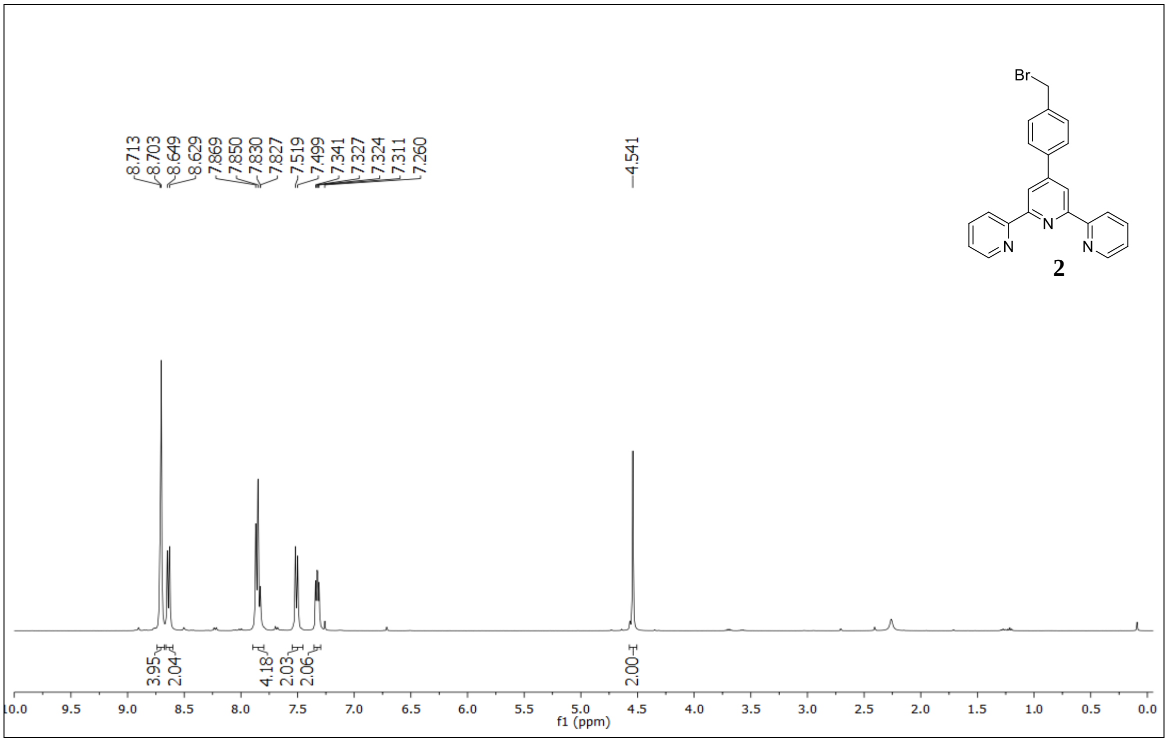


Fig. 3:^{1}H NMR ($CDCl_3$, 400 MHz) of **2**

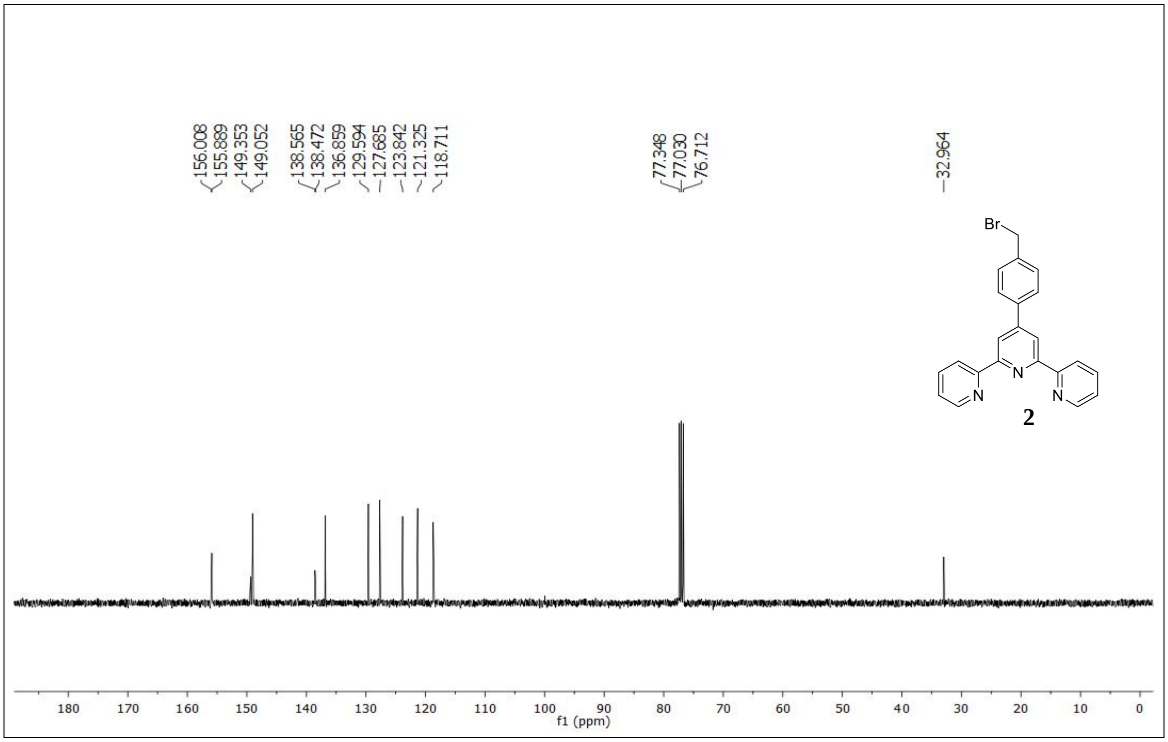


Fig. 4: ^{13}C NMR ($CDCl_3$, 100 MHz) of **2**

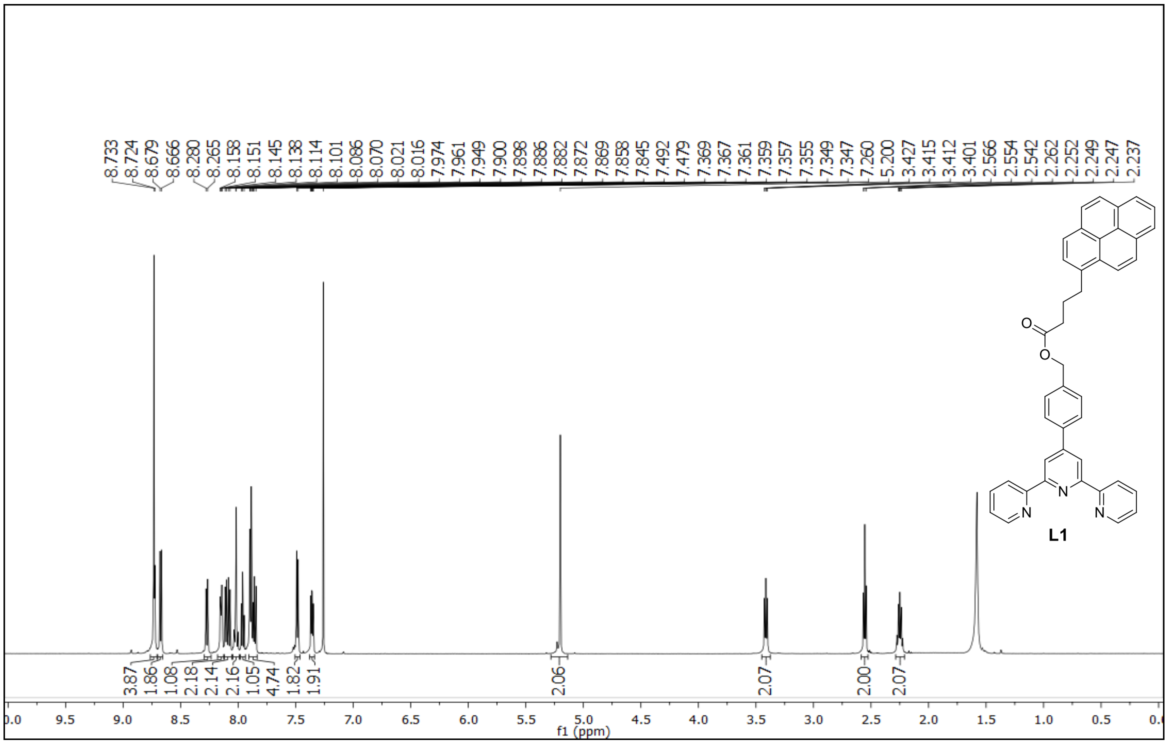


Fig. 5: ^{1}H NMR (CDCl$_3$, 600 MHz) of **L1**

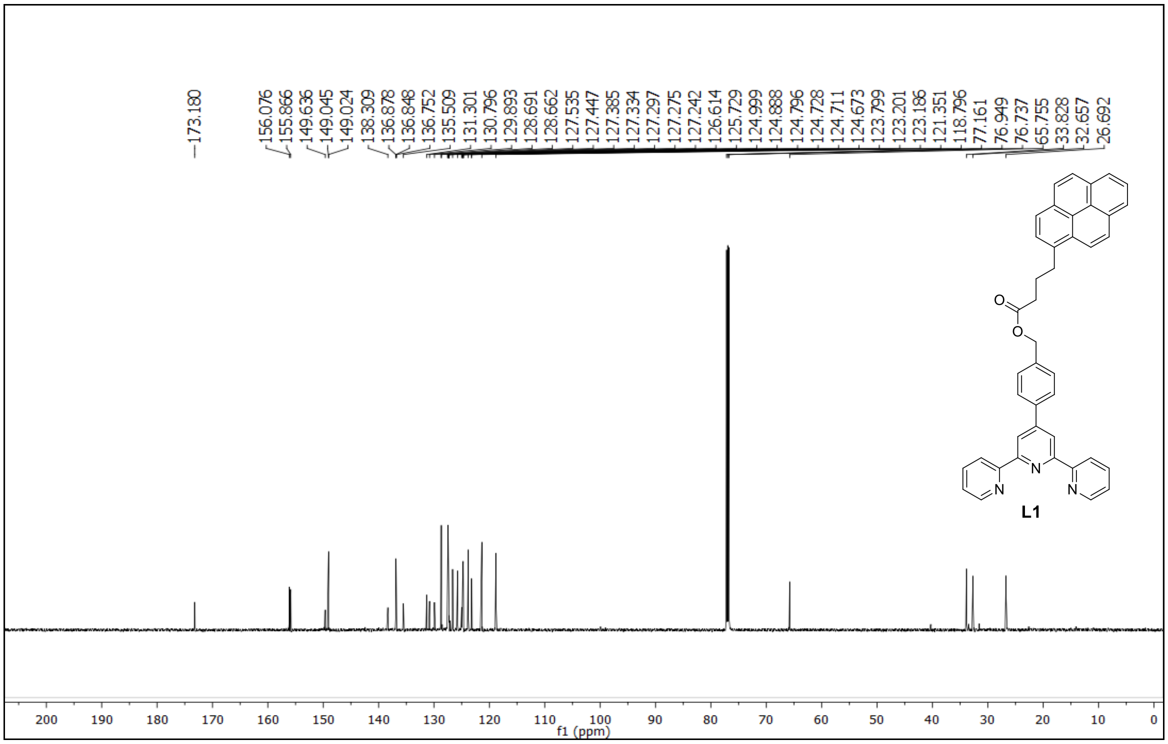


