Electronic Supplementary Material (ESI) for Chemical Communications

Naphthalimide based chemosensor for Zn^{2+}, pyrophosphate and H_2O_2: Sequential logic operations at the molecular level

Manoj Kumar,* Naresh Kumar and Vandana Bhalla

Department of Chemistry, UGC-Center for Advanced Studies-1,
Guru Nanak Dev University,
Amritsar-143005, Punjab, India
Email Address: mksharmaa@yahoo.co.in

Instruments and experimental procedures

General information

All reagents were purchased from Aldrich and were used without further purification. Acetonitrile (AR grade) was used to perform analytical studies. UV-vis spectra were recorded on a SHIMADZU UV-2450 spectrophotometer, with a quartz cuvette (path length, 1 cm). The fluorescence spectra were recorded with a SHIMADZU 5301 PC spectrofluorimeter (slit widths at excitation and emission of the spectrofluorimeter are 5-5). ^{1}H and ^{13}C NMR spectra were recorded on a JEOL-FT NMR-AL 300 MHz using $CDCl_3$ as solvent and tetramethylsilane $SiMe_4$ as internal standards. Data are reported as follows: chemical shifts in ppm (δ), multiplicity (s = singlet, d = doublet, q = quartet, br = broad singlet, m = multiplet, dd = doublet of doublet), coupling constants (Hz), integration, and interpretation. Fluorescence quantum yield[1] was determined by using optically matching solution of naphthalene (Φ_{fr} = 0.23 in ethanol) as standard at an excitation wavelength of 360 nm and quantum yield is calculated using the equation:

$$\Phi_{fs} = \Phi_{fr} \times \frac{1\text{-}10^{-ArLr}}{1\text{-}10^{-AsLs}} \times \frac{N_s^2}{N_r^2} \times \frac{D_s}{D_r}$$

Φ_{fs} and Φ_{fr} are the radiative quantum yields of sample and the reference respectively, A_s and A_r are the absorbance of the sample and the reference respectively, Ds and Dr the respective areas of emission for sample and reference. L_s and L_r are the lengths of the absorption cells of sample and reference respectively. N_s and N_r are the refractive indices of the sample and reference solutions (pure solvents were assumed respectively).

Procedure for sensing

UV-vis and fluorescence titrations were performed on 5 and 2 μM solution of ligand in CH_3CN/H_2O (8/2, v/v; buffered with HEPES, pH = 7.2; at 25 °C) mixture. Typically, aliquots of freshly prepared $M(ClO_4)_n$ (M = Hg^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, Co^{2+}, Cd^{2+}, Pb^{2+}, Fe^{3+}, Fe^{2+}, Ag^{+}, Mg^{2+},

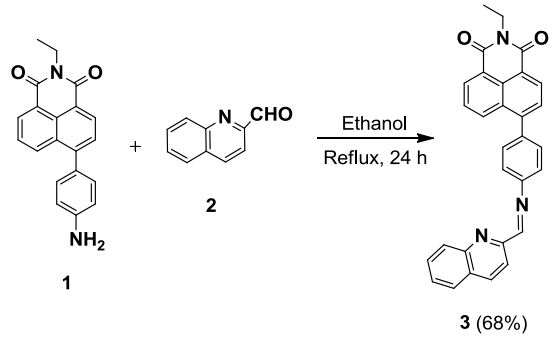
[1] J. N. Deams and G. A. Grosby, *J. Phys. Chem*., 1971, **75**, 991.

Ba^{2+}, Ca^{2+}, Li^{+}, K^{+} and Na^{+}; n = 1 or 2 or 3) and anions ($HP_2O_7^{-3}$, HPO_4^{-}, $H_2PO_4^{-}$, CN^{-}, F^{-}, Br^{-}, Cl^{-}, I^{-}, AcO^{-} and HSO_4^{-} as tetrabutylammonium salt; CO_3^{2-} as K_2CO_3) standard solutions (10^{-1} M to 10^{-3} M) were added to record the UV-vis and fluorescence spectra. Hydrogen peroxide (H_2O_2), *tert*-butyl hydroperoxide (TBHP), and hypochlorite (OCl^{-}) were delivered from 30%, 70%, and 5% aqueous solutions, respectively. Hydroxyl radical (•OH) and *tert*-butoxy radical (•O^{t}Bu) were generated by reaction of 1 mM Fe^{2+} with 100 μM H_2O_2 or 100 μM TBHP, respectively. In titration experiments, each time a 3 ml solution of ligand was filled in a quartz cuvette (path length, 1 cm) and spectra were recorded after the addition of appropriate analyte.

