Electronic Supplementary Material (ESI) for New Journal of Chemistry.

A Luminescent pH-Sensitive Lysosome targeting Eu(III) Probe

Kritika Gupta[a], Madhu Verma[b], Priyanka Srivastava[a], Sri Sivakumar[b] and Ashis K. Patra[a*]

[a]Department of Chemistry, Indian Institute of Technology Kanpur, Kanpur 208016, Uttar Pradesh, India

[b]Department of Chemical Engineering and Centre for Environmental Science and Engineering, Indian Institute of Technology Kanpur, Kanpur 208016, Uttar Pradesh, India

Materials and Methods: Reagents and solvents were purchased from Sigma Aldrich, Alfa Aesar, and TCI and were used without any further purification. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazoliumbromide (MTT), Dulbecco's modified eagle's medium (DMEM, Gibco® Life Technologies, Bengaluru, India), penicillin–streptomycin antibiotic, and gelatin (from cold water fish skin) were purchased from Sigma Aldrich (Bengaluru, India) and used for as received. LysoTracker® Blue DND-22 dye was obtained from ThermoFisher scientific India. The solvents used were either HPLC-grade or purified by standard procedures. ^{1}H and ^{13}C NMR spectra (chemical shifts in δ ppm) were recorded on a JEOL AL FT–NMR (400 and 500 MHz) spectrometer, using TMS as an internal standard. FT-IR spectra in KBr were recorded on a perkin elmer FT-IR spectrometer. Electrospray ionization mass spectral (ESI-MS) measurements were carried out using a WATERS Q-TOF Premier mass spectrometer. The UV-Vis absorption spectra were recorded on Perkin Elmer 1700 spectrophotometer using a quartz cuvette (path length = 1cm). The fluorescence and time-resolved luminescence spectra were recorded using Agilent Cary eclipse fluorescence spectrophotometer at 298 K. Stock solution of $[Eu(L^2)(H_2O)]$ (**1**) and $[Eu(L^5)(H_2O)]$ (**2**) (c = $1x10^{-3}$ M) were prepared in DMF-water (2:8, v/v). For the luminescence experiment at λ = 445 nm, slit width = 10, was used and data were recorded at normal PMT voltage. The lifetime measurements were performed under ambient conditions with $[Eu(L^2)(H_2O)]$ (**1**) in H_2O and D_2O, with a pulsed xenon lamp at λ_{ex} = 445 nm and λ_{em} = 615 nm with delay and gate time of 0.1 ms. The decay curves were fitted by the nonlinear least-squares method. The excited-state lifetime measurements in water and D_2O allowed the determination of the number of water molecules (q) directly coordinated to the Eu^{3+} ion with the modified Horrocks equation (1).[S1]

$$q_{Eu} = 1.11\left(\frac{1}{\tau_{H_2O}} - \frac{1}{\tau_{D_2O}} - 0.31\right) \quad \textbf{(1)}$$

The pH experiment in the range of pH = 2-10 was performed in phosphate buffer of different pHs calibrated with the help of pH meter (EUTECH instrument) solutions by mixing appropriate volumes of acid (HCL) and base (NaOH) components.

pKa calculations

The pKa value of $[Eu(L^2)(H_2O)]$ have been calculated using **Henderson-Hasselbalch equation** (2)

$$Log[\frac{Imax - I}{I - Imin}] = pH - pKa \qquad (2)$$

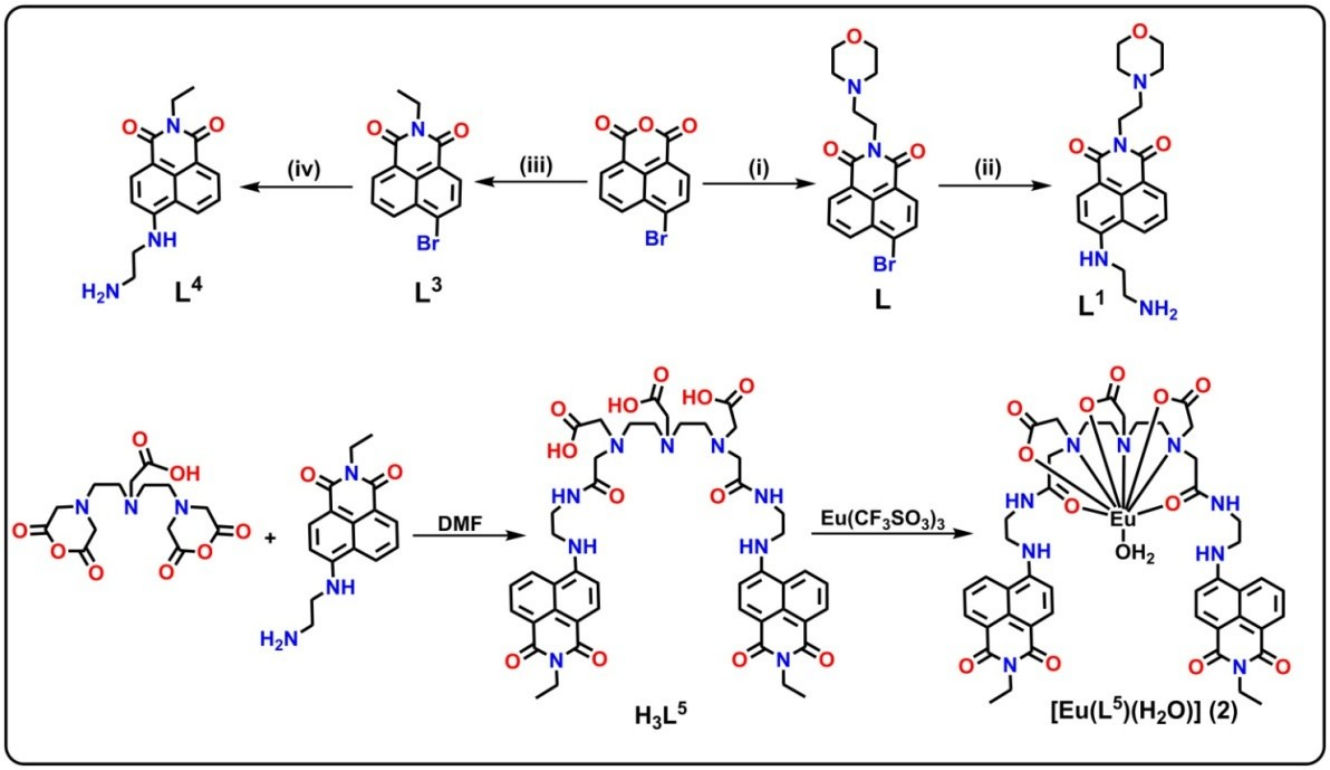
Where I is the emission intensity with variation in pH and I_{max}, I_{min} is the maximum and minimum emission intensity with the variation of pH.[S2]

Quantum yield estimation

The quantum yield of [Eu(L^2)(H_2O)] (**1**) were calculated utilizing equation (3)

$$\emptyset sample = \emptyset\, ref \left(\frac{Isample}{Iref}\right)\left(\frac{ODref}{ODsample}\right)\left(\frac{n2sample}{n2ref}\right) \qquad \textbf{(3)}$$

Where Φ represents the quantum yield, I, represent the integrated emission area and OD, the optical density of sample and reference (ref) fluorophore. The integrated emission area of Eu(III)-probe **1** was calculated at λ_{ex}= 445 nm.



Scheme S1. General Synthetic scheme for ligands and [Eu(L^5)(H_2O)] (**2**) (i) 4-(2-ethyl amine) morpholine, Ethanol, 50 °C (ii) Ethylenediamine/TEA/Pyridine, reflux, overnight (iii) Ethylamine, 1,4-Dioxane, Reflux, 7 h (iv) Ethylenediamine, 80 °C, 18 h.