Fig. 6: ^{13}C NMR (CDCl$_3$, 150 MHz) of **L1**

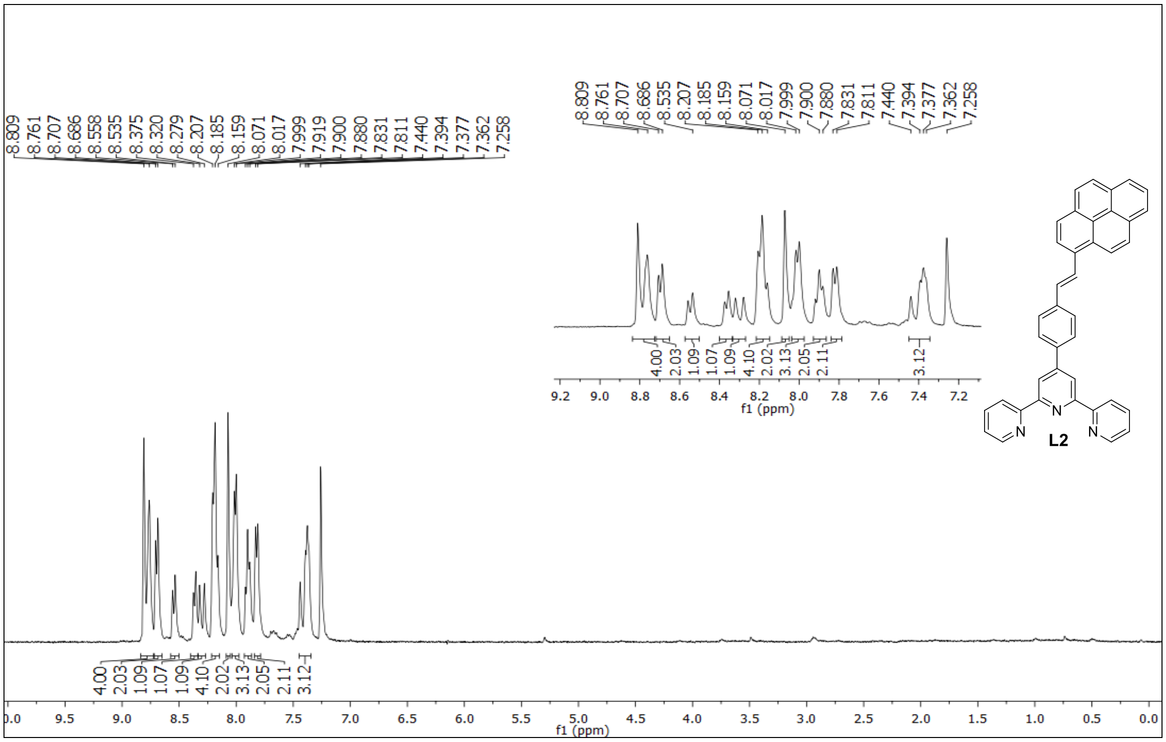


Fig. 7: ^{1}H NMR (CDCl$_3$, 400 MHz) of **L2**

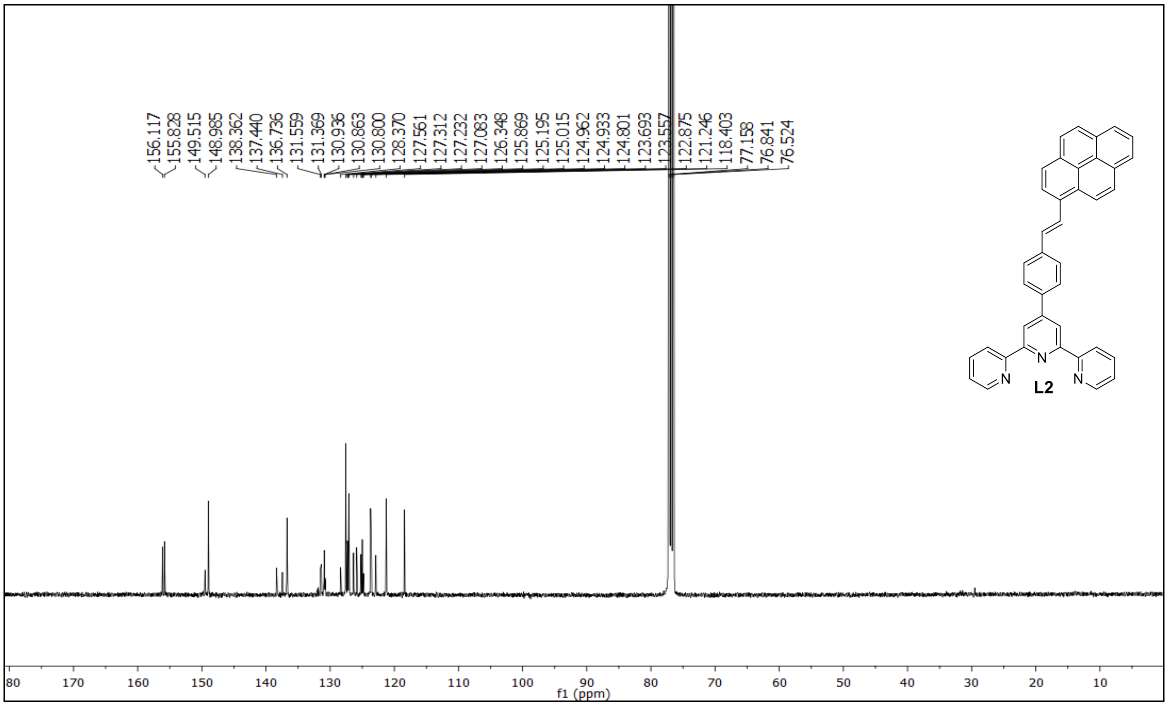


Fig. 8: ^{13}C NMR (CDCl$_3$, 100 MHz) of **L2**

3. LALDI Mass Spectra of ligand L1 when incubated with different metal perchlorate salts:

