Electronic Supporting Information (ESI)

Xanthone based Pb^{2+} selective turn on fluorescent probe for living cell staining

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Experimental

Materials

Fluoroglucinol (Aldrich, USA), β-resorcylic acid (Alfa Aesar, Germany), fused $ZnCl_2$ (Merck, India) and $POCl_3$ (Merck, India) have been used as received. All other chemicals and solvents are of analytical grade and used without further purification. Solvents used are of spectroscopic grade. Mili-Q Milipore® 18.2 MΩ cm^{-1} water has been used throughout all the experiments. The sources of Na^+, K^+, Ca^{2+}, Mg^{2+}, Ag^+, Mn^{2+}, Hg^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Cr^{3+} and Pb^{2+} ions are either their chloride or nitrate or perchlorate salts. All of these salts were of analytical grade (Merck, India).

Apparatus

^{1}HNMR spectra have been recorded in DMSO-d_6 with a Bruker Advance 300 MHz using TMS as the internal standard. Absorption and fluorescence spectra are recorded on Shimadzu Multi Spec 1501 absorption spectrophotometer and Hitachi F-4500 fluorescence spectrophotometer, respectively. Mass spectra are recorded in GCMS-SHIMADZU-QP5050A and QTOF Micro YA 263 mass spectrometer in ESI positive mode. IR spectra are recorded on a JASCO FTIR spectrophotometer (model: FTIR-H20).

Synthesis of 1, 3, 6- trihydroxy xanthone (L) (Scheme 1)

A mixture of β-resorcylic acid (154 mg, 1 mmol) and fluoroglucinol (126 mg, 1 mmol) are dissolved in $POCl_3$ under stirring condition and then fused $ZnCl_2$ (409.17 mg, 3 mmol) is added slowly with

continuous stirring for 2 h at 60^0C. Then, the reaction mixture is cooled to room temperature and slowly poured into ice water with continuous stirring to get red precipitate. The precipitate was washed with water followed by $NaHCO_3$ solution to obtain orange product (**L**), which was purified by column chromatography as yellow color 1,3,6- trihydroxy xanthone (**L**). Yield 78 %. Its molecular structure and purity were confirmed by QTOF –MS ES^+ (ESI, Fig. S1),^{1}H NMR (ESI, Fig. S2), FTIR (ESI, Fig. S3) and elemental analysis. QTOF –MS ES^+: $[M + Na]^+$ = 267.04 (100%) for $C_{13}H_8O_5$; ^{1}HNMR (300MHz, DMSO-d_6)**:** δ(ppm); 12.996 (s, 1H, -OH), 10.973 (s, 2H, -OH), 7.940 – 7.911 (d,1H, ArH), 6.868 – 6.832 (dd, 1H, ArH), 6.779 – 6.772 (d, 1H, ArH), 6.308 – 6.301 (d, 1H, ArH), 6.131 – 6.125 (d, 1H, ArH); FTIR (cm^{-1}): υ(OH) 3494.3, 3390.72 and 3200.36; elemental analysis as calculated for $C_{13}H_8O_5$ (%): C, 63.94; H, 3.30. Found (%): C,62.88; H,3.86.

Synthesis of Pb^{2+} complex of 1, 3, 6- trihydroxy xanthone, $Pb(L)_2$

A solution of $Pb(NO_3)_2$ (331.2 mg, 1 mmol) in DMF was added slowly in a DMF solution of **L** (244 mg, 1 mmol) under stirring condition and stirring was continued for another 30 minutes. Then the mixture was warmed at 75^0 C for 2.5 h to get a clear pale yellow solution. After vacuum distillation of the solvent, an off-white color compound was obtained. It was washed with cold ethanol and dried in vacuo over silica gel. Its molecular structure and purity have been confirmed by FTIR (ESI, Fig. S10), ^{1}H NMR (ESI, Fig.S2) and QTOF – MS ES^+ (ESI, Fig. S7). Yield: 25 %. QTOF –MS ES^+: 695.35 (m/z) assigned as $PbC_{13}H_8O_5$: FTIR (cm^{-1}): υ(OH) 3501, 3390 and 3200 (ESI, Fig. S8).

Cell studies

To detect Pb^{2+} ion, *Candida albicans* cells (obtained from exponentially growing culture in yeast extract glucose broth medium, pH 6.0, incubation temperature, 37^0C) and *Pollen* cells (obtained from matured buds extract glucose broth medium, pH 6.0, incubation temperature, 37^0C of *Tecoma stanf*, a common ornamental plant) have been centrifuged at 3000 rpm for 10 minutes, washed twice with 0.1 M HEPES buffer at pH 7.4. Then, these cells were incubated with **L (**5 μM in DMSO) for 40 minutes.

