

# A highly efficient PET *switch on-off-on* fluorescence receptor based on calix[4]arene for selective recognition of Cd<sup>2+</sup> and Sr<sup>2+</sup>

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## 1. Materials and methods

All the reagents and chemicals like DCC (N,N'-Dicyclohexylcarbodiimide), DMAP (4-Dimethylaminopyridine) and 8-Hydroxy quinoline used were of analytical grade purchased from sigma aldrich. Silica gel (Merck, 0.040-0.063 mm) was used for column chromatography. Metal salts used for the titrations were their perchlorate salts (Caution: since perchlorate salts are known to explode under certain conditions, these are to be handled carefully!) with formula,  $M(\text{ClO}_4)_2 \cdot x\text{H}_2\text{O}$ . Melting points were taken on Opti-Melt (Automated melting point system). The FT-IR spectra were recorded as KBr pellet on Bruker TENSOR-27 in the range of 4000-400  $\text{cm}^{-1}$ . Discover BenchMate system-240 V (CEM Corporation) microwave synthesizer was used for synthesis.  $^1\text{H}$  NMR spectra was scanned on 400 MHz FT-NMR Bruker Avance-400 in the range of 0.5 ppm -15 ppm and  $^{13}\text{C}$  NMR spectra was recorded on a Bruker DPX-300 spectrometer using internal standard tetramethylsilane (TMS) and deuterated DMSO as a solvent in the range of 0.5 ppm to 250 ppm. ESI Mass spectra were taken on a Shimadzu GCMS-QP 2000A. Emission spectrum was recorded on Horiba Jobin, Fluorolog, and Edinburgh F900. UV-Vis absorption spectra were acquired on a Jasco V-570 UV-Vis. spectrometer. Working standard solutions were prepared daily in deionized water. X-ray diffraction (XRD) experiments were performed on a SEIFERT-FPM (XRD7), using Cu  $K\alpha$  X-ray lines at 1.5406 Å as the radiation source at 40 kV and 30 mA power and also Make -Philips X'PERT MPD. Powder X-ray diffractometer.

## 2. Experimental

### 2.1 Synthesis of compound A: Microwave assisted synthesis of p-tert-butylcalix[4]arene

A mixture of p-tert-butyl phenol (4.0 g, 0.33 mM), sodium hydroxide (NaOH) (1 g) and formaldehyde(1.8 ml,0.18 mM) solution were taken in an open vessel and was irradiated with 50 W power in a microwave synthesizer Discover(CEM) by stirring for 3 min. Cooling for 10 min, resulted in yellow solid mass. Next, 4 ml of toluene and 30 ml of di phenyl ether was added in this yellow solid, again irradiated with microwave power of 100 W for 5 min with stirring and obtained a dark brown solution. Further, this solution was added in to 75 ml of ethyl acetate and kept for 2 h. Finally, white precipitate was obtained which was filtered and washed with ethyl acetate and finally dried. **Yield**, 3.5 g (96%). **Elemental analysis** for C<sub>44</sub>H<sub>56</sub>O<sub>4</sub>: Calcd.C;81.44%,H;8.70%,O;9.80%,Found:C;80.11%,H;8.261%,O;9.90%. **<sup>1</sup>HNMR**: δ<sub>H</sub> (DMSO,400 MHz): 1.18 (36H, t-butyl, s), 3.81 (8H,ArCH<sub>2</sub>Ar, s), 7.12 (8H,s,Ar-H), 9.71(4H, Ar-OH, s). **ESI-MS** (m/z) 648 (M+1).

**Synthesis of compound B and C were carried out according to previously reported method<sup>1</sup>.**

### 2.2 Synthesis of compound D:

A mixture of compound C (1 g, 0.001 mol) and 8-Hydroxyquinoline (0.6 g, 0.004 mol) were dissolved into anhydrous dichloromethane (20-25 ml) and stirred this solution for 15 minutes. Then N, N'-Dicyclohexylcarbodiimide (**DCC**) (0.93 g, 0.004 mol) and catalytic amount of 4-Dimethylaminopyridine (**DMAP**) were added into reaction mixture. This reaction mixture was stirred at room temperature for 48 hours. The reaction progress was monitored by thin layer chromatography (TLC) using mixture of chloroform: methanol (7:3). After the completion of reaction, solvent was evaporated. Then product was crystalized with dichloromethane. Yield