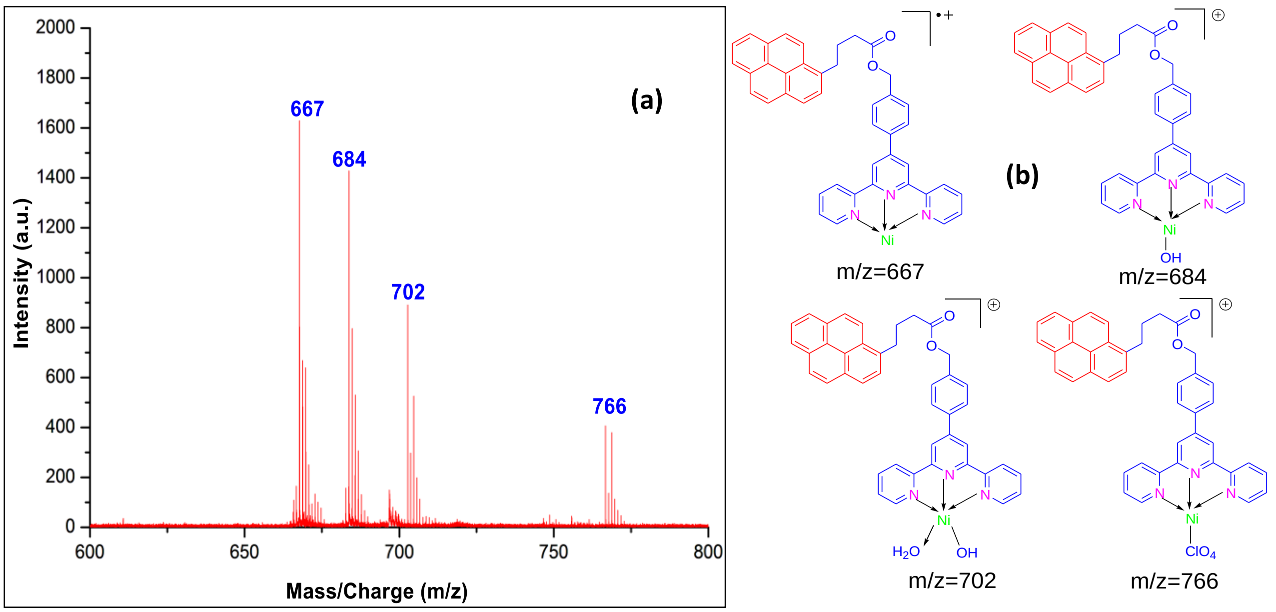


Fig. 9: (a) LALDI Mass Spectrum of ligand **L1**+ Ni(ClO_4)$_2$; (b) Tentative structures of the complexes for the peak appeared in LALDI MS of ligand **L1** and Ni(ClO_4)$_2$

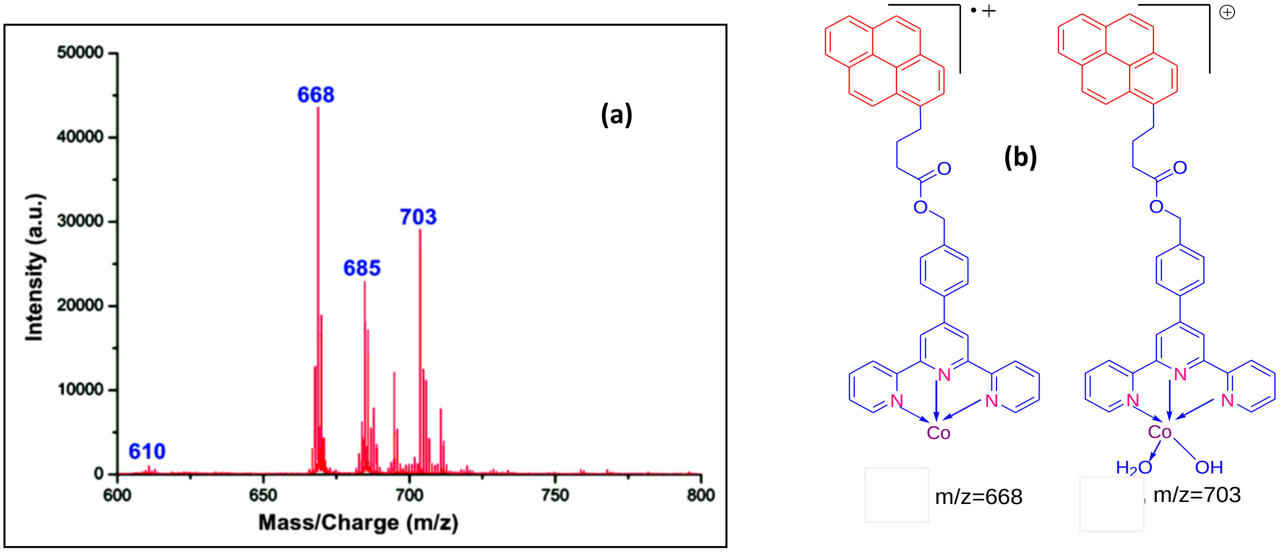