After incubation the cells were washed again with HEPES buffer at pH 7.4 and treated with 40 μM Pb^{2+} salt for 15 minutes in 0.1 M HEPES buffer (pH 7.4) and mounted on grease free glass slides to observe under fluorescence microscope equipped with blue filter.

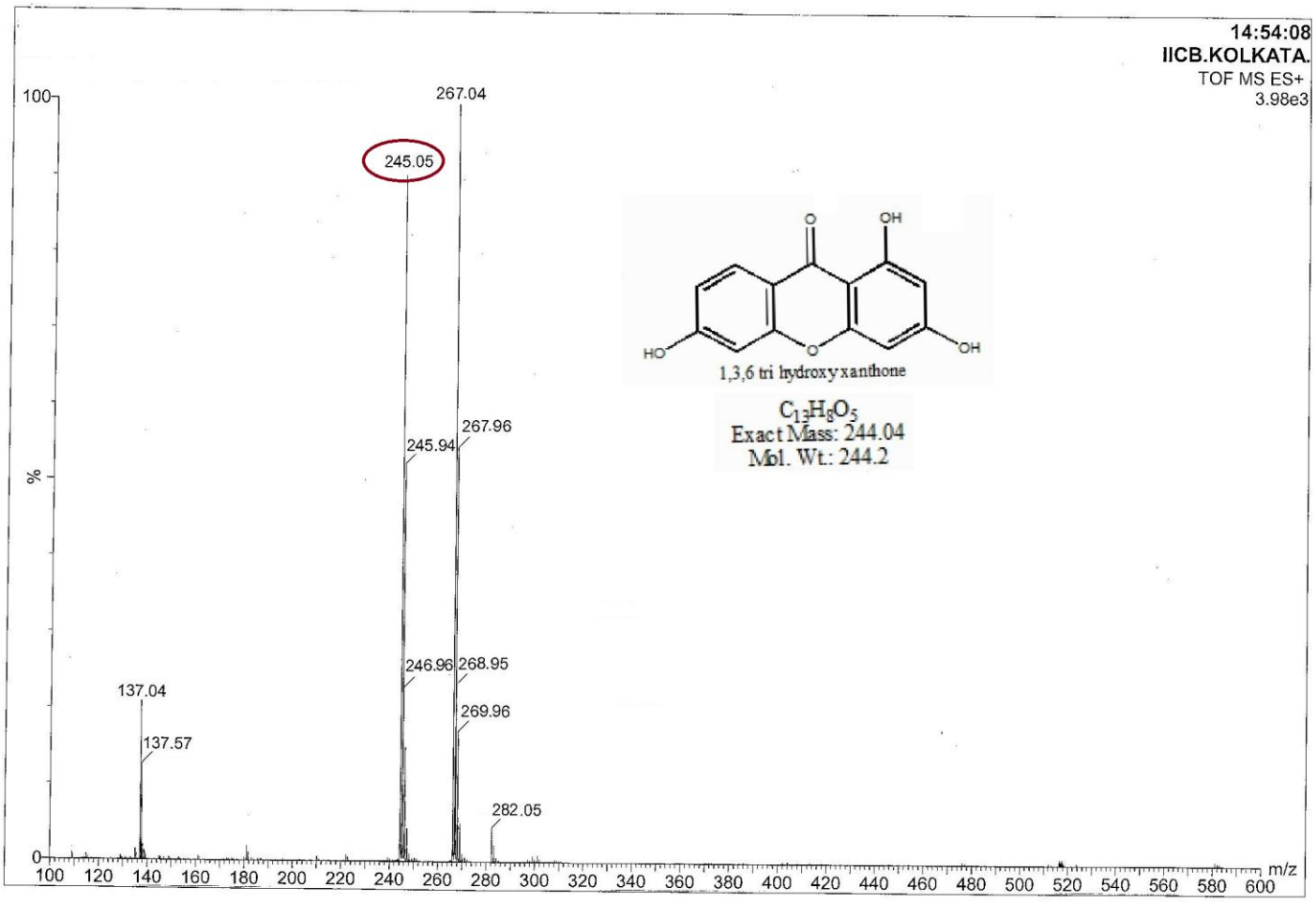