74%, mp >260<sup>0</sup> C. Anal.calc: C<sub>88</sub>H<sub>84</sub>N<sub>4</sub>O<sub>12</sub>: C, 76.06; H, 6.09; N, 4.03 % Found: C, 76.01; H, 5.98; N, 4.01 % **FT-IR (KBr)**  $\nu$ : 3340 cm<sup>-1</sup> (-CH), 1630 cm<sup>-1</sup> (-C=O). **<sup>1</sup>H NMR(DMSO)** 1.31 (s, C(CH<sub>3</sub>)<sub>12</sub>,36H), 3.82 (s, Ar-CH<sub>2</sub>-Ar, 8H),7.67(s, Ar-H, 8H), 4.09 (s, -OCH<sub>2</sub>, 8H), 8.10 (s, Ar-H, 4H), 8.06 (s, Ar-H, 4H),7.88 (s, Ar-H, 4H), 7.38 (s, Ar-H, 4H), 7.32 (s, Ar-H, 4H), 7.40(s, Ar-H, 4H). **<sup>13</sup>C NMR(DMSO)** 31.3 (-CH<sub>3</sub>), 67.2 (-CH<sub>2</sub>), 168.2, 152.3, 148.6, 136.9, 132.3, 128.9 (Ar-C), 135.9,128.2, 123.4,123.1, 118.2 ( Ar-CH), **ESI-MASS** : (m/z) 1390.1 (m+1).

### 3. Absorption and luminescence

Absorption spectra of compound lower rim calix[4]arene tetraamidoquinoline (TEQC) were recorded in acetonitrile and the data are given in experimental section. This compound show absorption band in the region between 290-390 nm, the band at 305 nm indicates  $\pi$ - $\pi^*$  transition of 8-Hydroxy quinoline system. This compound shows a strong luminescence band at 343 nm, 355 nm and 371 nm in acetonitrile with excitation at the absorption maxima ( $\lambda_{\max}$ ) of the quinoline moiety , which is at 300-310 nm.

### 4. Ion-binding study

Stock solutions of the complex TEQC (1 x 10<sup>-6</sup> M) and that of perchlorate salts (1 x 10<sup>-6</sup>M) of various metal ions (Zn<sup>2+</sup>, Cd<sup>2+</sup>, Fe<sup>2+</sup>, Hg<sup>2+</sup>, Na<sup>+</sup>, K<sup>+</sup>, Cu<sup>2+</sup>, Ni<sup>2+</sup>, Mn<sup>2+</sup>, Cr<sup>3+</sup>, Pb<sup>2+</sup>,Sr<sup>2+</sup>, Ce<sup>3+</sup>,Co<sup>2+</sup>, Ca<sup>2+</sup>, Mg<sup>2+</sup> and Fe<sup>3+</sup> ) were prepared in freshly purified acetonitrile. Then 2 mL stock solution of the complex and 2 mL stock solution of each metal salts were taken in a 5 mL volumetric flask, so that the effective concentration of the complex is 1 x 10<sup>-6</sup> M and that of the metal ions are 1 x 10<sup>-6</sup> M . The spectra of the cation added solutions was compared with that of the original solution to ascertain the interactions of the metal ions with the ionophore. For emission titration study, the same stock solutions of the complexes were used and the metal perchlorate solutions of desired