Fig. 10: (a) LALDI Mass Spectrum of ligand **L1** + Co(ClO_4)$_2$; (b) Tentative structures of the complexes for the peak appeared in LALDI MS of ligand **L1**and Co(ClO_4)$_2$

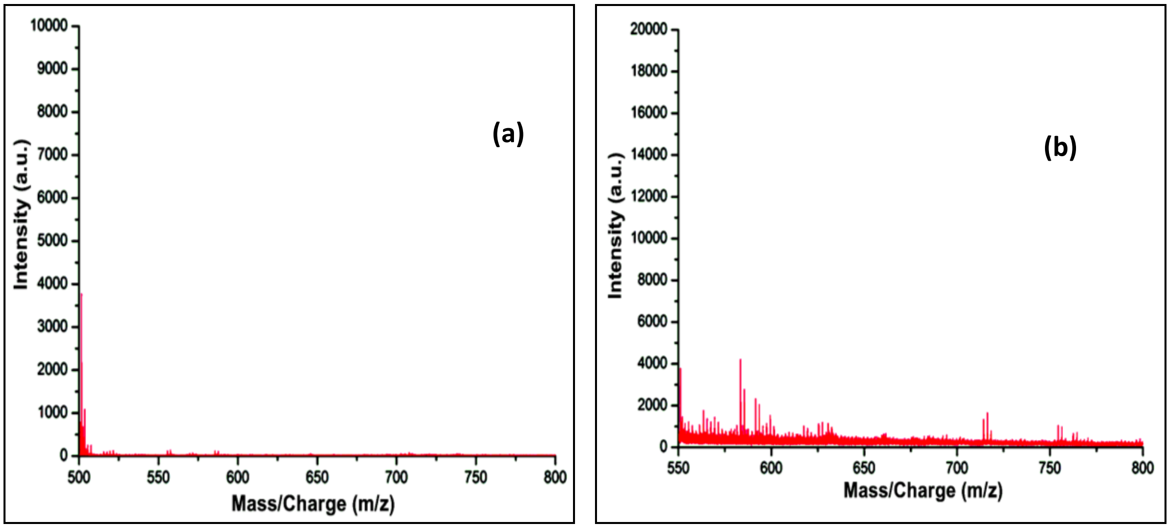


Fig. 11: (a) LALDI Mass Spectrum of ligand **L1**+ $Cu(ClO_4)_2$; (b) LALDI Mass Spectrum of ligand **L1**+ $Fe(ClO_4)_2$