Fig. S1. QTOF-MS spectrum of **L**

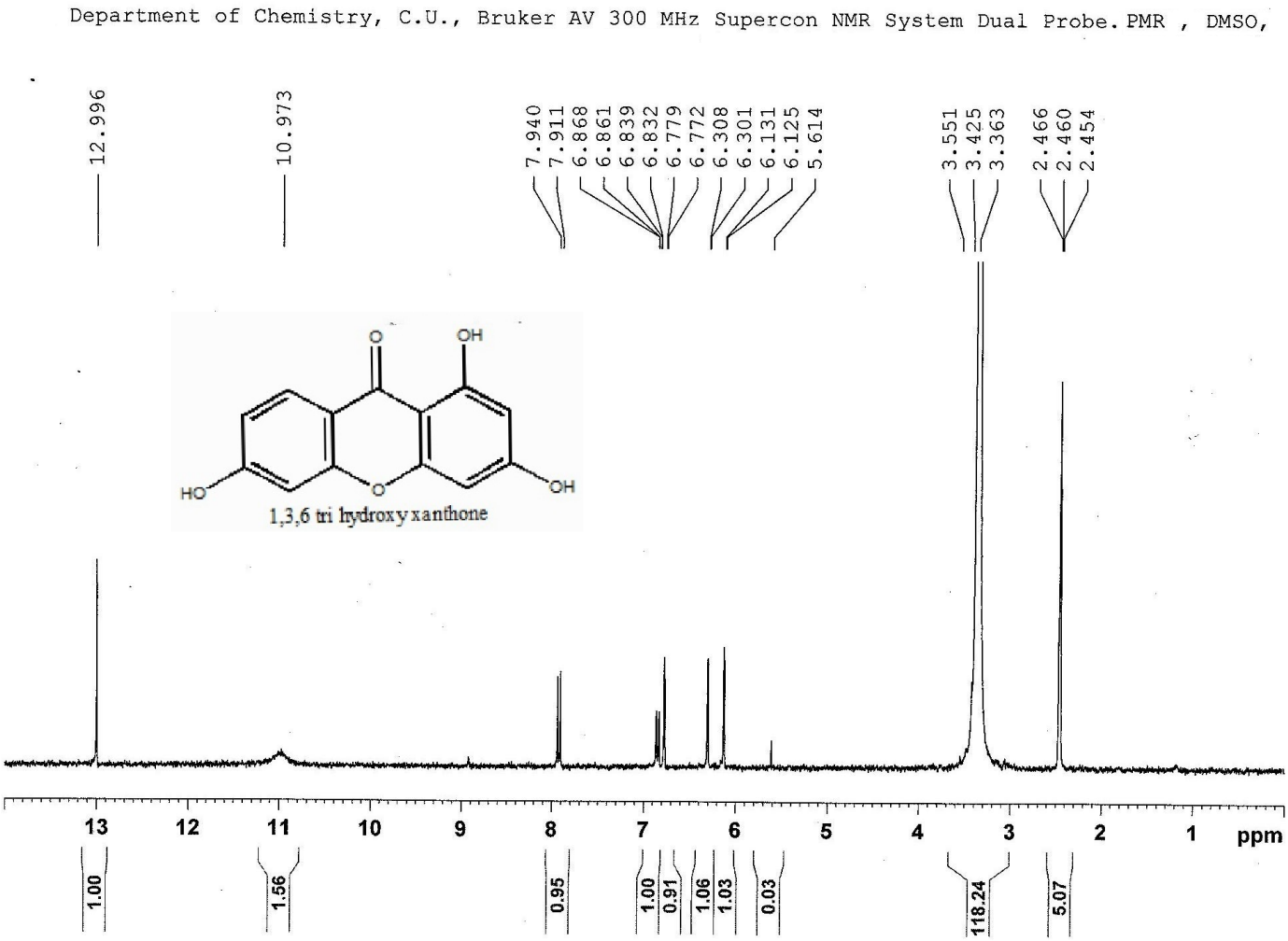

Fig. S2. ^{1}H NMR spectrum of **L**

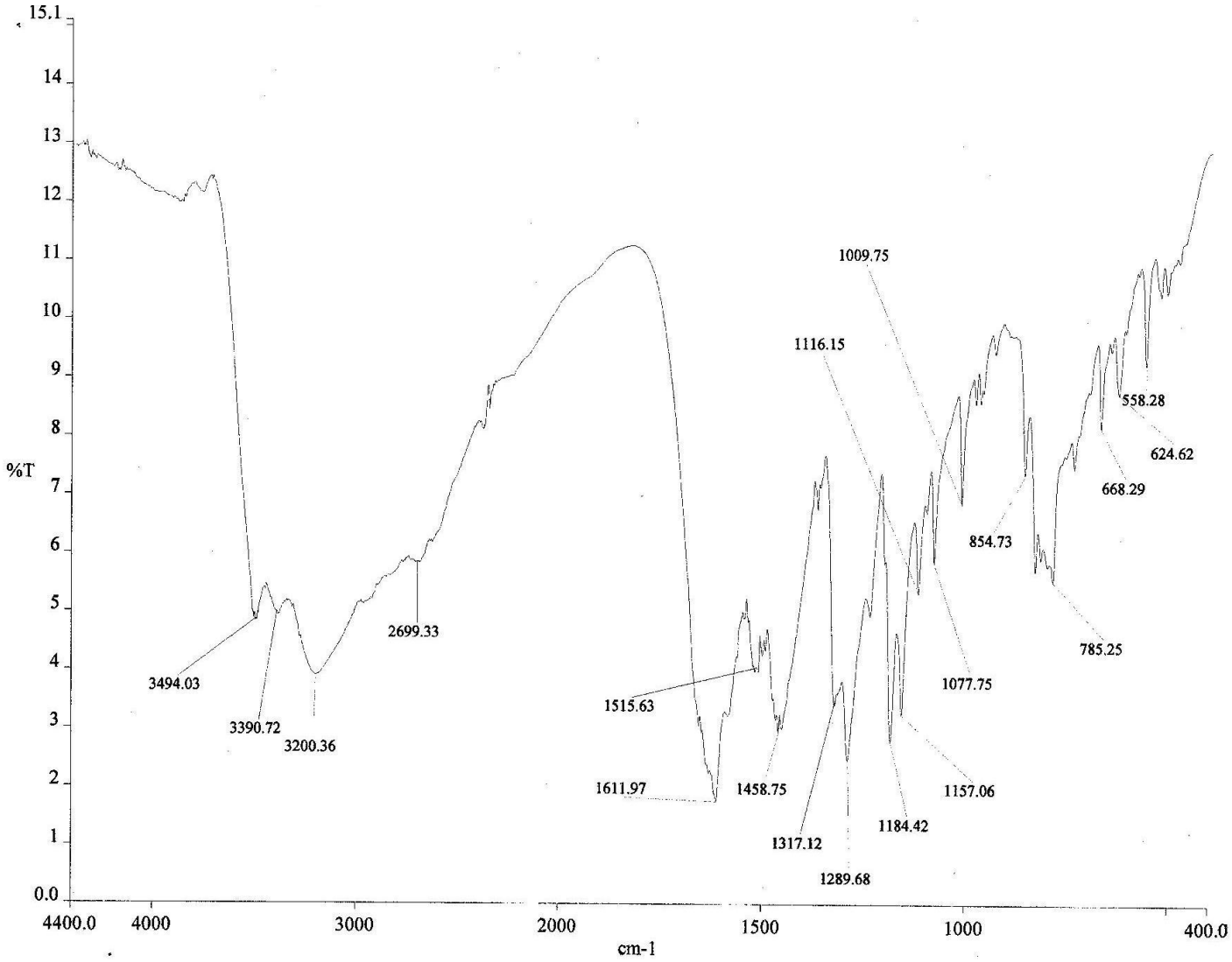

Fig. S3. FTIR spectrum of **L**

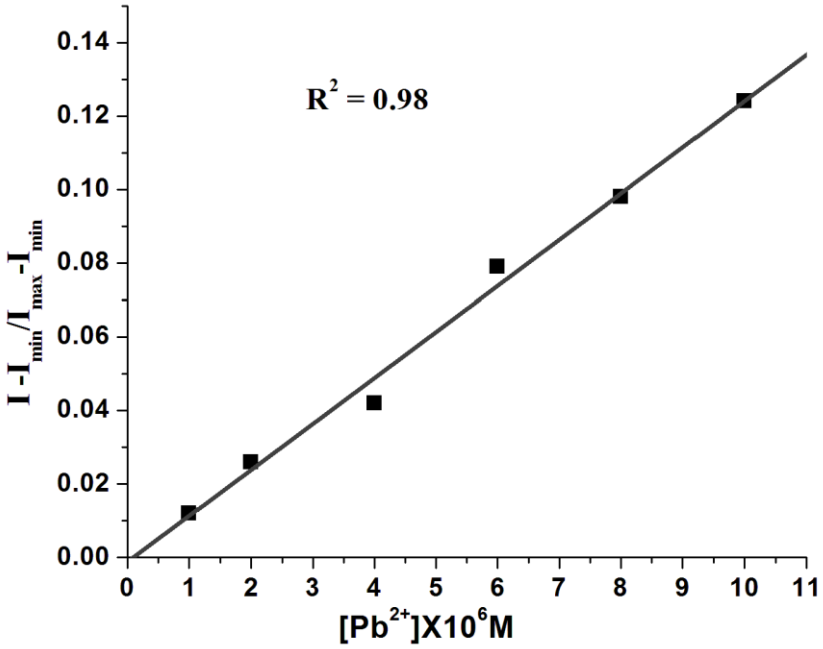

Fig. S4. Emission intensities of **L** (1 μM, λ_{em} = 510 nm)) at different concentrations of Pb^{2+} (1, 2, 3, 4, 5, 6, 7, 8, 9, 10 μM) are normalized between the minimum (0.0 μM Pb^{2+}) and the maximum emission intensity in DMSO – H_2O (2:1 ratio, v/v) solution. The best fit line cuts the X axis, known as detection limit having value 1.8×10^{-7} M.