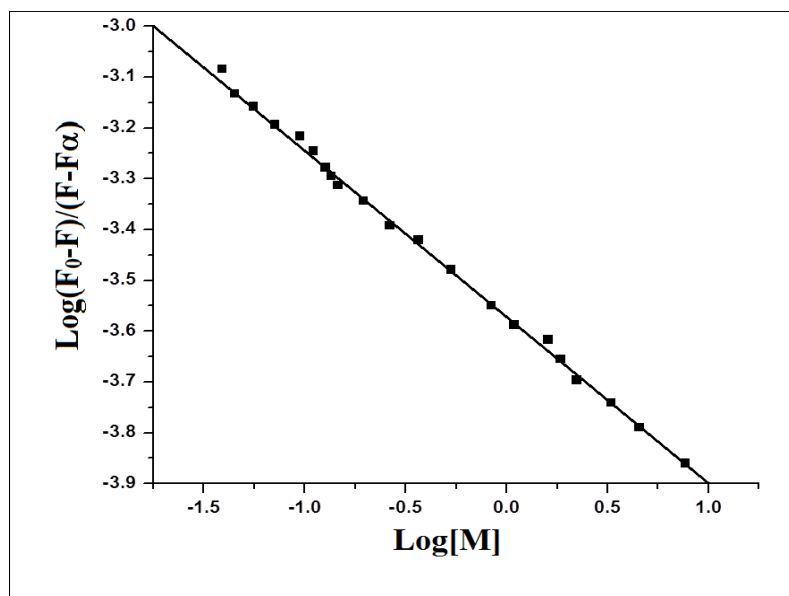
concentration (1.0 – 100.0 equivalents) were prepared by proper dilution of the stock solution. The ion-binding property of fluoroionophore TEQC was investigated with a large number of cations as their perchlorate salts ( $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Hg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Cu}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Cr}^{3+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Sr}^{2+}$ ,  $\text{Ce}^{3+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{Fe}^{3+}$ ) and anions ( $\text{F}^-$ ,  $\text{Cl}^-$ ,  $\text{Br}^-$ ,  $\text{I}^-$ ,  $\text{S}^{2-}$ ,  $\text{NO}_2^-$ ,  $\text{CH}_3\text{COO}^-$  and  $\text{H}_2\text{PO}_4^-$ ) as their tetrabutyl ammonium salts in acetonitrile. Then solution for  $\text{Cd}^{2+}$  and  $\text{Sr}^{2+}$  were prepared in the concentration range of (0-160 nM) and (0-100 nM) respectively with  $1 \times 10^{-8}$  M concentration of TEQC ligand. We have studied 0, 5, 10.0, 15.0, 20.0, 20.5, 30.0, 35.0, 40.0, 45.0, 50.0, 55.0, 60.0, 65.0, 70.0, 75.0, 80.0, 82.5, 85.0, 87.5, 90.0, 92.5, 95.0 and 100.0 nM of  $\text{Sr}^{2+}$  concentration with  $1 \times 10^{-8}$  M concentration of TEQC ligand. For  $\text{Cd}^{2+}$ , we have studied 0, 2.5, 5, 7.5, 10.0, 12.5, 15.0, 17.5, 20.5, 22.5, 25.0, 27.5, 30.0, 32.5, 35.0, 37.5, 40.5, 42.5, 45.0, 47.5, 50.5, 52.5, 55.0, 57.5, 60.0, 62.5, 65.0, 67.5, 70.0, 72.5, 75.0, 77.5, 80.0, 82.5, 85.0, 87.5, 90.0, 92.5, 95.0, 97.5, 100.0, 102.5, 105.0, 107.5, 110.0, 112.5, 115.0, 117.5, 120.5, 122.5, 125.0, 127.5, 130.0, 132.5, 135.0, 137.5, 140.0, 142.5, 145.0, 147.5, 150.5, 152.5, 155.0, 157.5, 160.0 nM with  $1 \times 10^{-8}$  M concentration of TEQC ligand.

The ion-recognition process was monitored by luminescence, UV-Vis and ESI-Mass spectral changes. The binding constants were calculated by fluorescence titration data. Here, we have shown representative spectra showing the changes observed in emission intensities upon the addition of increasing concentration of ions are shown in the figure. According to this procedure, the fluorescence intensity ( $F$ ) scales with the metal ion concentration ( $[M]$ ) through  $(F_0 - F)/(F - F_\infty) = ([M]/K_{\text{diss}})^n$ . The binding constant ( $K_s$ ) is obtained by plotting  $\text{Log}[(F_0 - F)/(F - F_\infty)]$  vs.  $\text{Log}[M]$ , where  $F_0$  and  $F_\infty$  are the relative fluorescence intensities without addition of guest metal ions and with maximum concentration of metal ions (when no further change in emission intensity takes

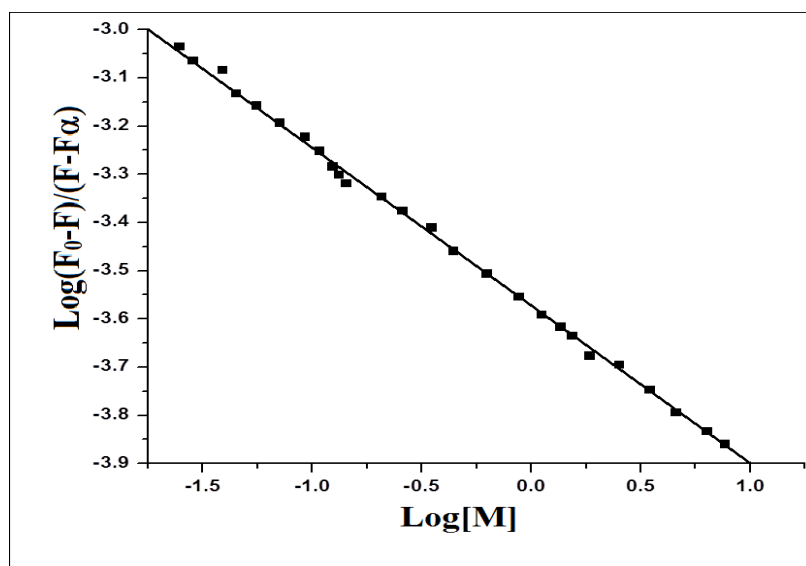
place), respectively. The value of  $\text{Log } [M]$  at  $\text{Log } [(F_0-F)/(F-F_\infty)] = 0$  gives the value of  $\log (K_{\text{diss}})$ , the reciprocal of which is the binding constant ( $K_s$ ).