4. UV-VIS titration spectra of the ligand L1 in presence of metal ions

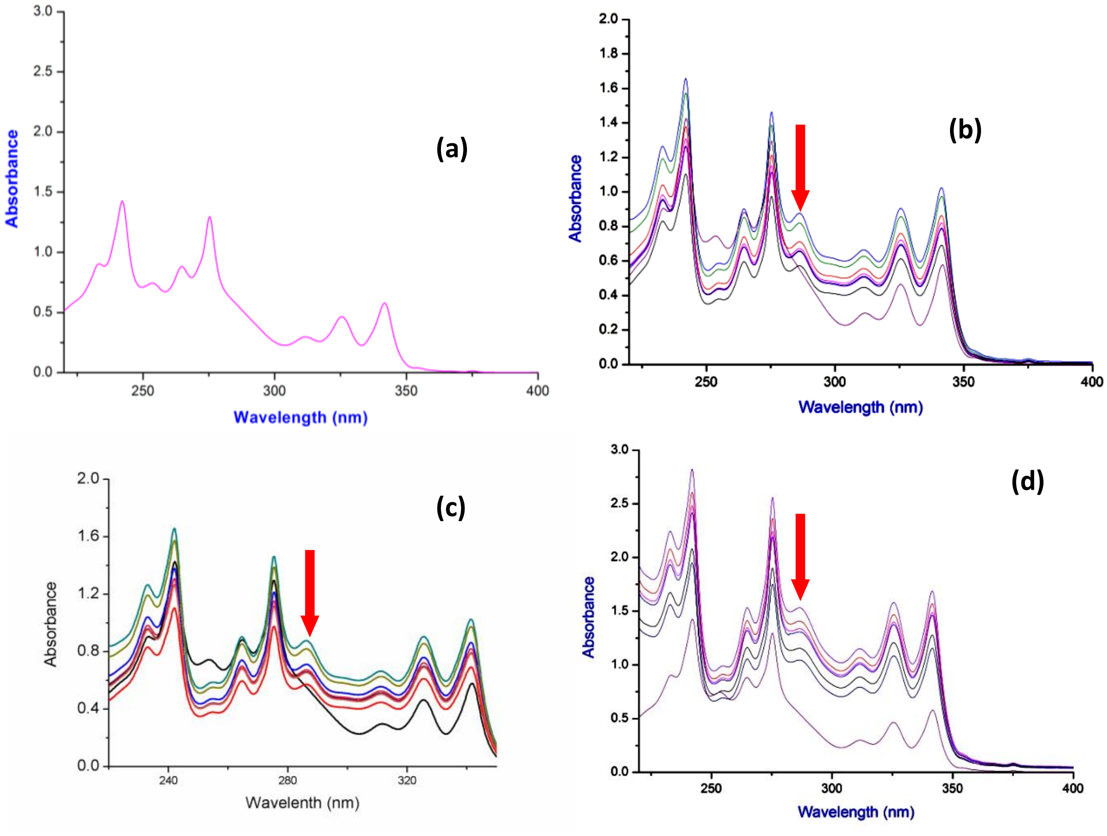


Fig. 12: UV-VIS titration spectra of ligand **L1** (10^{-5}M) with varying concentration of metal ions: (a) ligand **L1**; (b) $Co(ClO_4)_2$ (c) $Zn(ClO_4)_2$ and (d) $Ni(ClO_4)_2$

5. Fluorescence behaviour of ligand L1 in presence of metal ions

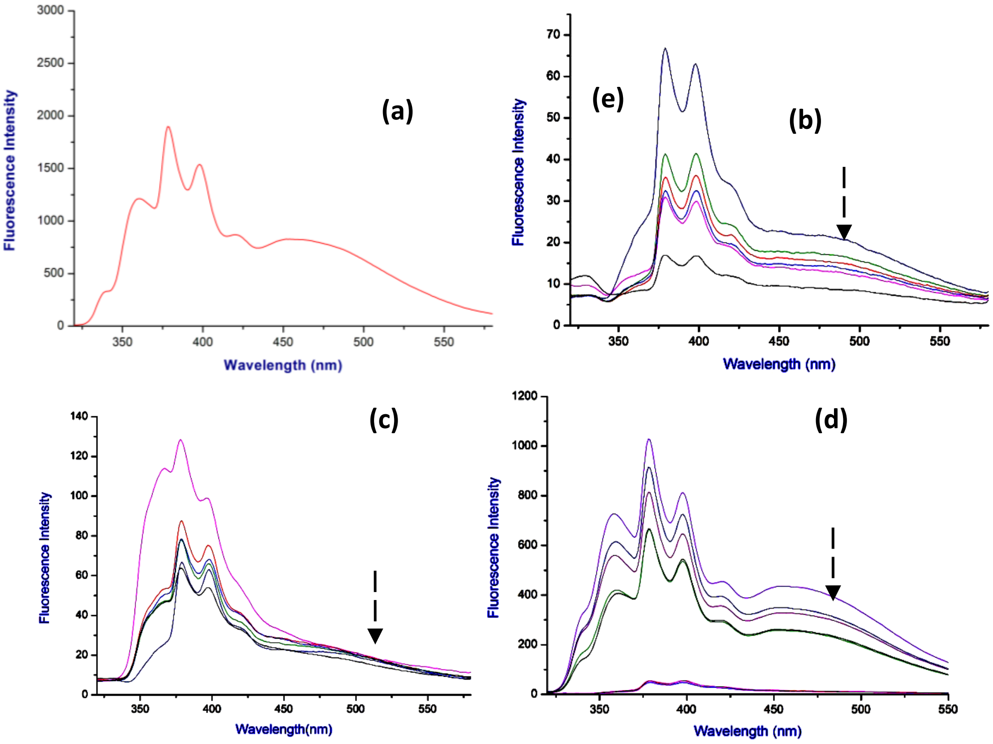


Fig. 13: Fluorescence titration spectra of ligand (10^{-5}M) with varying concentration of metal ions: (a) ligand **L1**; (b) Co(ClO_4)$_2$; (c) Ni(ClO_4)$_2$; and (d) Cu(ClO_4)$_2$

[⇢ indicates increasing concentration]