4-Bromo-N-(2-morpholinoethyl)-1,8-naphthalimide (L):

The suspension of 4-bromo-1,8-naphthalic anhydride (0.554 g, 2 mmol) was taken in anhydrous ethanol (10 ml) and 4-(2-ethyl amine) morpholine (325 µl, 2.5 mmol) was added. The reaction mixture was stirred at 50°C for 5 h. After complete reaction (monitored on TLC), cold water (25 ml) was added to the reaction mixture and filtered. The precipitate was washed with 10% aqueous Na_2CO_3 solution followed by water and dried to obtain a light yellow colour crystalline solid. Yield: 80% (0.62 g, 1.70 mmol). ESI-MS in DMF (*m/z*): $[M+H]^+$ calcd for $C_{18}H_{18}BrN_2O_3$: 389.0501 (100%), 391.0480 (97.3%), Found: 389.0508 (100%), 391.0485 (97%). ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.64-8.63 (d, 1H, *J* = 6 Hz), 8.57-8.53 (d, 1H, *J* = 6.8 Hz), 8.40-8.38 (d, 1H, *J* = 7.6 Hz), 8.04-8.02 (d, 1H, *J* = 8.4 Hz), 7.86-7.82 (m, 1H, *J1* = 8.4 Hz; J_2 = 7.6 Hz) 4.33-4.30 (m, 2H, *J* = 6.8 Hz), 3.66-3.64 (m, 4H), 2.70-2.66 (m, 2H, J_1J_2=6.8Hz*)* 2.58-2.56 (m, 4H). ^{13}C NMR (400 MHz, $CDCl_3$): 163.71, 133.40, 132.13, 131.32, 131.21, 130.76, 130.40, 129.14, 123.16, 122.29, 67.14, 56.17, 53.90, 37.43.

4-(2-aminoethylamino)-N-(2-morpholinoethyl)-1,8-naphthalimide (L^1):

A solution of L (0.5 g, 1.28 mmol) was taken in anhydrous pyridine (10 ml), Ethylene diamine (2.56 ml, 38.4 mmol) and triethylamine (200 µl) were added and reaction mixture was refluxed for overnight. After the completion of reaction (as monitored on TLC), the solvent was evaporated under reduced pressure and cold water was added to the reaction mixture to precipitate out the desired product, which was filtered and dried in air. Pure compound was obtained by column chromatography (elution with methanol) to afford an orange colour compound of L^1. Yield: 81% (0.41 g, 1.03 mmol). ESI-MS in DMF (*m/z*): $[M+H]^+$ calcd for $C_{20}H_{25}N_4O_3$: 369.1927 (100%). Found: 369.1921 (100%).^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm): 8.56-8.54 (m, 1H, *J* = 6.1 Hz), 8.42-8.40 (d, 1H, *J* = 8.2 Hz), 8.22-8.20 (d, 1H, *J*= 8.2 Hz), 7.65- 7,62 (m, 1H, J_1 = 7.7 Hz, J_2 = 7.9Hz), 6.22 (s, -NH), 4.32-4.29 (m, 2H, J_1, J_2 = 7,3 Hz), 3.83-3.80 (m, 2H), 3.79-3.67 (m, 4H), 2.70-2.66 (m, 2H), 2.59 (m, 4H). ^{13}C NMR (400 MHz, $CDCl_3$): 164.28, 163.40, 151.34, 134.76, 131.22, 129.88, 129.25, 122.32, 120.75, 108.14, 104.41, 66.76, 56.32, 53.96, 46.23, 38.96, 36.98. UV-vis (in 10 mM PBS buffer, 298 K), λ_{max}, nm (ε, M^{-1} cm^{-1}): 437 (18006).

N,N''-bis-(4-(2-aminoethylamino)-N-(2-morpholinoethyl)-1,8-naphthalimide)-diethylenetriamine-N,N',N''-triacetic acid (H_3L^2):

To the solution of DTPA-bis(anhydride) (0.179 g, 0.5 mmol) in dry DMF (30 mL), L^1 (0.368 g, 1.0 mmol) was added and the reaction mixture was stirred overnight at 80 °C under inert atmosphere. The solvent was then removed under reduced pressure. The solid was washed with acetonitrile (3×15 mL) and diethyl ether (3×15 mL) three times and then 10 mL of distilled water was added to it and pH was adjusted to 8.0 with NaOH solution. The aqueous phase was extracted with chloroform (3×15 mL). Aqueous layer was then evaporated in vacuo and finally dried in vacuum overnight to afford a orange powder product (yield: 0.465 g, 85%). ESI-MS in H_2O (*m/z*): $[M+H]^+$ calcd for $C_{54}H_{68}N_{11}O_{14}$: 1094.4947 (100%). Found: 1094.4945 (100%).^{1}H-NMR (500 MHz, DMSO-d_6) δ ppm: 8.63 (d, 2H), 8.23 (d, 2H), 8.05 (d,2H), 7.48 (t, 2H), 7.12 (d, 2H), 7.42 (s, 2H), 6.65 (d, H), 3.96 (t, 4H), 3.55 (t, 4H), 3.46 (t, 4H), 3.28 (s, 6H), 3.21 (s, 4H), 3.14 (t, 4H)), 3.05 (d, 4H), 2.97 (d, 4H), 2.77 (t, 4H), 2.72 (t, 4H). FT-IR (KBr pellet, ν_{max}, cm^{-1}): 3230 (w), 1632 (s, $\nu_{C=O}$ of COOH), 1573 (s, $\nu_{C=O}$ of CONH), 1446 (w), 1399 (s), 1253 (m), 1176 (m), 1095 (m), 985 (m), 758 (m). (vs, very strong; s, strong; m, medium; w, weak; br, broad). UV-vis (in 10 mM PBS buffer, 298 K), λ_{max}, nm (ε, M^{-1} cm^{-1}): 443 (22400).

4-Bromo-N-(ethyl)-1,8-naphthalimide (L^3) and 4-(2-aminoethylamino)-N-(ethyl)-1,8-naphthalimide (L^4):

Reaction of 4-bromo-1,8-naphthalic anhydride (1) with ethylamine gave L^2, which on further reaction with 1,2-diaminoethane *via* a known procedure yield L^3. [S3]

N,N''-bis-(4-(2-aminoethylamino)-N-(2-ethyl)-1,8-naphthalimide)-diethylenetriamine-N,N',N''-triacetic acid (H_3L^5):

H_3L^5 was synthesized according to the above procedure of **H_3L^2** using DTPA-bis(anhydride) (0.179 g, 0.5 mmol), L^3 (0.283 g, 1.0 mmol). (yield: 0.36 g, 78%). ESI-MS in CH_3OH (*m/z*): $[M+H]^+$ calcd for $C_{46}H_{54}N_9O_{12}$: 924.3892 (100%). Found: 924.3830 (100%). ^{1}H-NMR (400 MHz, DMSO-d_6) δ ppm: 8.55 (d, 2H), 8.37 (d, 2H), 8.20 (d, 2H), 8.04 (s, 1H), 7.63 (t, 2H), 6.78 (d, 2H), 3.97 (t, 4H), 3.59-3.51 (m, 8H), 3.43-3.39 (m, 10H), 3.08 (q, 6H), 2.58 (t, 8H), 1.12 (t, 4H)). FT-IR (KBr pellet, ν_{max}, cm^{-1}): 3240 (w), 1640 (s, $\nu_{C=O}$ of COOH), 1577 (s, $\nu_{C=O}$ of CONH), 1455 (w), 1362 (s), 1247 (m), 1170 (m), 1092 (m), 983 (m), 775 (m). (vs, very strong; s, strong; m, medium; w, weak; br, broad).