## 5. Real sample preparation

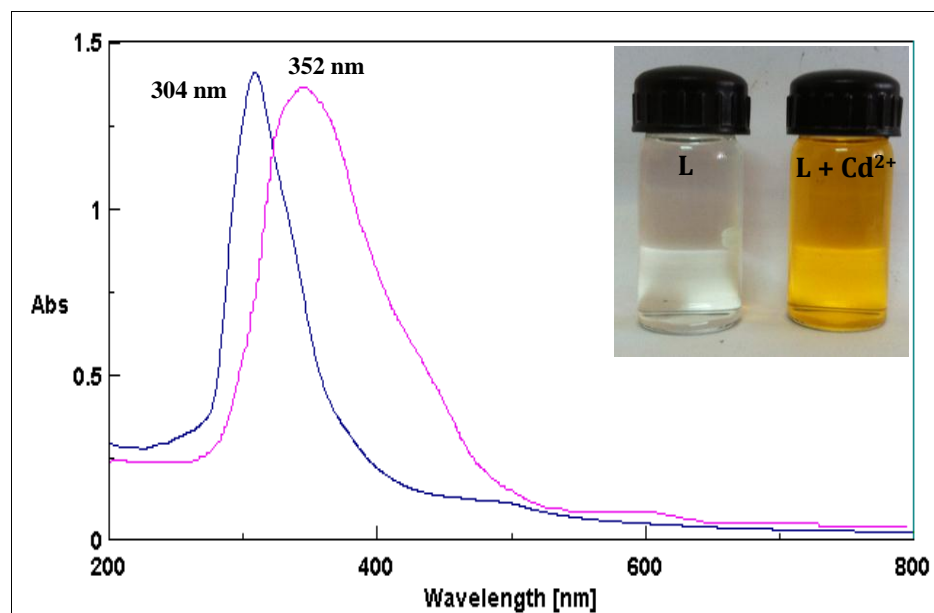
For the analytical application of proposed fluorescence probe we have applied this probe for real sample analysis in industrial waste water for  $\text{Cd}^{2+}$  and  $\text{Sr}^{2+}$ . The proposed sensor was successfully used in the determination of  $\text{Cd}^{2+}$  and  $\text{Sr}^{2+}$  in spiked water samples. The waste water samples (100 ml) were collected from industrial water (vatava). The  $\text{Cd}^{2+}$  and  $\text{Sr}^{2+}$  containing water sample was subjected for extraction procedure. Our compound was soluble in chloroform as well as in acetonitrile but as acetonitrile is miscible with, the TEQC was dissolved in chloroform to prepare solution for analysis. Then in separating funnel, we took 60 ml of ligand solution and 40 ml of water sample and shake for half an hour. The organic layer was separated and dried with anhydrous sodium sulphate. The organic extract was made up to 100 ml and measured the fluorescence intensity to find out the concentration of  $\text{Cd}^{2+}$  and  $\text{Sr}^{2+}$  in organic layer. The results of real sample analysis are given in **Table S1-S2**.