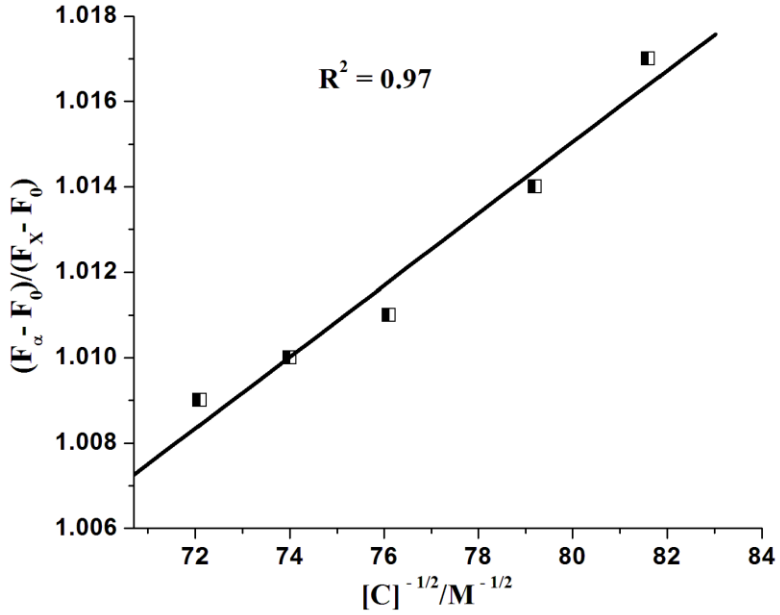

Fig. S5. Benesi-Hildebrand plot for determination of binding constant (λ_{em} = 510 nm)