Synthesis of [Eu(L^2)(H_2O)] (1):

The Eu(III) probe were prepared by following a general synthetic procedure. To a 5 mL MeOH solution containing $Eu(CF_3SO_3)_3$ (0.138 g, 0.23 mmol) was added dropwise to an aqueous solution (10 mL) of

H_3L^2 (0.252 g, 0.23 mmol) pre-treated with NaOH (0.027 g, 0.67 mmol) for 15 min. The reaction mixture was stirred at 40 °C for 5 h to obtain an orange precipitate. The precipitate was filtered and washed successively with cold methanol (3×5 mL) and diethyl ether (3×5 mL) and finally dried in a vacuum over P_4O_{10} (yield: 0.310 g, 79 %). Anal. calcd for $C_{54}H_{65}EuN_{11}O_{14}$: C, 51.43; H, 5.28; N, 12.05. Found: C, 52.12; H, 5.44; N, 12.28. ESI-MS in H_2O (m/z): $[M\text{-}H_2O+H]^+$ calcd for $C_{54}H_{65}EuN_{11}O_{14}$(relative abundance): 1244.392 (100.0%), 1242.391 (91.6%), 1245.395 (58.4%), 1243.394 (53.5%), 1246.399 (16.7%). Found: 1244.392 (100.0%), 1242.393 (74.0%), 1245.397 (60.0%), 1243.392 (55.5%), 1246.397 (25.5%). FT-IR (KBr pellet, ν_{max}, cm^{-1}): 3221 (w), 1635 (s, $\nu_{C=O}$ of COOH), 1582 (s, $\nu_{C=O}$ of CONH), 1440 (w), 1397 (s), 1248 (m), 1174 (m), 1091 (m), 983 (m), 757 (m).). (vs, very strong; s, strong; m, medium; w, weak; br, broad). UV-vis (in 10 mM PBS buffer, 298 K), λ_{max}, nm (ε, M^{-1} cm^{-1}): 445 (25350).

Synthesis of [Eu(L^5)(H_2O)] (2):

The title probe was synthesized according to the above procedure using **H_3L^5** (0.179 g, 0.5 mmol), $Eu(CF_3SO_3)_3$ (0.138 g, 0.23 mmol), and NaOH (0.027 g, 0.67 mmol). (yield: 0.24 g, 76%). FT-IR (KBr pellet, ν_{max}, cm^{-1}): 3221 (w), 1639 (s, $\nu_{C=O}$ of COOH), 1578 (s, $\nu_{C=O}$ of CONH), 1445 (w), 1397 (s), 1239 (m), 1170 (m), 1091 (m), 979 (m), 777 (m).). (vs, very strong; s, strong; m, medium; w, weak; br, broad). UV-vis (in 10 mM PBS buffer, 298 K), λ_{max}, nm (ε, M^{-1} cm^{-1}): 438 (14330).

Cytotoxicity assay and uptake studies

Cytotoxicity of the samples was determined by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay using MCF-7 (breast cancer), PC3 (prostate cancer), A498 (kidney cancer) and NRK-49F (rat kidney fibroblast cells) non-cancerous cell line.[S4] In brief, all the cells were cultured in DMEM medium supplemented with FBS (10%, v/v) and antibiotic (penicillin/streptomycin 1%, v/v) at 37 °C in an incubator containing 5% CO_2 atmosphere. 10^5 cells were seeded in a 96-well plate and incubated at 37 °C with 5% CO_2 till cells get adhere and obtain their morphology. Then the cells were treated with the [Eu(L^2)(H_2O)] (**1**) with different concentrations (10, 20, 40, 60, 80 μM) for the next 24 h. Further, MTT (200 μl, 0.5 mg/ml) was added to each well and incubated for next 4 h, then MTT was removed from wells and 200 μl of DMSO was added to each well after 20 minutes the plate was measured using a microliter plate reader (MultiSkan UV-Vis spectrometer, Thermo Scientific) at a wavelength of 570 nm. Formation of purple colored formazan (absorbance at 570 nm) represents the

viable cells, considering the control (untreated) cells as 100% viable. All readings were taken in triplicates.

Cellular uptake studies

To study the cellular uptake studies, cells were cultured in 24 well plate having coverslip coated with gelatin with Dulbecco's minimum essential medium (DMEM) medium supplemented with fetal bovine serum (FBS) (10%, v/v) and antibiotic (penicillin/streptomycin 1%, v/v) at 37 °C in an incubator containing 5% CO_2 atmosphere. 10^5 cells were seeded in a 96-well plate and incubated at 37 °C with 5% CO_2 till cells get adhere and obtain their morphology. Further, the cells were incubated with the [Eu(L^2)(H_2O)] (**1**) at 20 μM concentration for 5 h. Then the media was removed and each well was washed thrice with PBS. For nucleus staining, Hoechst 33258 dye (10 μg/mL) was used for 20 min, after staining each well was washed thrice with PBS. 4% formaldehyde was used for fixing the cells and after washing each well with PBS, mounted the coverslip on the glass slides.

Next for the co-localization studies, after treatment with [Eu(L^2)(H_2O)] (**1**) (20 μM) cells were incubated with LysoTracker® Blue DND-22 (5 μM for 45 min), cells were washed thrice with PBS. 4% formaldehyde was used for fixing the cells and after washing each well with PBS, mounted the coverslip on the glass slides. Slides were observed under confocal microscopy. All the staining protocols were used as provided by the respective suppliers. The slides were observed and images were clicked using a Carl Zeiss LSM780NLO confocal laser scanning microscope (CLSM) using appropriate filters for blue and red emission from Hoechst 33258, Eu(III)-probe **1** with suitable filters for blue (blue ch1: λ = 371–500 nm) emission from Hoechst 33258 and LysoTracker® Blue DND-22, and red (red ch2: λ = 562–736 nm) emission from [Eu(L^2)(H2O)] (**1**) for Eu^{III} probe **1** using Chameleon tunable multiphoton laser and a DPSS green laser. Pearson's coefficient for the acquired images was measured by ImageJ colocalization analysis software.

To study the in vitro pH effect on probe **1**, 10^5 MCF-7 cells were seeded in 24 well plate having coverslip coated with gelatin in DMEM medium supplemented with fetal bovine serum (FBS) (10%, v/v) and antibiotic (penicillin/streptomycin 1%, v/v) at 37 °C in an incubator containing 5% CO2 atmosphere till cells get adhere and obtain their morphology. Cells were first incubated with probe (20 μM) for 5 h at 37 °C. Each well was washed three times with PBS buffer, and then chloroquine was added (at concentrations 100 μM (pH 6.4) and 200 μM (pH 6.6)) to further change the cellular pH. Cover-slips were mounted on the glass slides and observed under confocal microscope.

References:

S1. W. D. Horrocks and D. R. Sudnick, *J. Am. Chem. Soc*., **1979**, *101*, 334-340; W. D. Horrocks, Jr. and D. R. Sudnick , *Acc. Chem. Res.*, **1981**, *14* , 384; R. M. Supkowski and W. D. Horrocks, Jr. , *Inorg. Chim. Acta*, **2002**, *340*, 44-48.

S2. L. J. Henderson, Am J Physiol. **1908**, 21, 173; H. N. PoN, M. Senozan, J Chem Educ. **2001**, 78, 1499–503; L. P. Hammett, J Am Chem Soc. **1928**, 50, 2666; A. G. Hills, The Am. J. Med., **1973**, 55, 2, 131-133; M. H. Lee, N. Park, C. Yi, J. H. Han, J. H. Hong, K. P. Kim, D. H. Kang, J. L. Sessler, C. Kang, J. S. Kim, *J. Am. Chem. Soc*. **2014**, *136*, 14136−14142.

S3. T. Gunnlaugsson, P. E. Kruger, P. Jensen, J. Tierney, H. D. P. Ali, G. M. Hussey, J. Org. Chem., **2005**, *70*, 26, 10875-10878; O. Sahin, M. Yilmaz, Tetrahedron Lett., **2012**, *53*, 18, 2319-2324.

S4. T. Mosmann, J. Immunol. Methods, **1983**, *65*, 55-63.