6. LALDI Mass Spectra of ligand L2 when incubated with different nickel perchlorate salts:

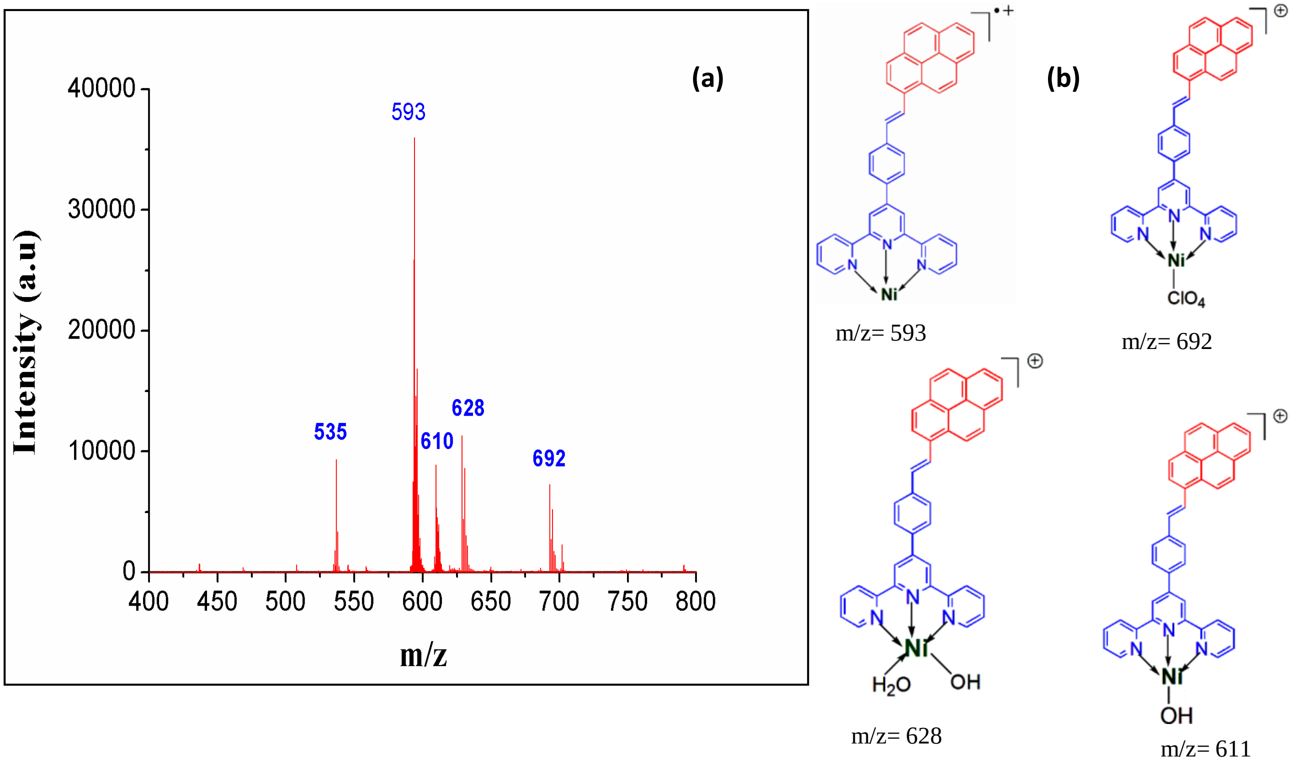


Fig. 14: (a) LALDI Mass Spectrum of ligand **L2** + Ni(ClO_4)$_2$; (b) Tentative structures of the complexes for the peak appeared in LALDI MS of ligand **L2** and Ni(ClO_4)$_2$

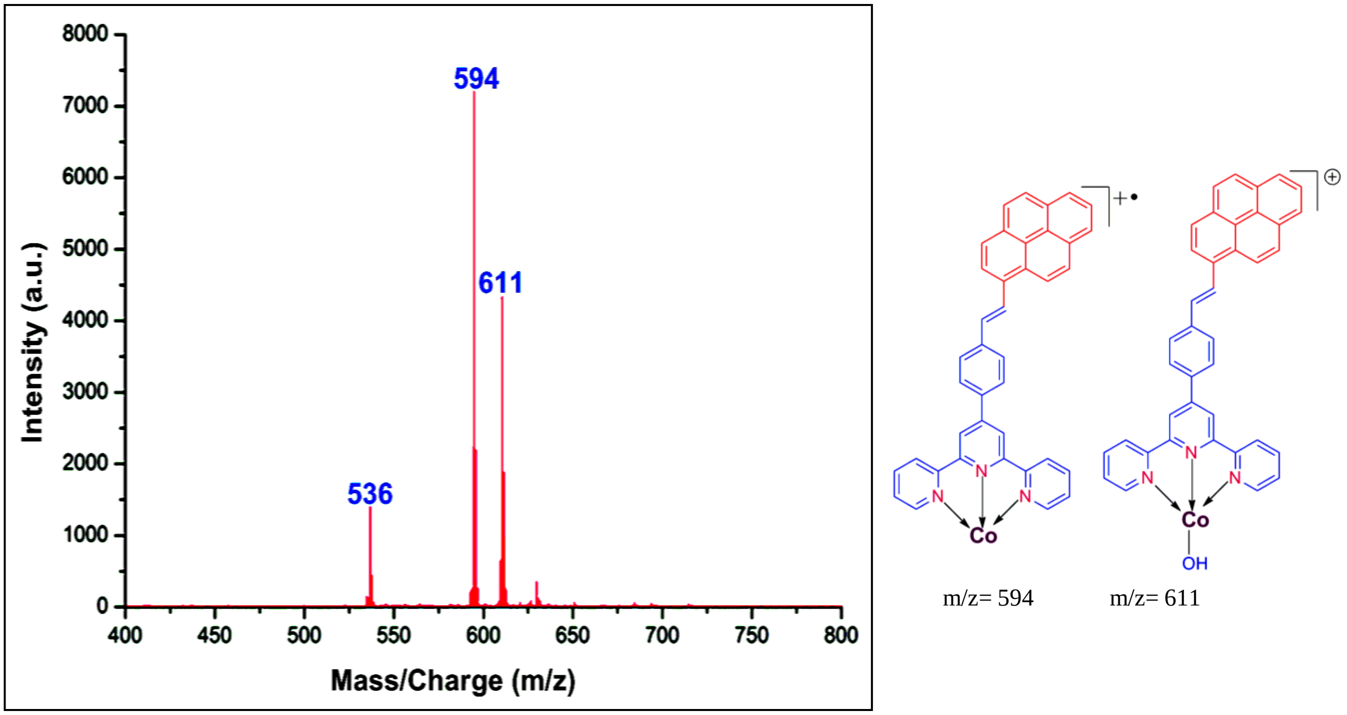


Fig. 15: (a) LALDI Mass Spectrum of ligand **L2** + Co(ClO_4)$_2$; (b) Tentative structures of the complexes for the peak appeared in LALDI MS of ligand **L2** and Co(ClO_4)$_2$