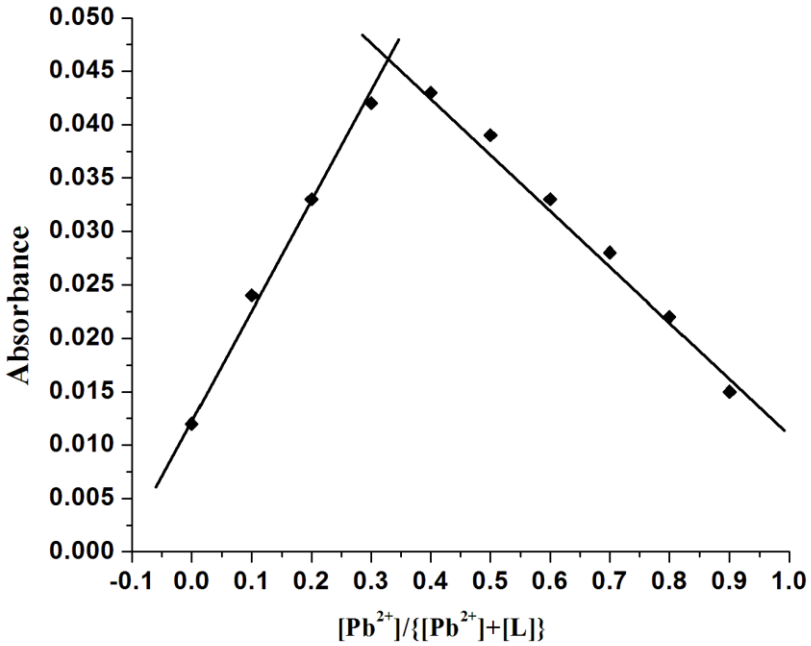

Fig.S6. Job's plot in DMSO – H_2O (2:1, v/v) solution

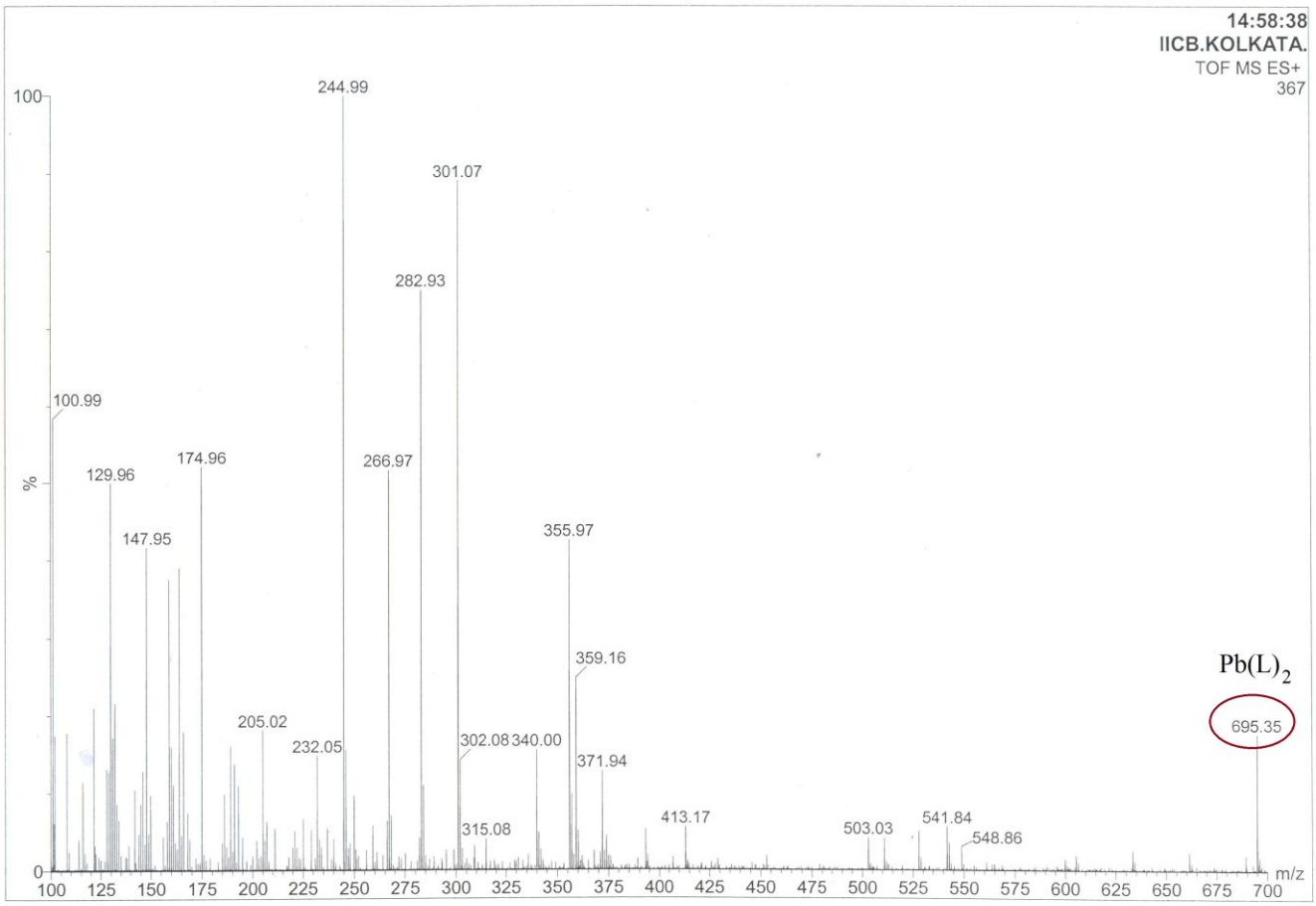

Fig. S7. QTOF-MS spectrum of Pb(**L**)$_2$ complex

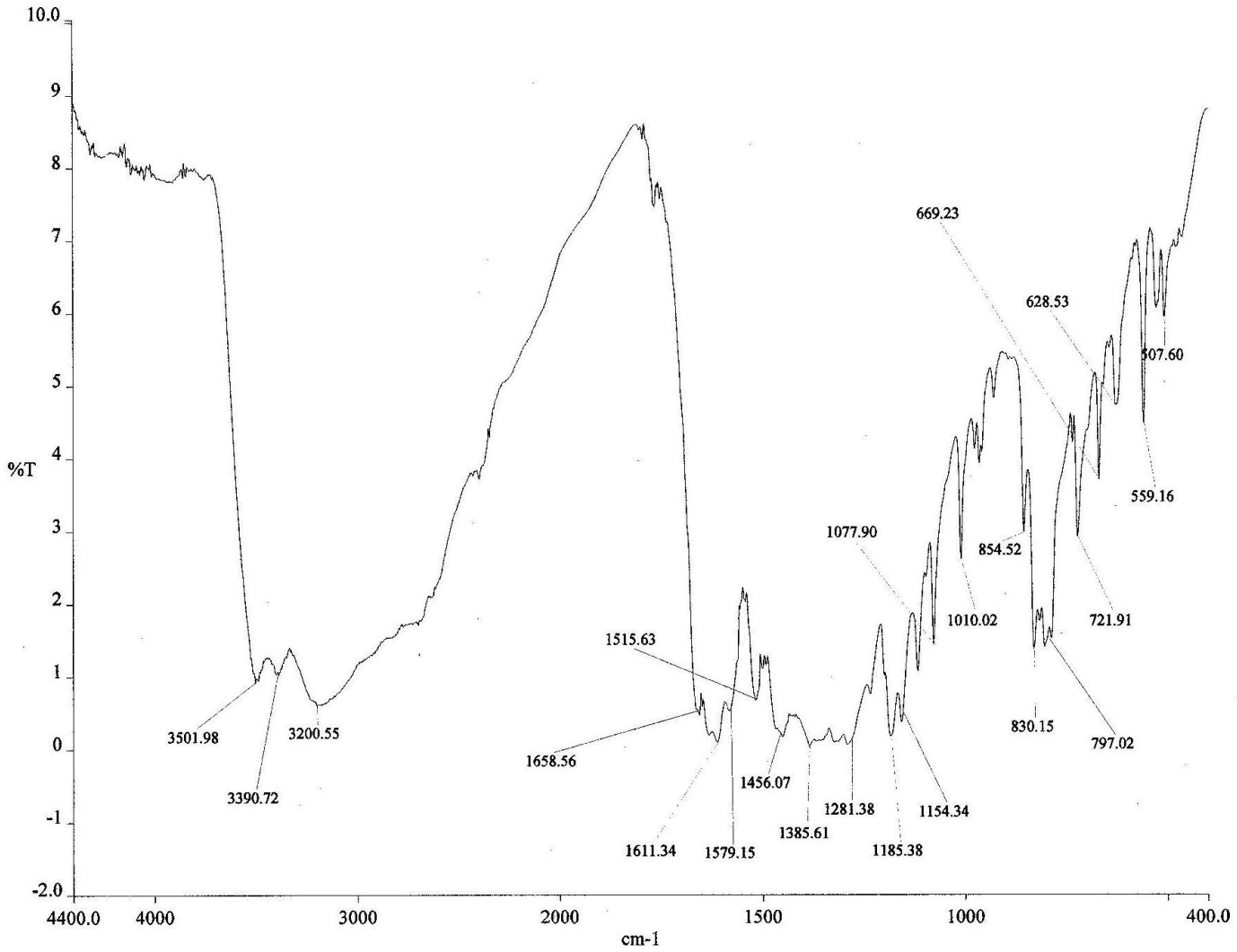

Fig. S8. FTIR spectra of Pb(**L**)$_2$ complex

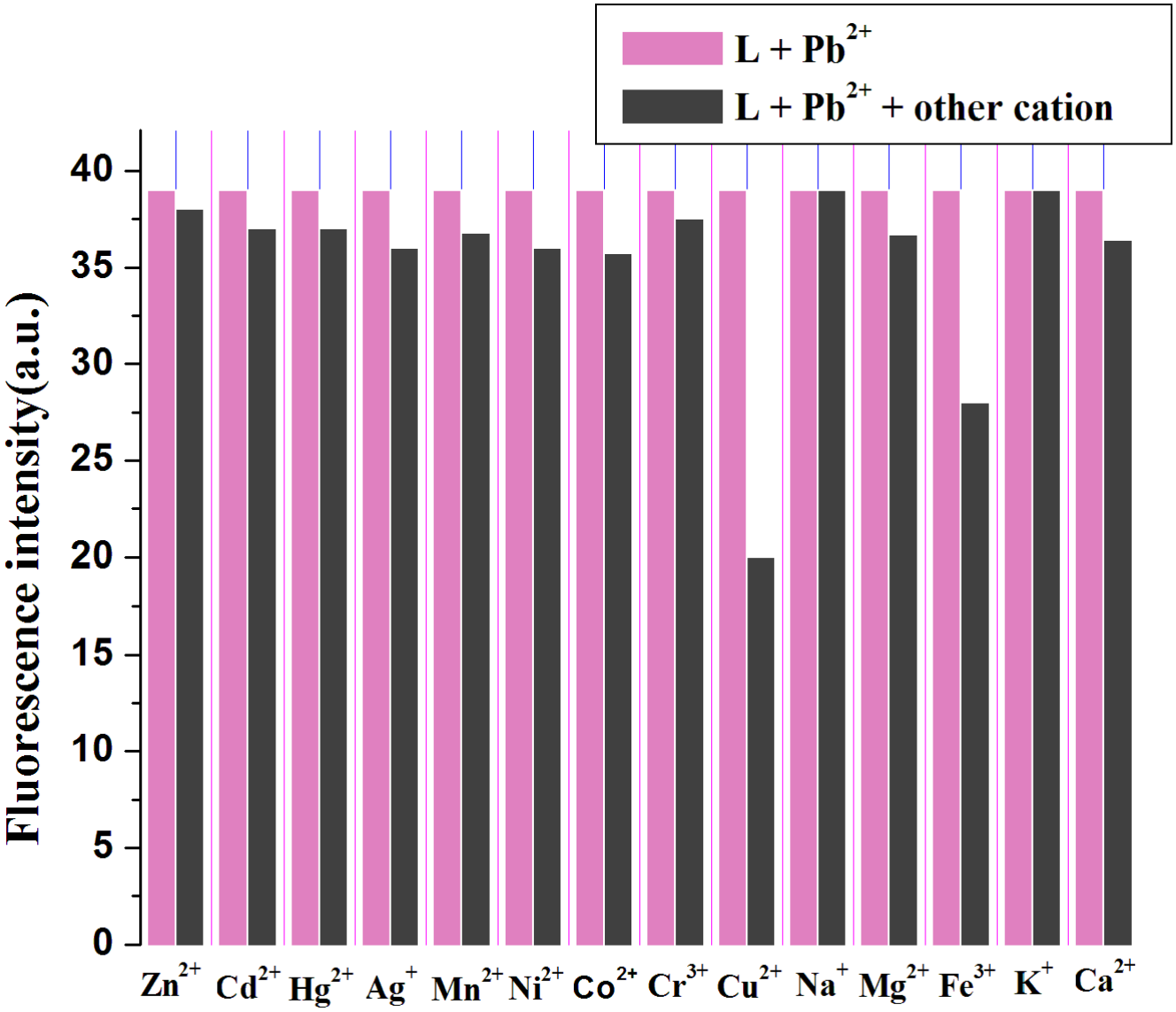

Fig.S9. Effect of common cations (20 μM) on the emission intensity of [**L** (1 μM) + Pb^{2+} (10 μM)] system in DMSO – H_2O (2:1, v/v) solution

Table S1. Comparison of the present probe with some other selected Pb^{2+} sensors

Probe with reference	**LOD**	**Association (K_a)/ dissociation constant (K_d)**	**Interference**	**Living cell imaging**
Diaminoanthraquinone- linked polyazamacrocycles[1]	0.1 μM	1×10^{8} (K_a)	Al^{3+}, Zn^{2+}, Ni^{2+}, Cd^{2+}	-