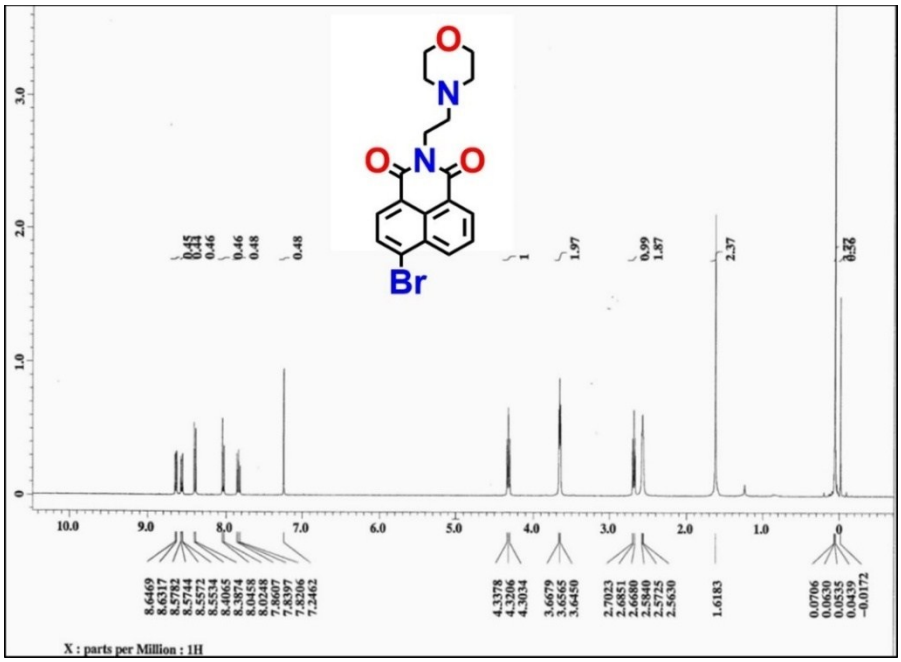


Figure S1. ^{1}H NMR spectrum of compound (**L**) in $CDCl_3$.

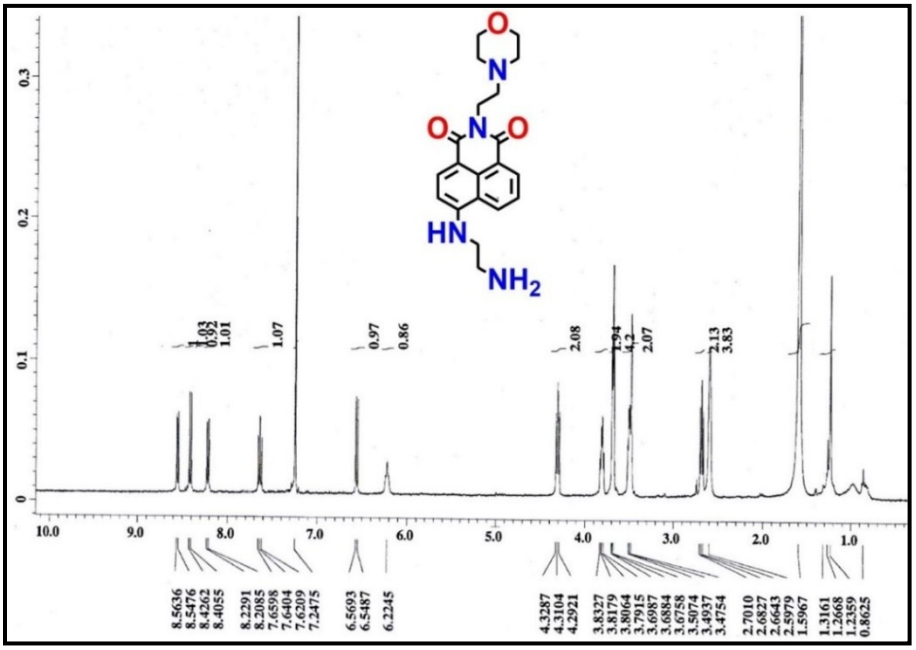


Figure S2. ^{1}H NMR spectrum of compound (**L^{1}**) in $CDCl_3$.

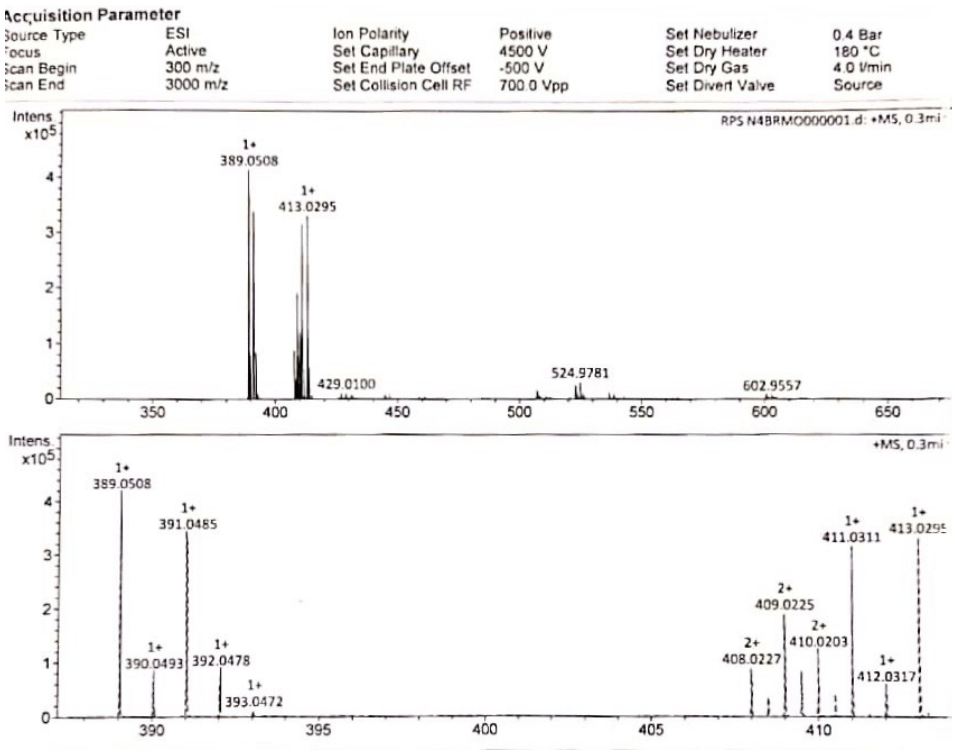


Figure S3. ESI-MS spectra of compound (**L**) in ethanol showing molecular ion $[M+H]^+$ peak at *m/z* 389.0508 with matching isotopic distribution pattern.

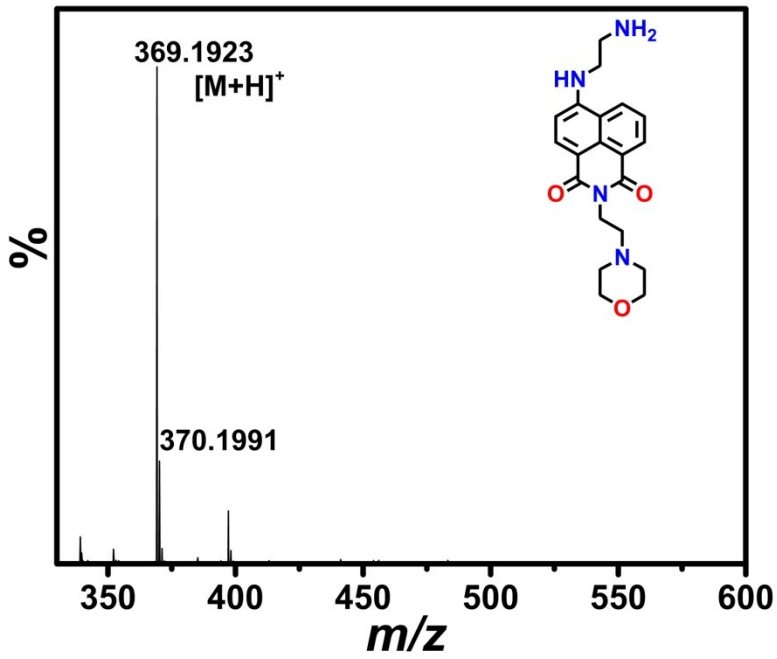


Figure S4. ESI-MS spectra of compound ($\mathbf{L^1}$) in ethanol showing molecular ion peak with isotopic distribution pattern.