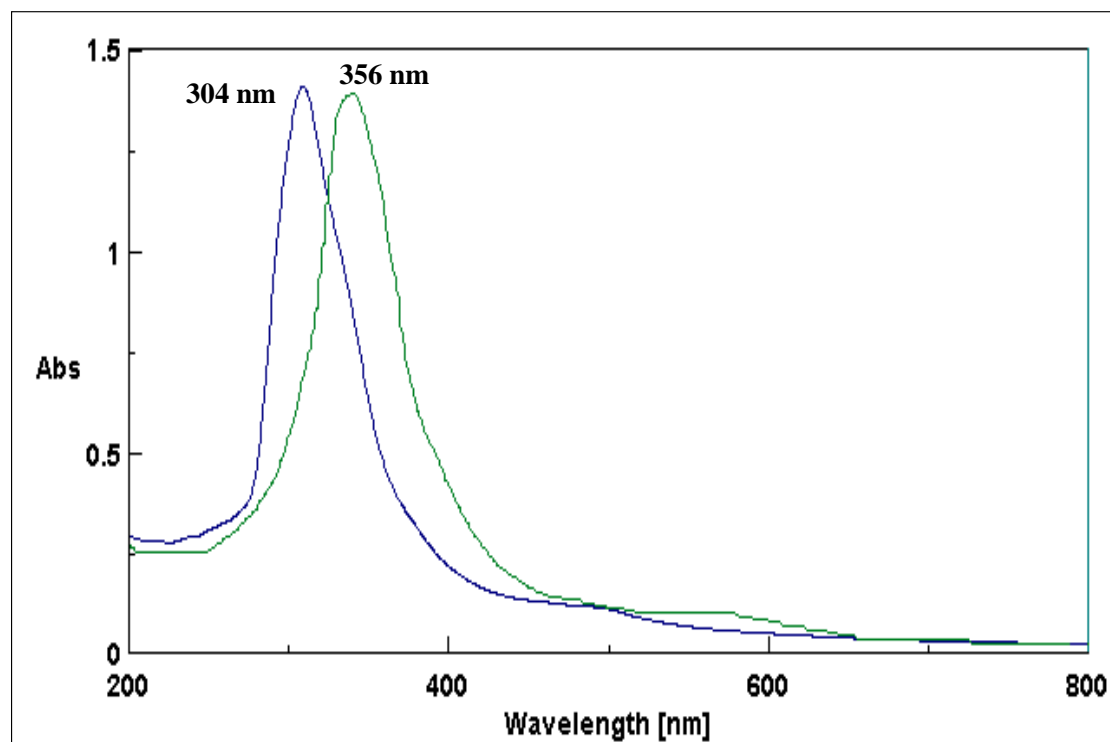
**Fig. S1** Binding constant plot for  $\text{Cd}^{2+}$  with TEQC ligand from emission titration.



**Fig. S2** Binding constant plot for  $\text{Sr}^{2+}$  with TEQC ligand from emission titration.

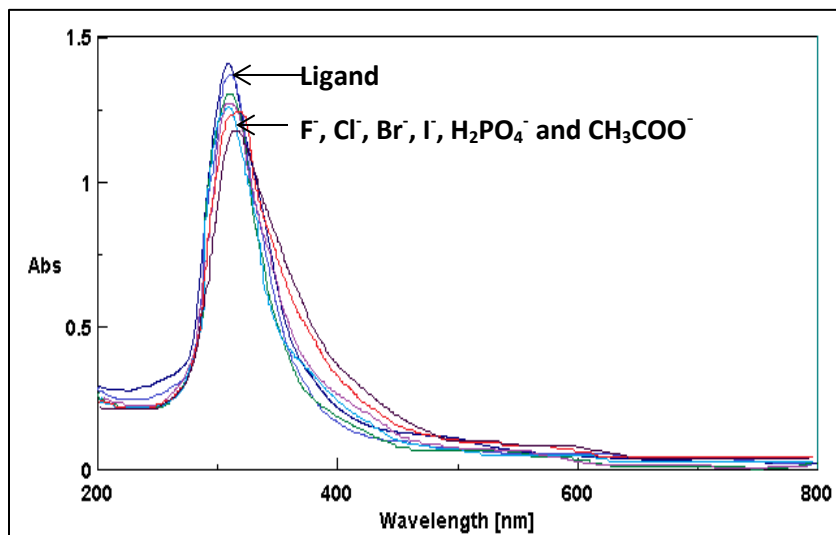


**Fig. S3** Absorption spectral changes of TEQC ( $1 \times 10^{-6}$  M) ligand in the presence of  $\text{Cd}^{2+}$  ( $1 \times 10^{-6}$  M).