Procedure for fluorescence imaging

The prostate cancer (PC3) cell lines were incubated with receptor **3** (5 µM in CH_3CN/H_2O; 8/2, v/v buffered with HEPES, pH = 7.2) in RPMI-1640 medium for 20 min at 37ºC and washed with phosphate buffered saline (PBS) buffer (pH 7.4) to remove excess of receptor **3**. The cells pre-treated with **3** were then treated with Zn^{2+} ions (10 µM) in RPMI-1640 medium and incubated for further 20 min at 37ºC and washed with PBS buffer. The confocal fluorescence images were scanned on a Nikon eclipse TiE inverted fluorescence microscope equipped with a Nikon AiR laser scanning confocal microscope system. The imaging was done with excitation wavelength of 405 nm and fluorescence images are recorded at blue (470 ± 20 nm) and red channels (570 ± 20 nm).

Synthetic routes and characteristic data



Scheme 1 Synthesis of compound **3**.

Compound **1**[2] was synthesized according to the literature procedure.

Synthesis of (3):

A mixture of compound **1** (0.1 g; 0.31 mmol) and 2-quinolinecarboxaldehyde **2** (0.04 g; 0.31 mmol) in ethanol was refluxed for 24 h. After the completion of the reaction, solvent was evaporated and the residue left was crystallized from $CHCl_3/CH_3OH$ to give compound **3** in 68% yield; m.p. > 250 °C. ^{1}H NMR ($CDCl_3$, 300 MHz): δ = 1.37 (t, J = 6 Hz, 3 H, CH_3), 4.25-4.33 (q, 2 H, CH_2), 7.52-7.64 (m, 5 H, Ar-H), 7.71-7.80 (m, 3 H, Ar-H), 7.90 (d, J = 9 Hz, 1 H, Ar-H), 8.19 (d, J = 9 Hz, 1 H, Ar-H), 8.32 (t, J = 9 Hz, 2 H, Ar-H), 8.41 (d, J = 9 Hz, 1 H, Ar-H), 8.65-8.68 (m, 2 H, Ar-H), 8.90 (s, 1 H, HC=N), ^{13}C NMR ($CDCl_3$, 300 MHz): δ = 13.76, 35.92, 115.38, 121.43, 121.66, 121.73, 123.35, 125.19, 125.37, 126.86, 127.18, 127.88, 128.15, 129.14, 129.25, 130.31, 130. 57, 131.11, 131.27, 131.37, 131.51, 133.33, 136.32, 147.68, 157.00, 159.06, 164.49 and 164.69 ppm. MS ES+, *m/z*: = 456.18 $[M+H]^+$. $C_{30}H_{21}N_3O_2$: calcd. C 79.10, H 4.65, N 9.22; Found C 79.02, H 4.78, N 9.47.

[2] M. Kumar, N. Kumar, V. Bhalla, P. R. Sharma and T. Kaur, *Org. Lett.*, 2011, **13**, 1422.

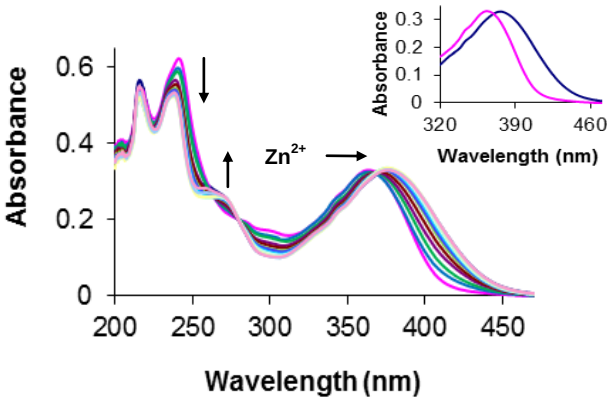


Figure S1 UV-vis spectra of **3** (5.0 μM) with Zn^{2+} ions (15 equiv) in H_2O:CH_3CN (2:8, v/v) buffered with HEPES, pH = 7.0. Inset showing the UV-vis spectra of free **3** (pink line) and **3** + Zn^{2+} ions (blue line).