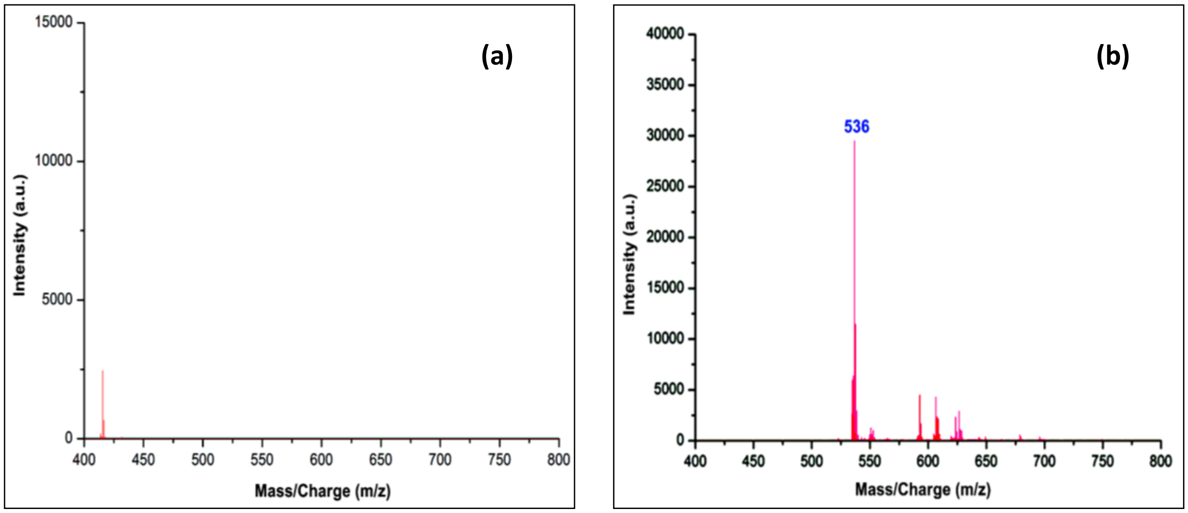
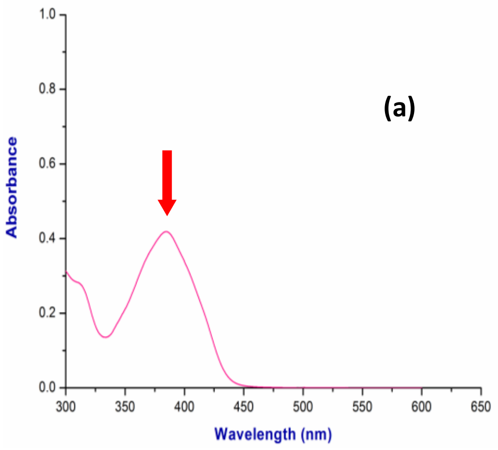


Fig. 16: (a) LALDI Mass Spectrum of ligand **L2** + $Cu(ClO_4)_2$; (b) LALDI Mass Spectrum of ligand **L2** + $Fe(ClO_4)_2$

7. UV-VIS titration spectra of the ligand L2 in presence of metal ions



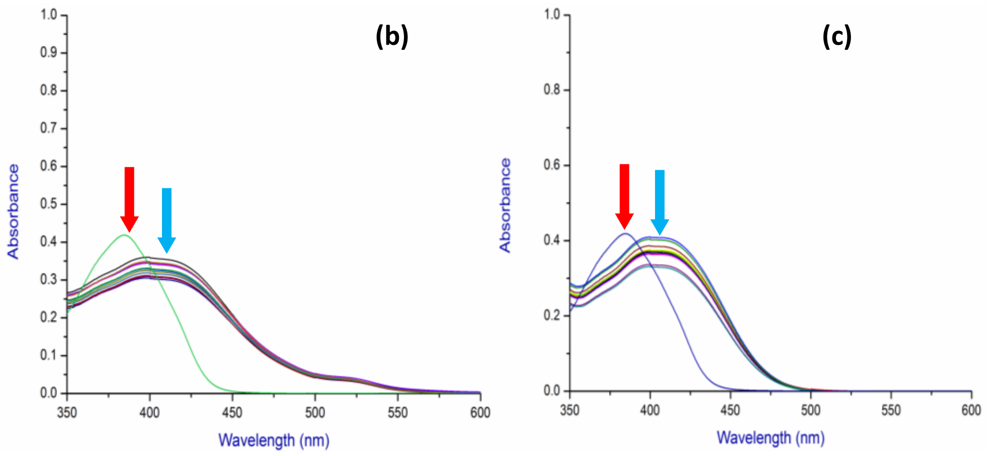


Fig. 17: UV-VIS titration spectra of ligand **L2** (10^{-5}M) with varying concentration of metal ions: (a) free ligand **L2**; (b) $Co(ClO_4)_2$; (c) $Ni(ClO_4)_2$; The red arrow indicates the absorption maxima for ligand **L2** and the blue arrow indicates the shift of absorption maxima for metal-complexes

8. Fluorescence behaviour of the Ligand 2 in presence of metal ions:

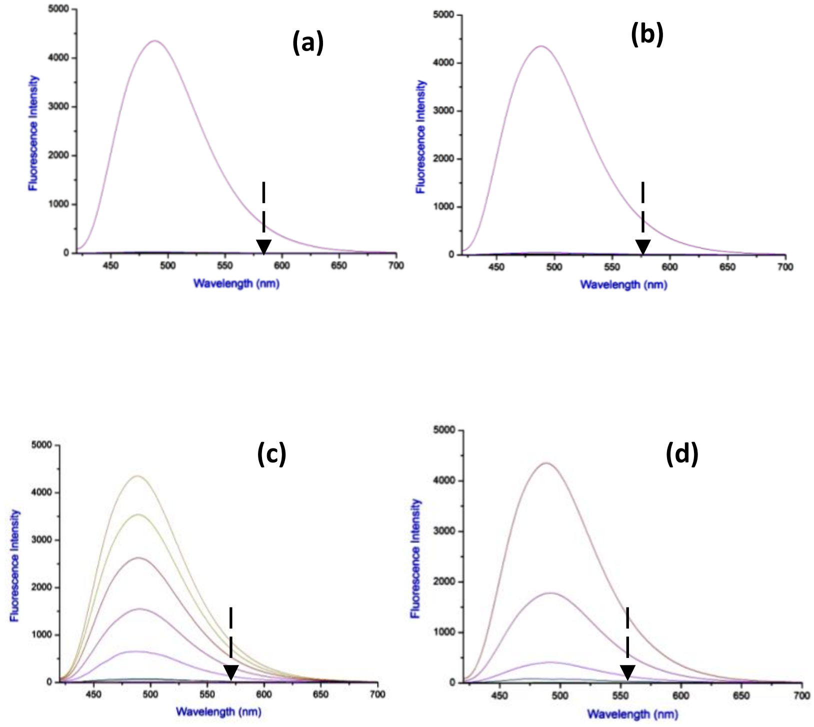


Fig. 18: Fluorescence titration spectra of ligand **L2** (10^{-5}M) with varying concentration of metal ions: (a) $Ni(ClO_4)_2$; (b) $Co(ClO_4)_2$; (c) $Cu(ClO_4)_2$ and (d)$Fe(ClO_4)_2$ [--▸ indicates increasing concentration]