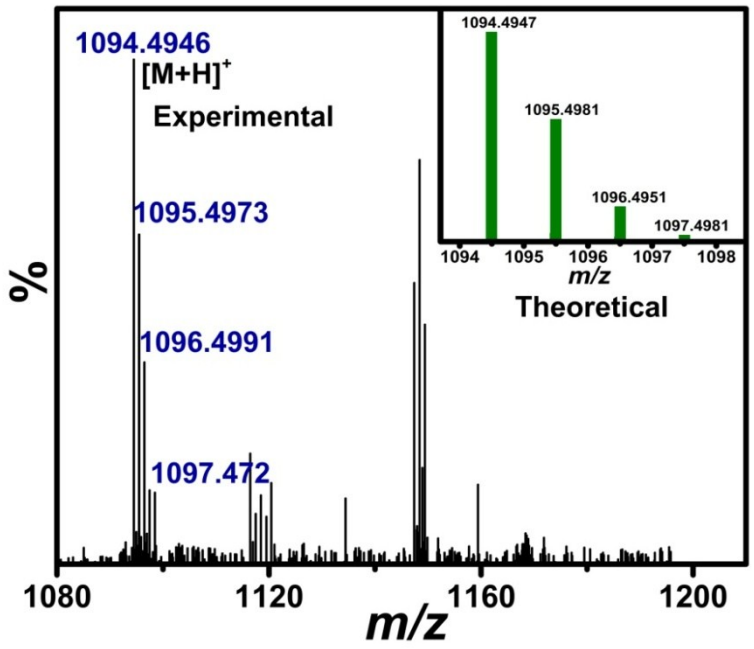


Figure S5. ESI-MS spectra of H_3L^2 in water showing isotopic distribution pattern. Inset shows the theoretically simulated isotopic distribution patterns of the observed $[M+H]^+$ peaks at *m/z* 1094.494 and a peak 1148.4 correspond to $[M+CH_3OH+H_2O]$.

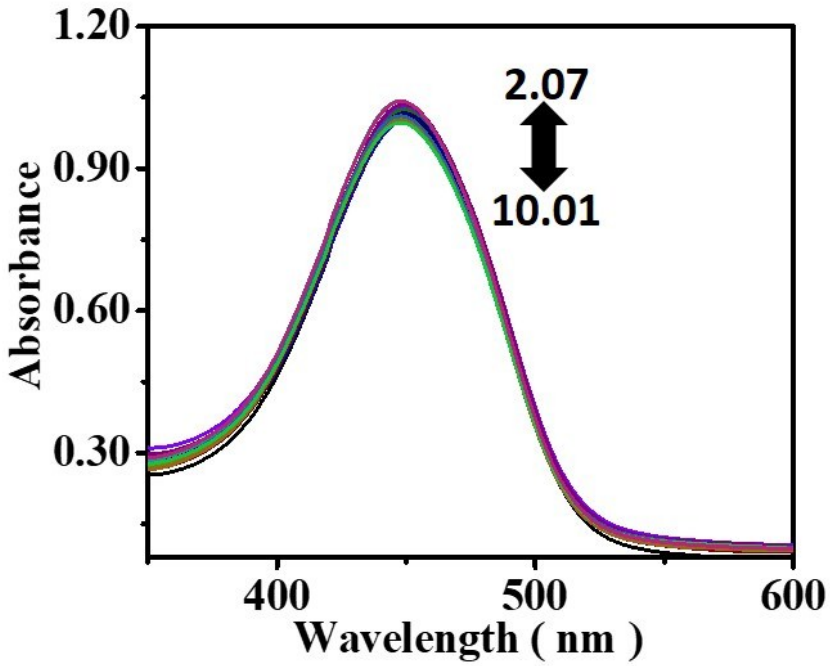


Figure S6. Absorption spectra of $[Eu(L^2)(H_2O)]$ (**1**) complex under different pHs from 2.07 - 10.01 in 10 mM phosphate buffer at RT.

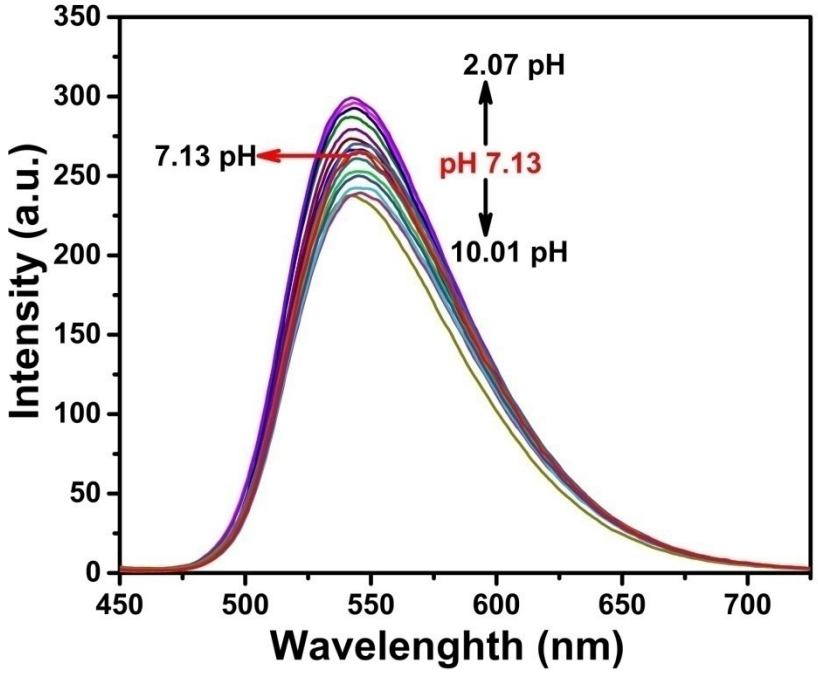


Figure S7. Steady state fluorescence emission spectra of [Eu(L^2)(H_2O)] (**1**) (10 μM) at λ_{ex}= 445 nm under different pHs from 2.07 - 10.01 in 10 mM phosphate buffer at 298 K.

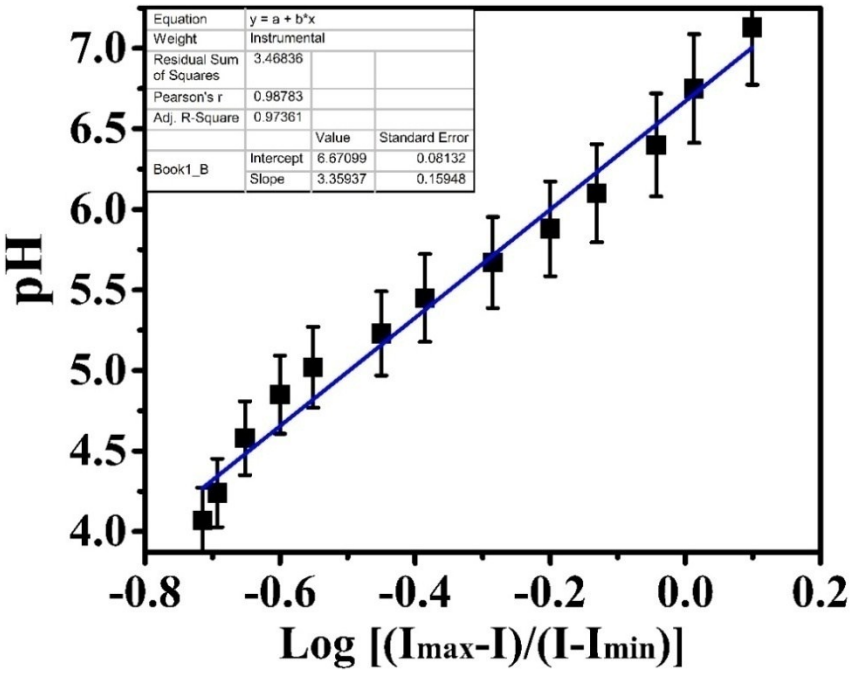


Figure S8. Plot of pH vs. log[(I_{max}-I)(I-I_{min})], where I is the emission intensity at 615 nm band with variation in pH and I is the observed ratio of fluorescence intensity of [Eu(L^2)(H_2O)] (**1**) at λ_{ex}= 445 nm.