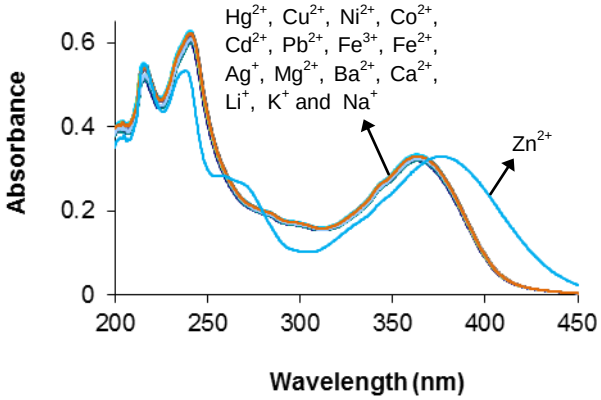


Figure S2 UV-vis spectra of **3** (5.0 μM) with different metal ions (15 equiv each) in CH_3CN:H_2O; (8:2, v/v) buffered with HEPES, pH = 7.0.

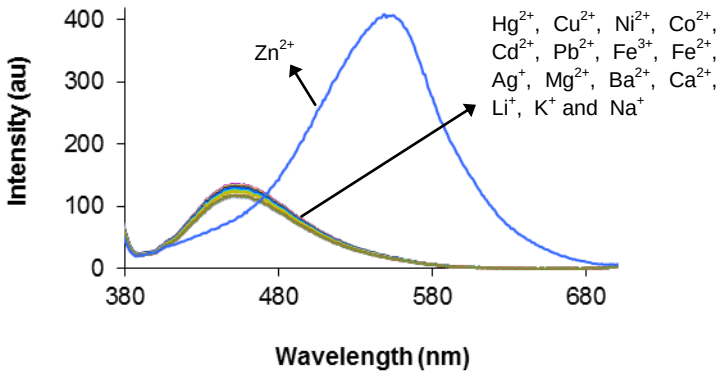


Figure S3 Fluorescence spectra of **3** (2 μM) in H_2O:CH_3CN (2:8, v/v) buffered with HEPES, pH = 7.0; at 25 °C; λ_{ex} = 360 nm in the presence of different metal ions (26 μM each).

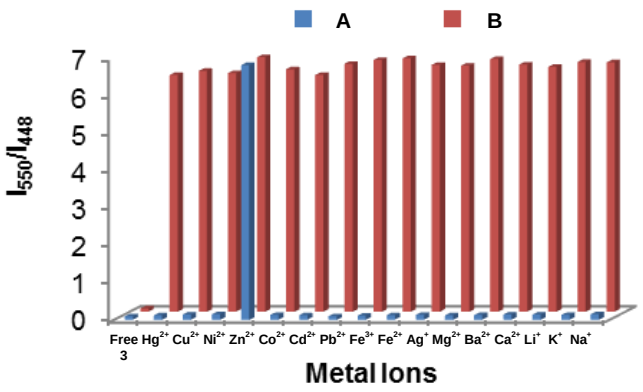


Figure S4 (A) Fluorescence selectivity (I_{550}/I_{448}) of **3** (2 μM) in the presence of various metal ions (26 μM each). (B) Competitive fluorescence selectivity (I_{550}/I_{448}) of receptor **3** towards Zn^{2+} ions (26 μM) in the presence of other metal ions (26 μM each) in H_2O:CH_3CN (2:8, v/v) buffered with HEPES, pH = 7.0 (at 25 °C; λ_{ex} = 360 nm).

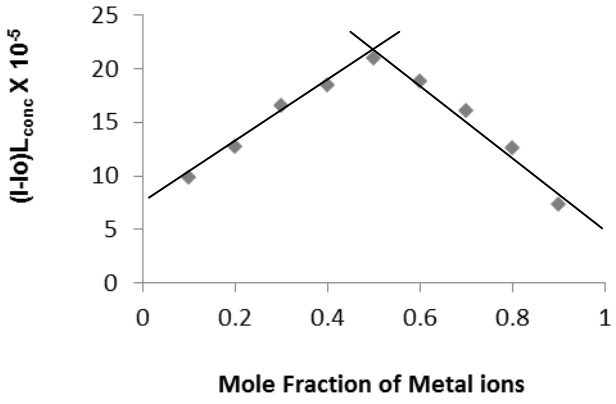


Figure S5 Job's plot for determining the stoichiometery of **3** and Zn^{2+} ions.