4,4-Difluoro-4-bora-3a,4a-diaza-*s*-indacene (BODIPY) Derivatives[2]	1 μM	1×10^{5} (K_a)	Nil	-
5-[*p*-N,N-Bis(2-pyridylmethyl)amino-phenyl]-10,15,20-tris(4-tert-butylphenyl) porphyrin (porphyrin-1-DPA)[3]	0.31 µM	2.1×10^{4} (K_a)	Cu^{2+}	-
Ag-rhodamine 6G hybrid nanorods[4]	0.24 µM	-	Cu^{2+}, Zn^{2+}, Ni^{2+}, Fe^{3+}	-
N-[4(1-Pyrene)-butyroyl]-L-tryptophan (PLT)[5]	0.15 µM	1.09×10^{6} (K_a)	Zn^{2+}, Cd^{2+}, Cr^{3+}	-
Triazole-Modified Calix[4] crown[6]	-	3.71×10^{4} (K_a)	Cu^{2+}, Cr^{3+}, Hg^{2+}	-
4,4-Difluoro-4-bora-3a,4adiaza-s-indacene (BODIPY) derivative[7]	-	1.05×10^{5} (K_a)	Cu^{2+}, Li^{+}, Na^{+}, Mg^{2+}, K^{+}, Ca^{2+}, Zn^{2+}, Ag^{+}, Cd^{2+}, Hg^{2+}	+ ve