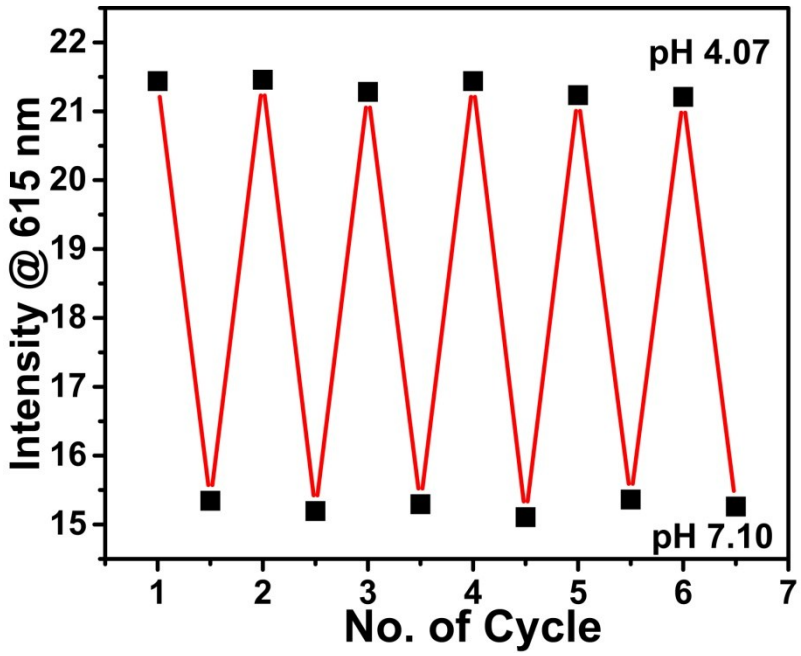


Figure S9. Reversibility of the luminescence response at 615 nm of [Eu(L^2)(H_2O)] (**1**) (30 μM) between pH 7.10 and 4.07 (λ_{ex}= 445 nm).

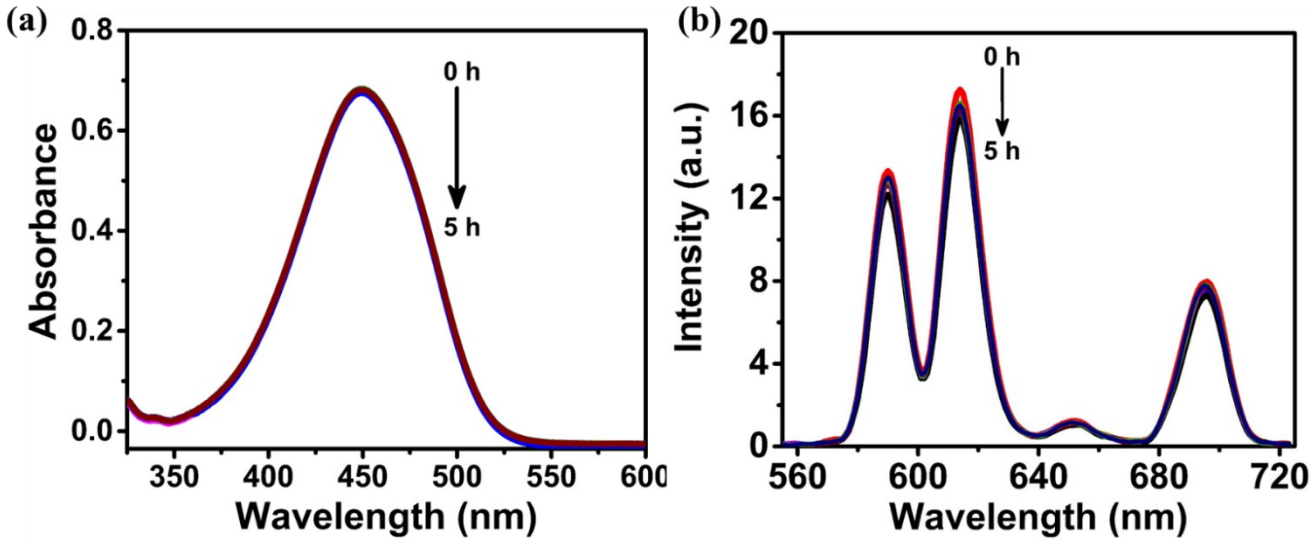


Figure S10. Time-dependent absorption and luminescence spectral traces of [Eu(L^2)(H_2O)] (**1**) (30 μM) monitored for 5 h in 10 mM phosphate buffer at 25 °C to access the solution state stability of the [Eu(L^2)(H_2O)] (**1**).

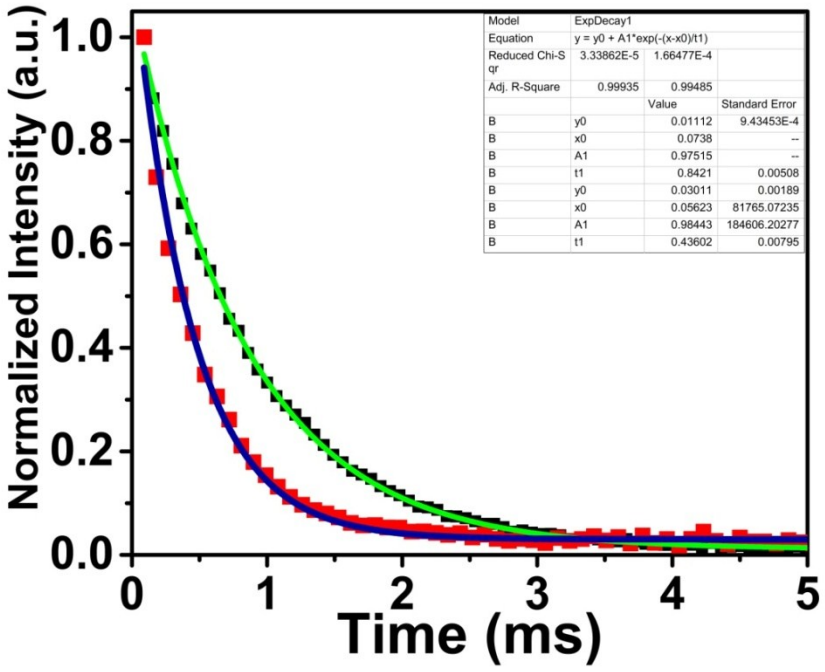


Figure S11. Luminescence decay profile from $^5D_0$ state at 615 nm for [Eu(L^2)(H_2O)] (**1**) (30 μM) in H_2O (red) τ = 0.436 ms and D_2O (black) τ = 0.842 ms at 298 K (delay time = gate time = 0.1 ms). Data is fitted by single exponential fit.