Calculations for detection limit:

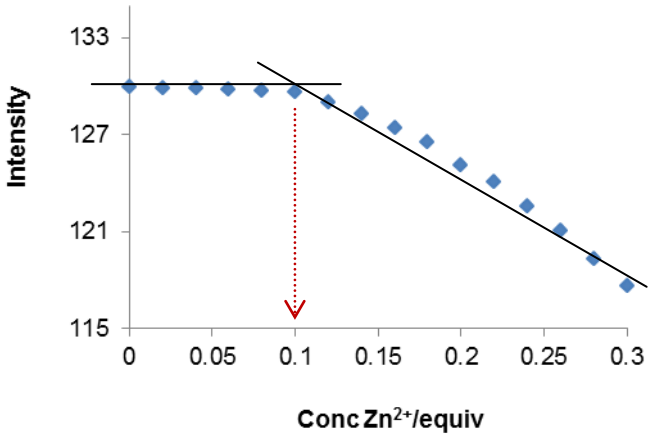


Figure S6. Figure showing the fluorescence intensity at 448 nm as a function of Zn^{2+} ions concentration.

To determine the detection limit, fluorescence titration of compound **3** with Zn^{2+} ions was carried out by adding aliquots of ferric solution of minimum concentration and the fluorescence intensity as a function of Zn^{2+} ions added was then plotted. From this graph the equivalents used at which there was a sharp change in the fluorescence intensity multiplied with the concentration of receptor **3** gave the detection limit.

Equation used for calculating detection limit (DL):

$DL = C_L \times E_T$

C_L = Conc. of Ligand; E_T = Equiv. of Titrant at which change observed.

Thus;

$DL = 2 \times 10^{-6} \times 0.1 = 0.2 \times 10^{-6} = 20 \times 10^{-8}$ mol L^{-1}

Similar procedure was utilized for calculating the detection limits of pyrophosphate and H_2O_2.

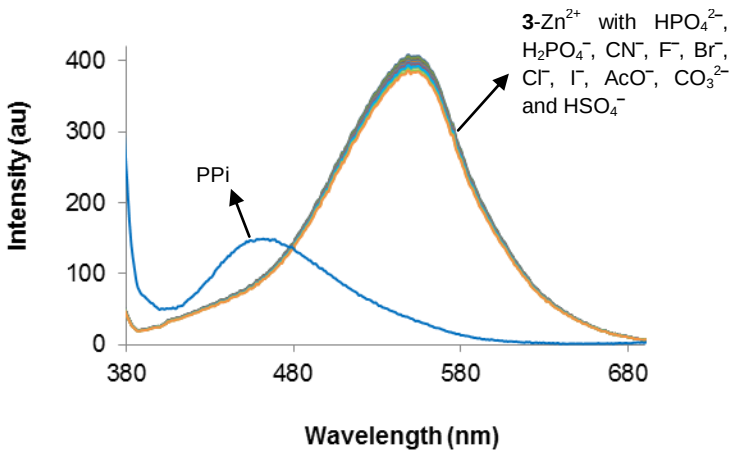


Figure S7. Fluorescence spectra of **3** (2 μM) upon addition of various inorganic anions (60 μM each) to the **3**-Zn^{2+} complex in H_2O:CH_3CN (2:8, v/v) buffered with HEPES, pH = 7.0; λ_{ex} = 360 nm.

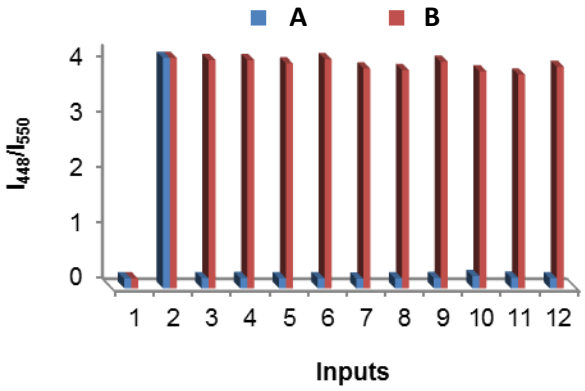


Figure S8. Fluorescence response of **3**-Zn^{2+} complex to various anions (60 μM each) H_2O:CH_3CN (2:8, v/v) buffered with HEPES, pH = 7.0 (at 25 °C; λ_{ex} = 360 nm). Bars represent fluorescence selectivity (I_{448}/I_{550}) of **3**-Zn^{2+} complex upon addition of different anions. (A) Blue bars represent selectivity of **3**-Zn^{2+} complex upon addition of different anions; (B) Red bars represent competitive selectivity of receptor **3**-Zn^{2+} complex towards PPi (60 μM) in the presence of other anions (60 μM). 1, free; 2, PPi; 3, HPO_4^{2-}; 4, $H_2PO_4^-$; 5, CN^-; 6, F^-; 7, Br^-; 8, Cl^-; 9, I^-; 10, AcO^-; 11, CO_3^{2-} and 12, HSO_4^-.