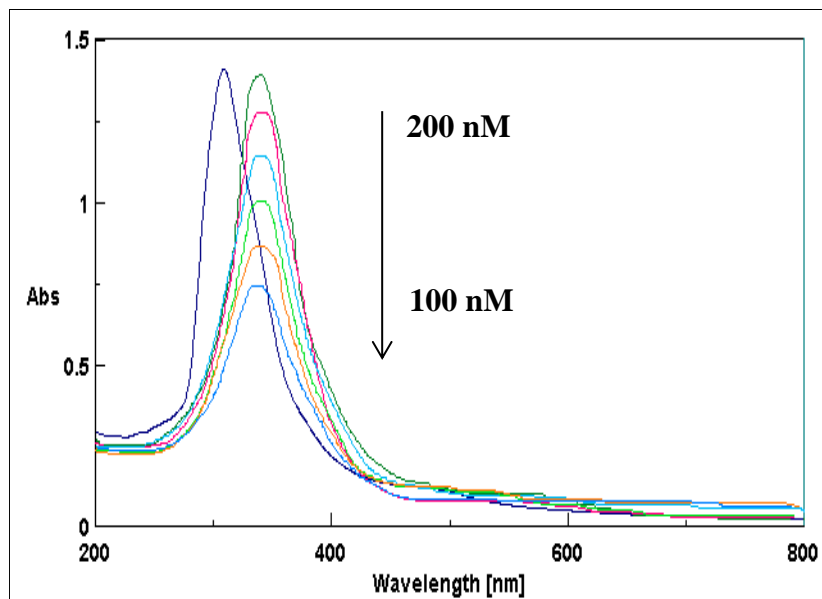


**Fig. S4** Absorption spectral changes of TEQC ( $1 \times 10^{-6}$  M) ligand in the presence of  $\text{Sr}^{2+}$  ( $1 \times 10^{-6}$  M).