4,4-Dimethyl-4H-5-oxa-1,3-dithia-6,11-diazacyclopenta[a]anthracen-2-one[8]	0.5 µM	0.021 μM (K_d)	Nil	+ ve
Glutathione (GSH)-capped quantum dots (QDs)[9]	0.02 µM	1.28×10^{6} (K_a)	Ag^{+} and Cu^{2+}	-
Calixarene bearing four dansyl groups (Calix-DANS4)[10]	0.025 µM	1×10^{10} (K_a)	Nil	+ ve
Aza crown ether appended hetarylazo dye[11]	5 µM	$1\times10^{5.4}$ (K_a)	-	-
Rhodamine–phenylurea conjugate (RPU)[12]	0.007 µM	7.4×10^{7} (K_a)	Nil	-
Leadfluor-1 (LF1)[13]	<0.075 µM	23 ± 4 μM (K_d)	Cu^{2+}	+ ve
2-Ferrocenylimidazo[4,5-*b*]pyridine[14]	0.013 µM	6.1×10^{5} (K_a)	Hg^{2+}	-
Probe with bis(2-pyridylmethyl)amine unit[15]	0.64 µM	--	Cu^{2+}, Hg^{2+}	-
1,3,6- trihydroxy xanthone present work	0.18 µM	1.25×10^{3} (K_a)	Cu^{2+}, Fe^{3+}	+ ve

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