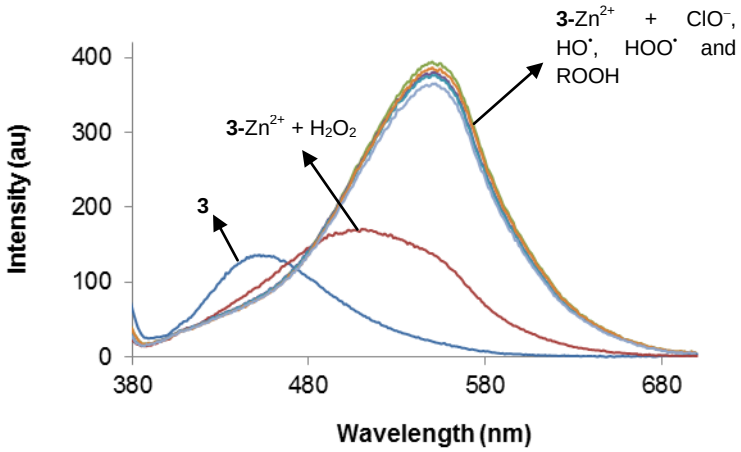


Figure S9. Fluorescence spectra of **3** (2 μM) upon addition of various reactive oxygen species (80 μM each) to the **3**-Zn^{2+} complex in H_2O:CH_3CN (2:8, v/v) buffered with HEPES, pH = 7.0; λ_{ex} = 360 nm. Hydrogen peroxide (H_2O_2), *tert*-butyl hydroperoxide (TBHP), and hypochlorite (OCl^-) were delivered from 30%, 70%, and 5% aqueous solutions, respectively. Hydroxyl radical (•OH) and *tert*-butoxy radical (•O^{t}Bu) were generated by reaction of 1 mM Fe^{2+} with 100 μM H_2O_2 or 100 μM TBHP, respectively.