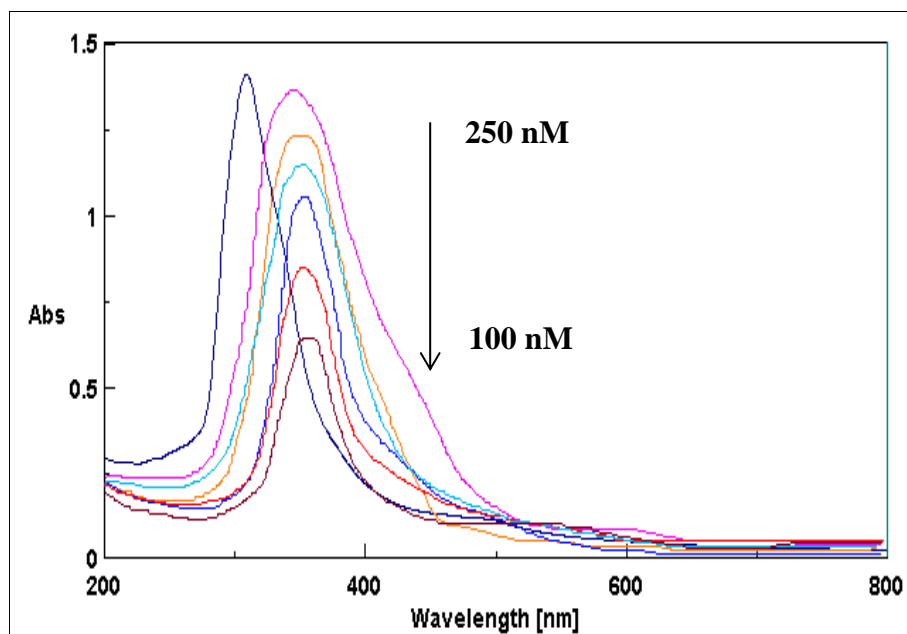




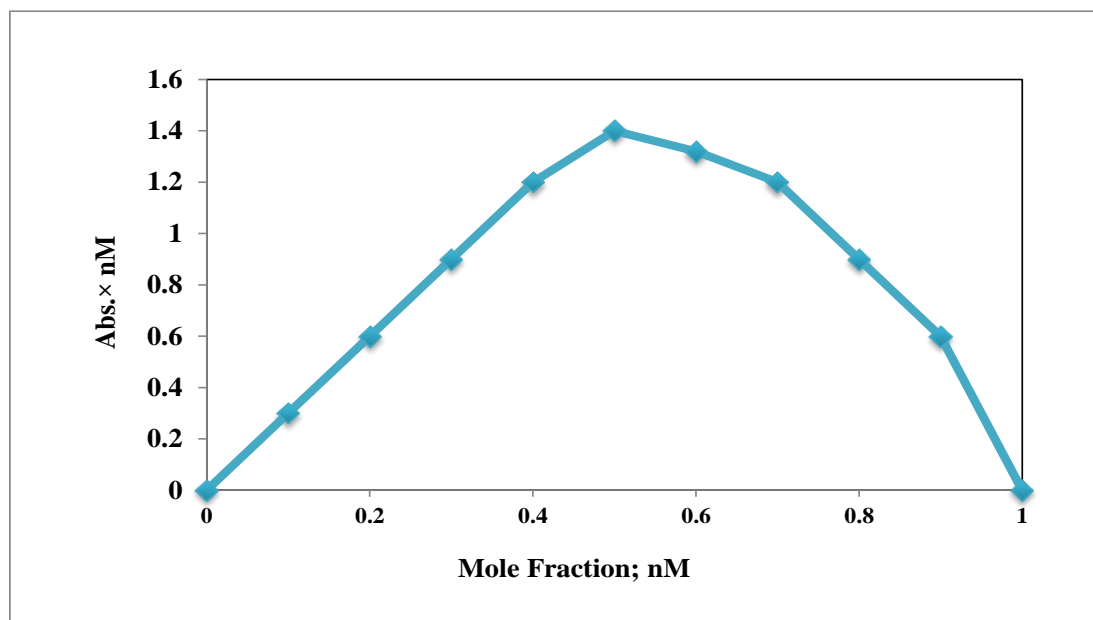
**Fig. S5** Absorption spectral changes of TEQC ( $1 \times 10^{-6}$  M) ligand in the presence of various anions (F<sup>-</sup>, Cl<sup>-</sup>, Br<sup>-</sup>, I<sup>-</sup>, H<sub>2</sub>PO<sub>4</sub><sup>-</sup> and CH<sub>3</sub>COO<sup>-</sup>) ( $1 \times 10^{-6}$  M).



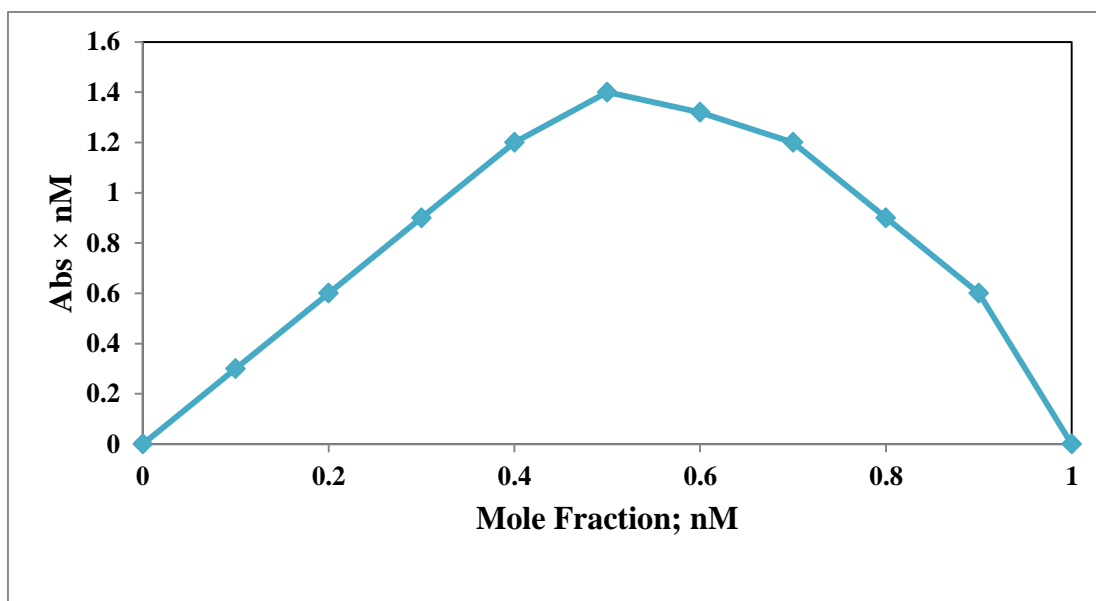
**Fig. S6** The plot demonstrates the absorption spectral changes of TEQC in the presence of different concentrations of Cd<sup>2+</sup> (200 nM, 180 nM, 160 nM, 140 nM, 120 nM and 100 nM).