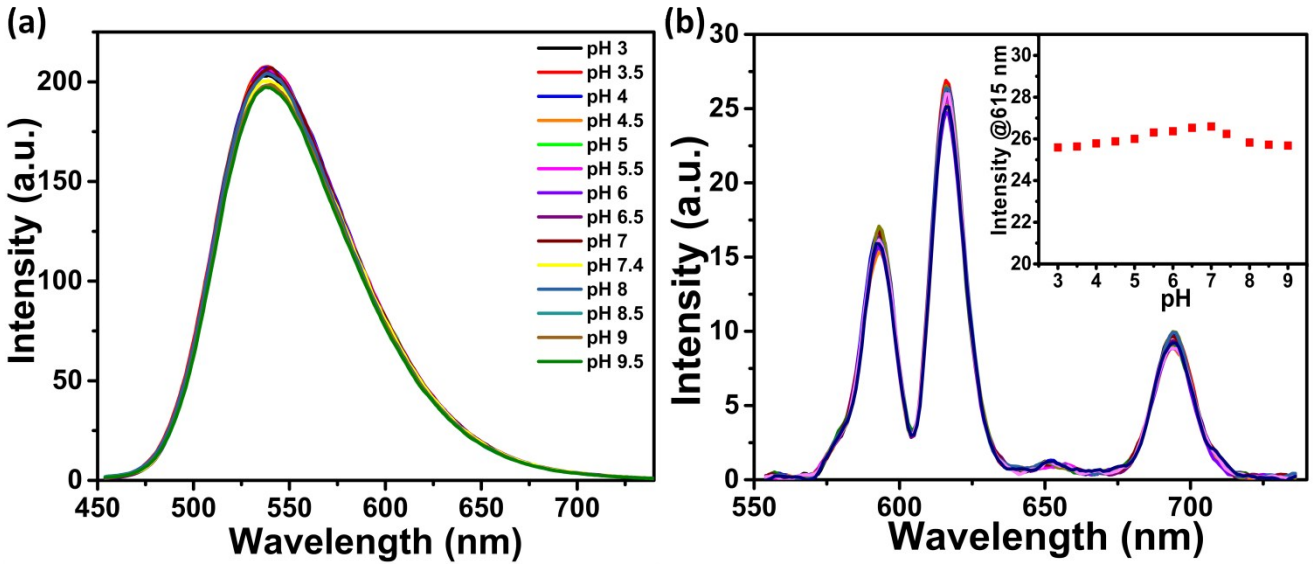


Figure S12: (a) Steady-state fluorescence and **(b)** Time-resolved luminescence spectral traces (inset changes in $^5D_0 \rightarrow {}^7F_2$ transition at 615 nm) of [Eu(L^5)(H_2O)] (**2**) (20μM) under different pHs from 3.05 - 9.07 at λ_{ex} = 438 nm in 10 mM phosphate buffer at 298 K.

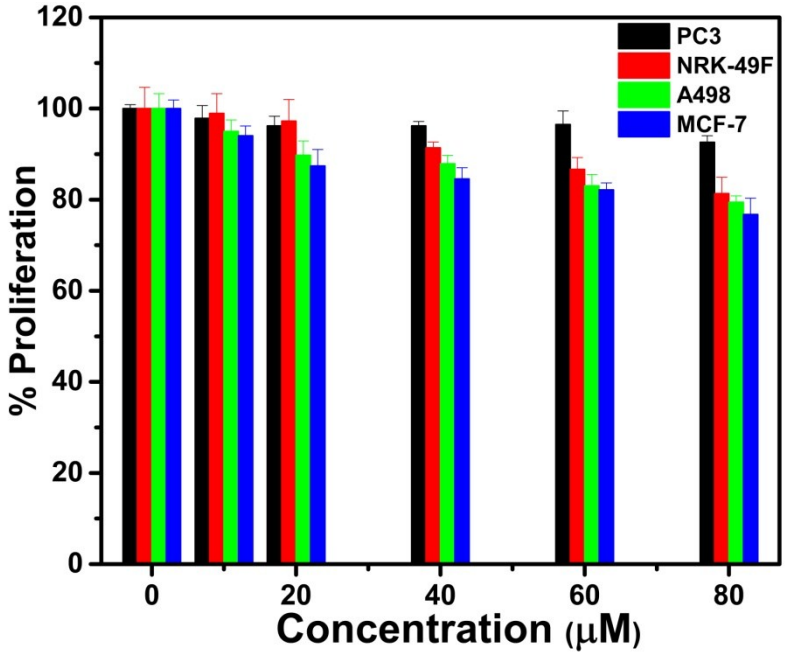


Figure S13. MTT cytotoxicity assay of [Eu(L^2)(H_2O)] (**1**) at various concentration with MCF-7, PC3, A498, and NRK-49F cells upon incubation for 24 h.

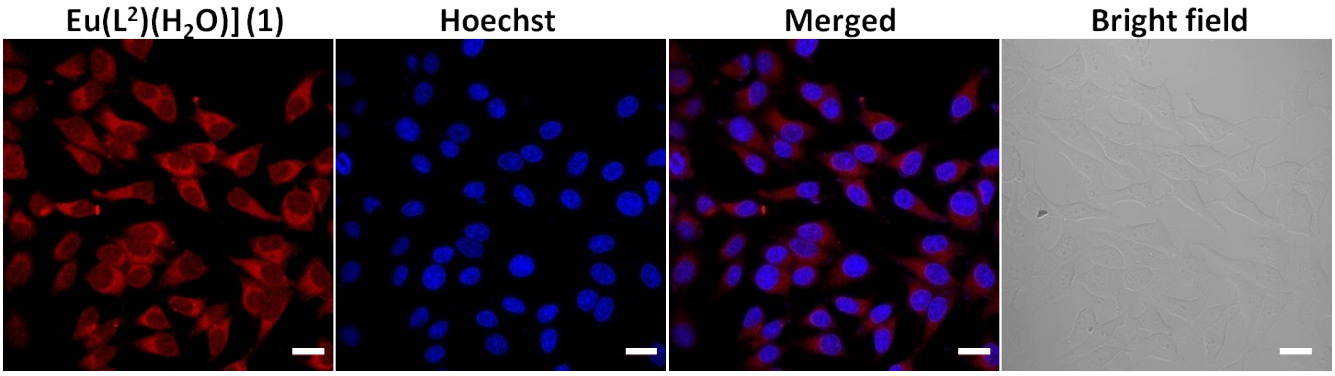


Figure S14. Confocal fluorescence microscopic images of the MCF-7 cells on treatment with the [Eu(L^2)(H_2O)] (**1**) and nuclei stained with Hoechst 33258 dye, merged images showing nuclear localization of the probe **1** (red for Eu emission, and blue for Hoechst 33258 dye); (20 μM for probe **1** λ_{ex} = 405, λ_{em} = 562-736 nm, , laser = 561 nm; 10 μg mL^{-1} for Hoechst 33258 blue dye, λ_{em} = 371-500 nm, laser = 730 nm. Scale bar = 20 μm.