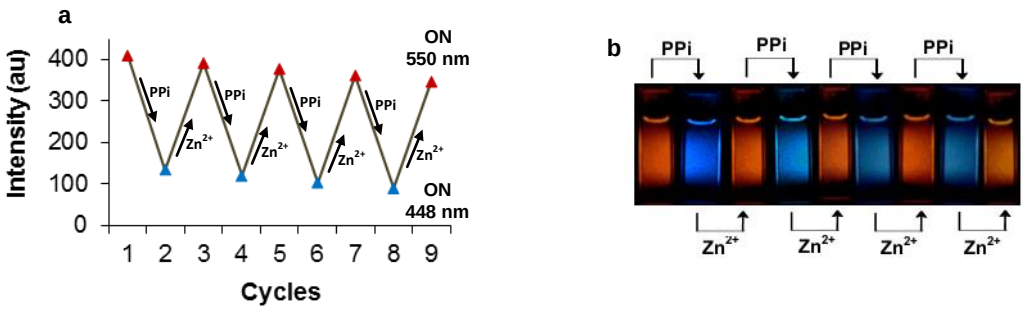
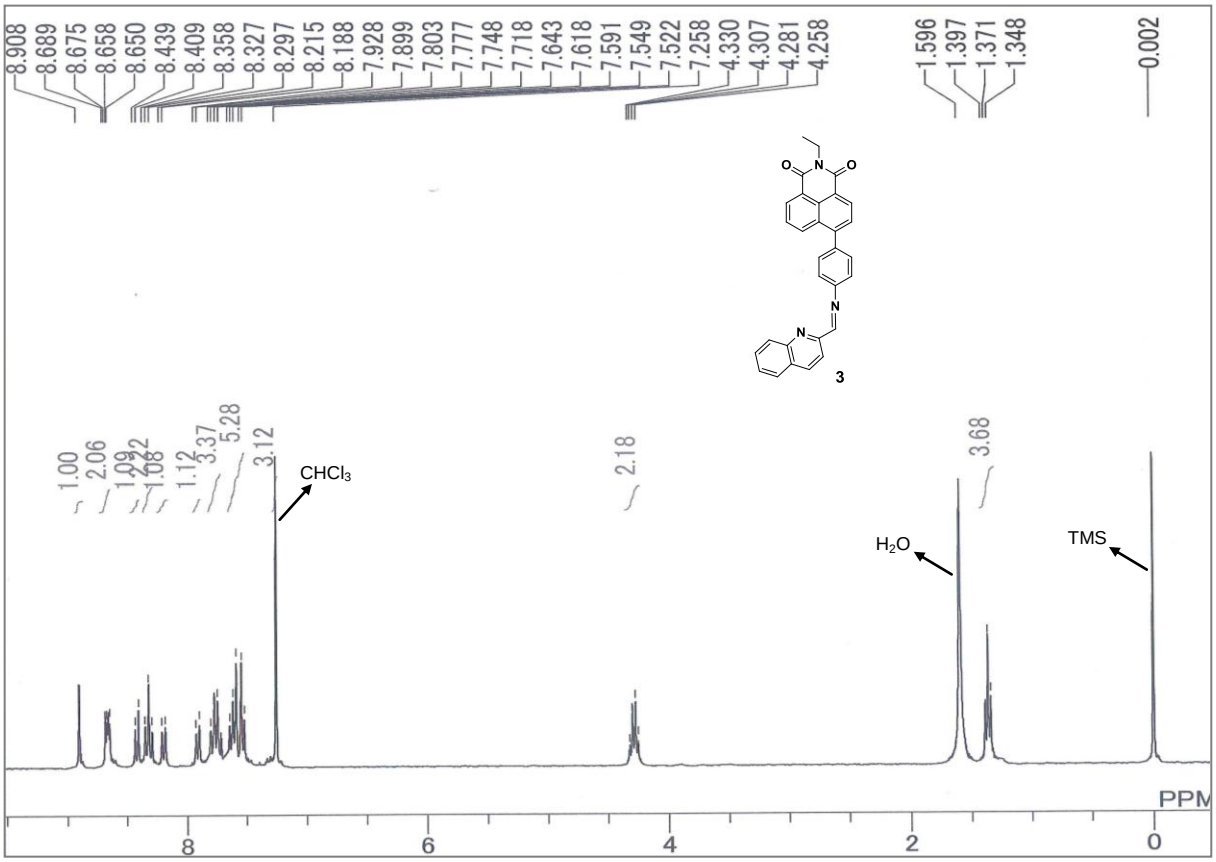