9. *In vitro* cell culture assay method

HaCaT cells (Human keratinocyte cell line) were grown under 5% C02 and 37°C (under humidified incubator) in DMEM-F12(Dulbecco's Modified Eagle's Medium) with 10% Fetal Bovine Serum (FBS), L-glutamine (0.365gm) and 1% Antimycotic antibiotic. Subsequently the cells were seeded on lysine coated cover slips (18 mm x 18 mm) containing DMEM-F12 medium and incubated under ambient culture condition (37°C, 5% CO2). After that the cells were treated as follows:

i) 0.5 mg/ml $ZnClO_4$ for 2 hours; ii) 0.5 mg/mL ligand **L2** for 2 hours; iii) 0.5 mg/ml $ZnClO_4$ and 0.5 mg/ml ligand **L2** for 2 hours

Prior to assay, the cells were washed by Phosphate Buffered Saline (pH = 7.4) for three times to remove the debris (dead cell or cell secretions). Then the treated cells were counter stained by DAPI (4′,6-diamidino-2-phenylindole) (stain for nucleus) for 5-10 min at room temperature in dark. Microphotography of the labelled cells was performed by fluorescence microscopy under 200X magnification.

10.Stern-Volmer treatment:-

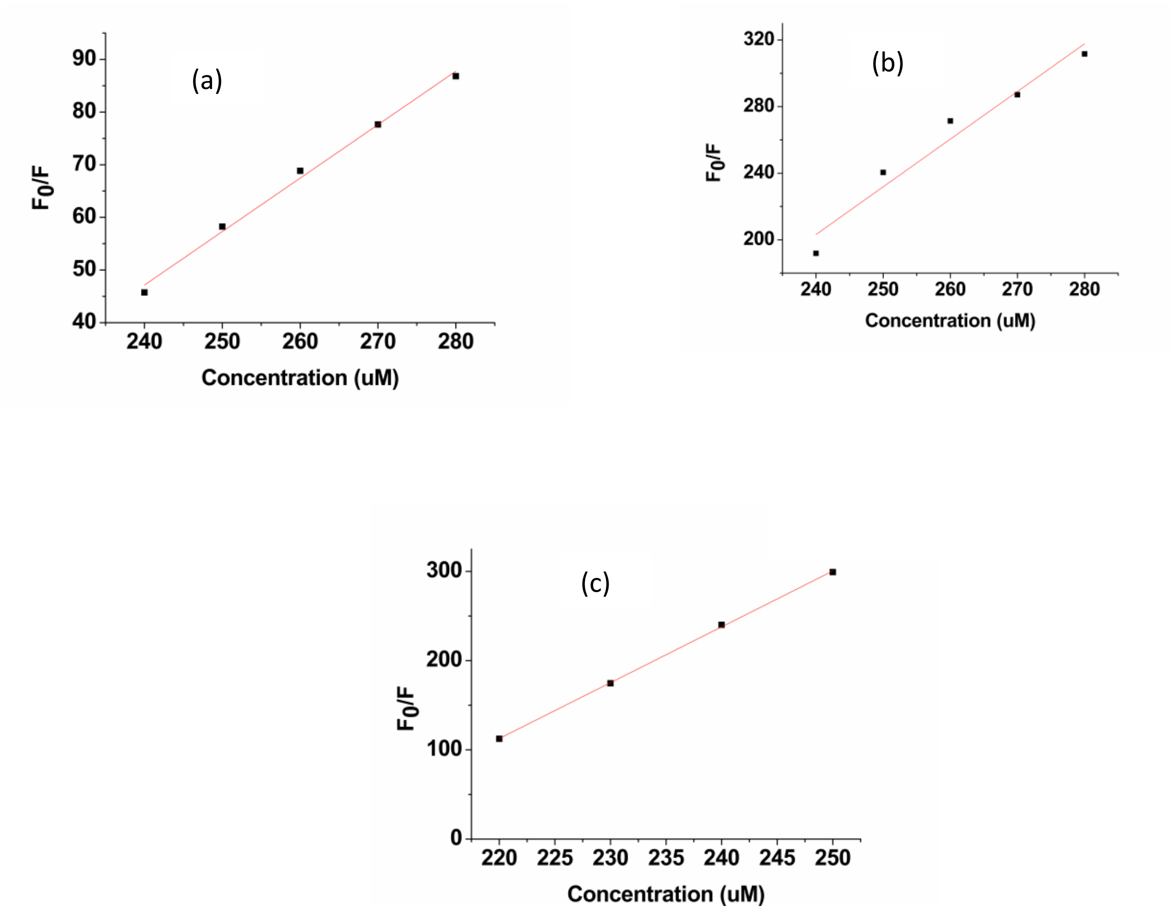
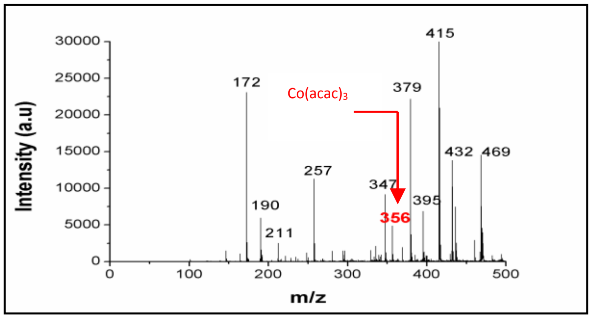


Fig. 19: Stern-Volmer plot of ligand L2 with (a)Co^{2+} (b)Ni^{2+} (c)Zn^{2+}

Table 1- K_{SV} of Zn^{2+}, Ni^{2+}, Co^{2+} from Stern-Volmer equation

Metal ion	**K_{SV} (μM^{-1})**
Zn^{2+}	6.25
Ni^{2+}	2.86
Co^{2+}	1.01

11.MALDI mass spectra of the cobalt(III) acetylacetonate ion (m/z= 356) using CHCA matrix



MALDI mass spectra of the cobalt(III) acetylacetonate ion (m/z= 356) using CHCA matrix