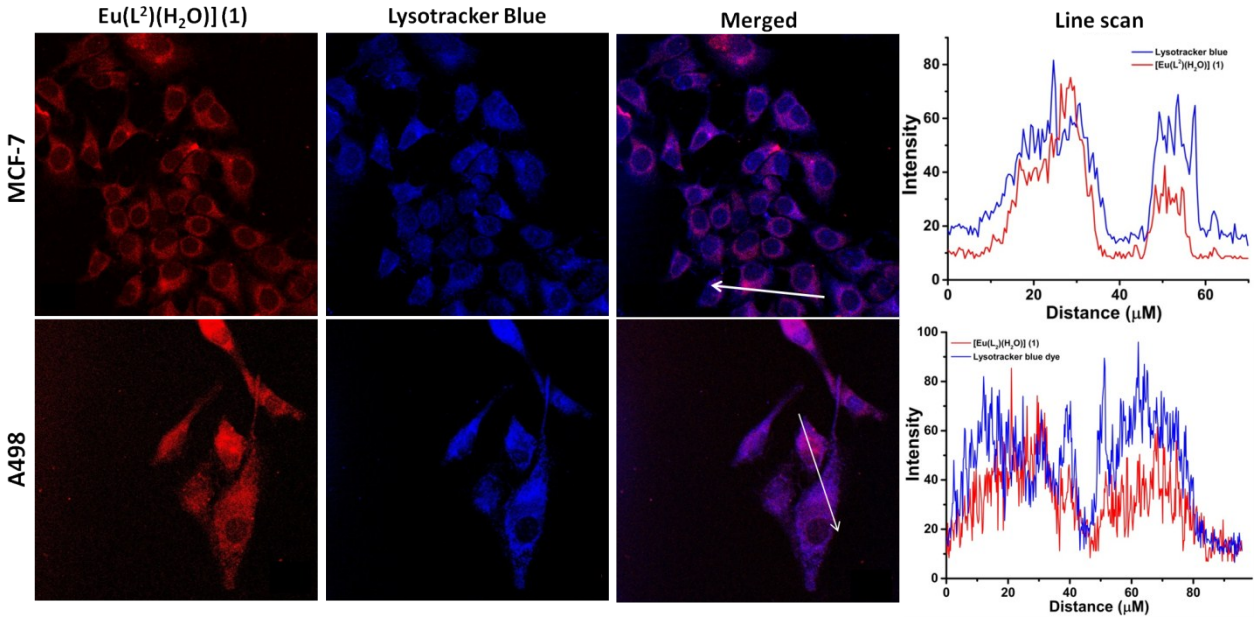


Figure S15. Line scan intensity profile of merged image of MCF-7 and A498 cells treated with the [Eu(L^2)(H$_2$O)] (**1**) (20 μM) after 4 h of incubation, merged images showing lysosomal localization of the [Eu(L^2)(H$_2$O)] (**1**) with a Pearson coefficient of 0.70 and 0.79 for MCF-7 and A498 cell line respectively; Scale bar = 20 μm.

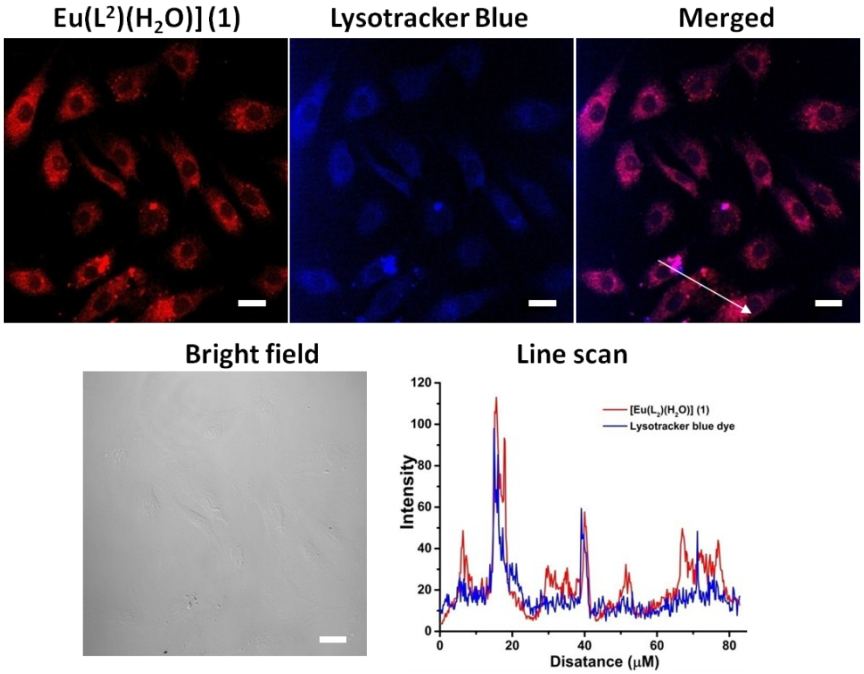


Figure S16. Confocal fluorescence microscopic images with line scan intensity plot of NRK-49F cells treated with the [Eu(L^2)(H$_2$O)] (**1**) (20 μM) and LysoTracker® Blue (5 μM) after 5 h of incubation predominant lysosomal co-localisation with a Pearson coefficient of 0.60 (red for Eu emission, and blue

for LysoTracker® blue dye); (20 μM for probe **1** λ_{ex} = 405, λ_{em} = 562-736 nm, , laser = 561 nm; 5 μM for blue lysotracker, λ_{em} = 371-500 nm, laser = 730 nm). Scale bar = 20 μm.

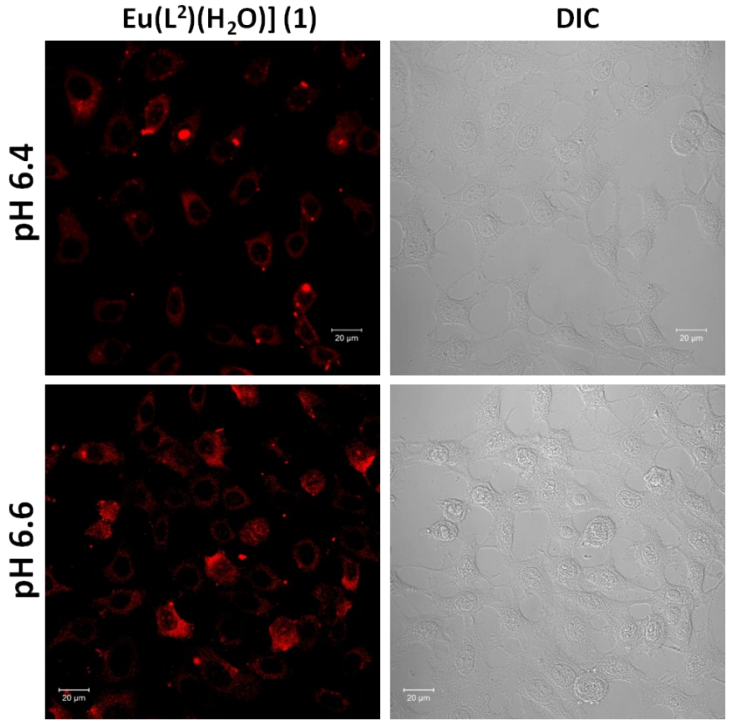


Figure S17. Confocal microscopy images observing the lysosomes of MCF-7 cells (1h incubation) on treatment with the [Eu(L^2)(H_2O)] (**1**) at pH 6.4 (top) and pH 6.6 (bottom) at 20 μM (Left).Corresponding DIC images were shown (right).

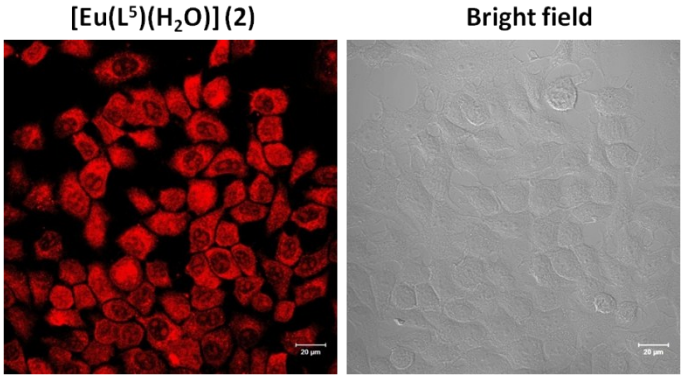


Figure S18. Confocal fluorescence microscopic images of the MCF-7 cells on treatment with the [Eu(L^5)(H_2O)] (**2**) with DIC of the probe **2** (red for Eu emission); (20 μM for probe **1** λ_{ex} = 405, λ_{em} = 562-736 nm, , laser = 561 nm; 10 μg mL^{-1}. Scale bar = 20 μm.