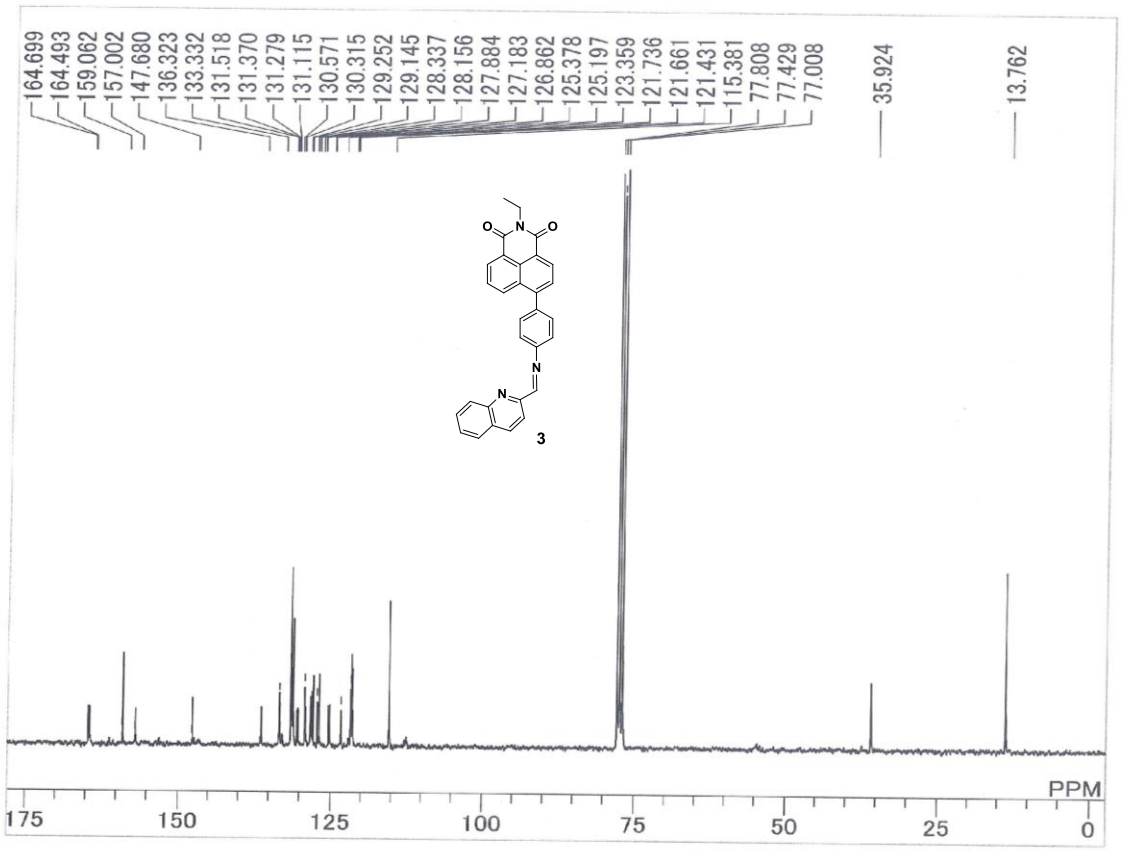


Figure S10. Figure showing the reversibility and reusability of the receptor **3** for sensing of Zn^{2+} and PPi by alternate addition: (a) fluorescence intensity obtained during the titration of **3**-Zn^{2+} with PPi followed by the addition of Zn^{2+} (**3**-Zn^{2+}) = 2 μM; λex = 360 nm; (b) fluorescence changes after each addition of PPi and Zn^{2+} sequentially.

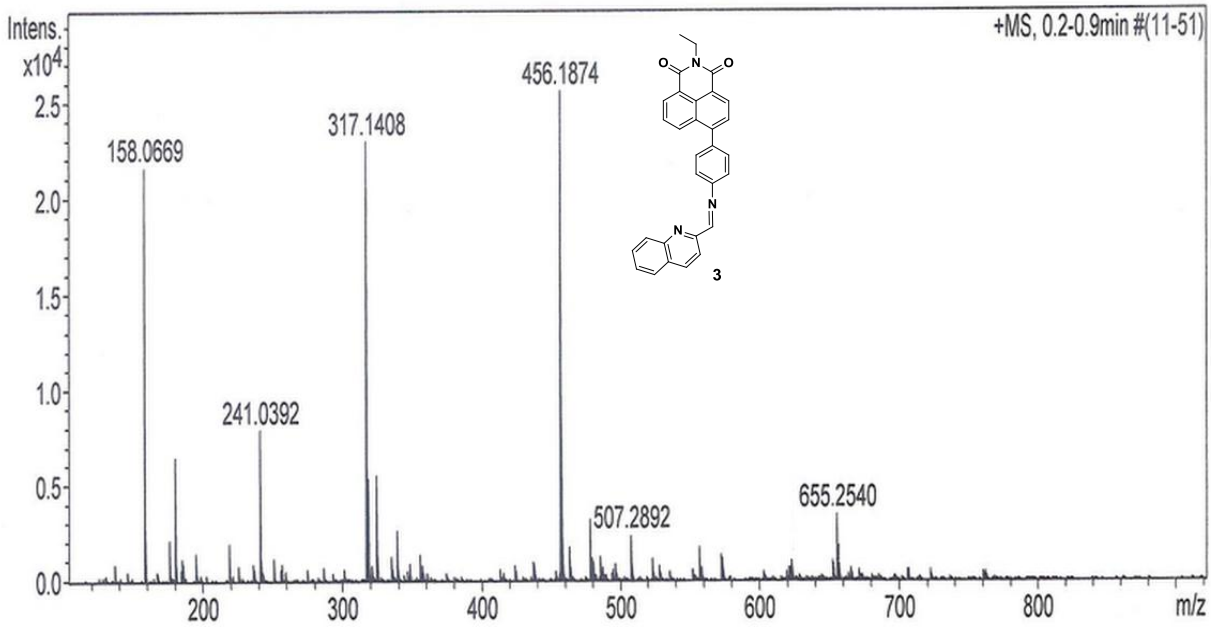
^{1}H NMR spectrum of compound **3**



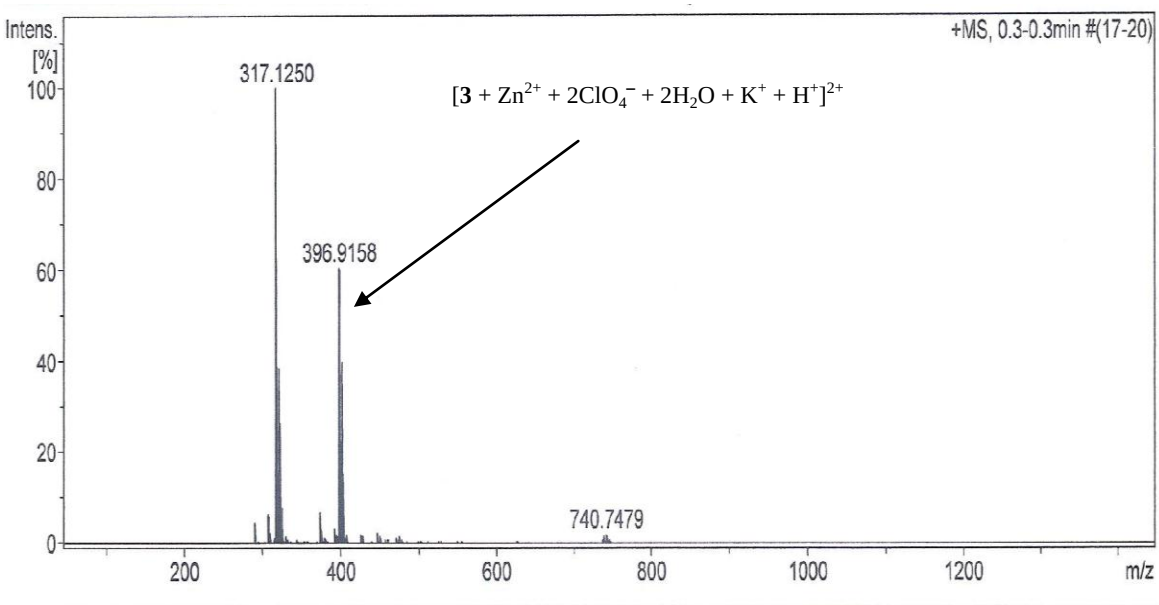
^{13}C NMR spectrum of compound **3**

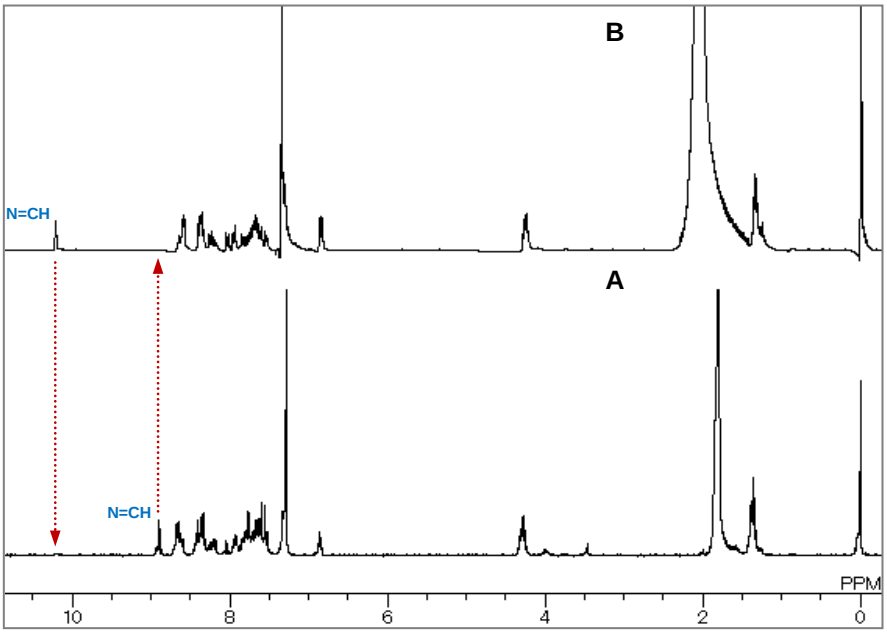


Mass spectrum of compound **3**



Mass spectrum of compound **3**-Zn^{2+} complex





^{1}H NMR (300 MHz) spectra of **3** in $CDCl_3/CD_3CN$ (9:1); (A) Free ligand; (B) Free ligand + 1.0 equiv. of zinc perchlorate.