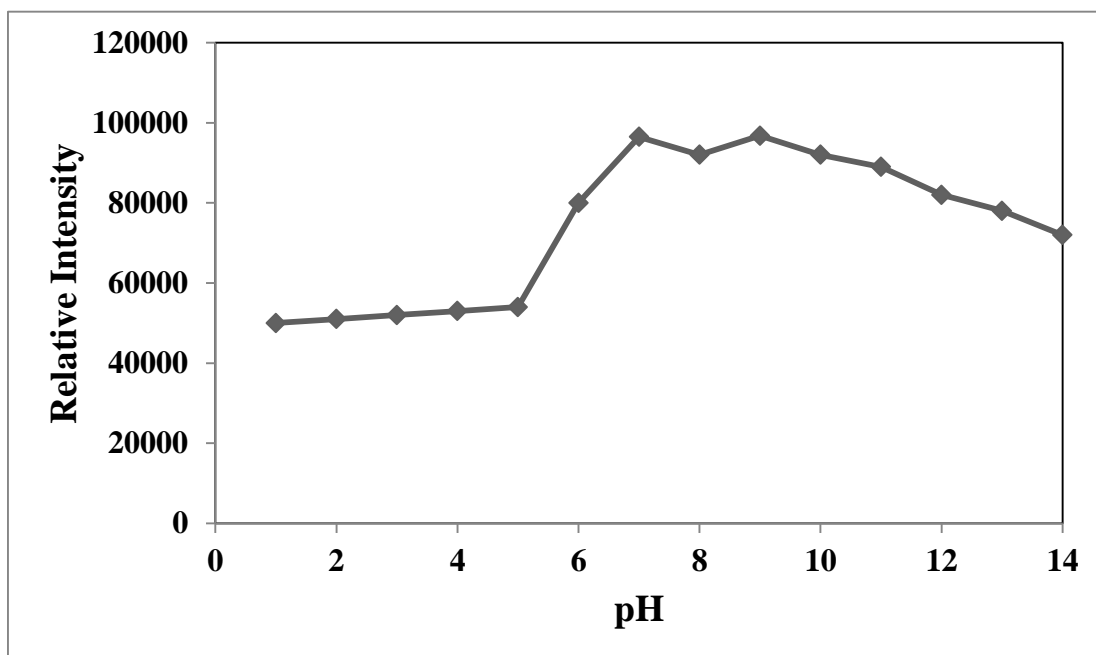
**Fig. S7** The plot demonstrates the absorption spectral changes of TEQC in the presence of different concentrations of Sr<sup>2+</sup> (250 nM, 225 nM, 200 nM, 175 nM, 150 nM, 125 nM and 100 nM).



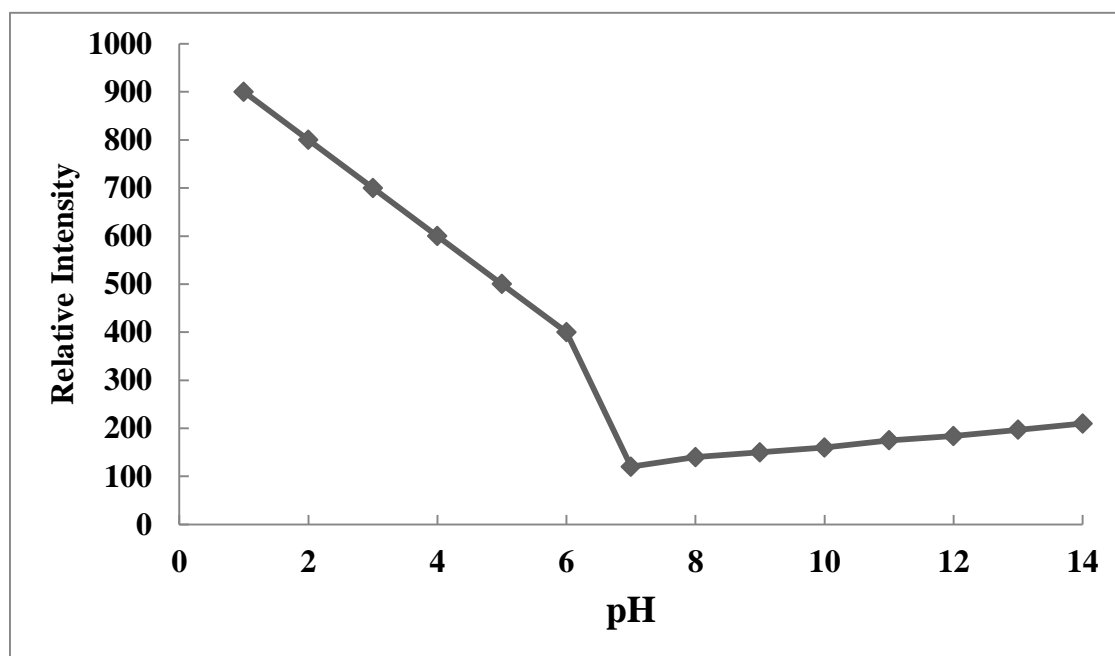
**Fig.S8** Job's plot obtained from the absorption titration of TEQC with Cd<sup>2+</sup>



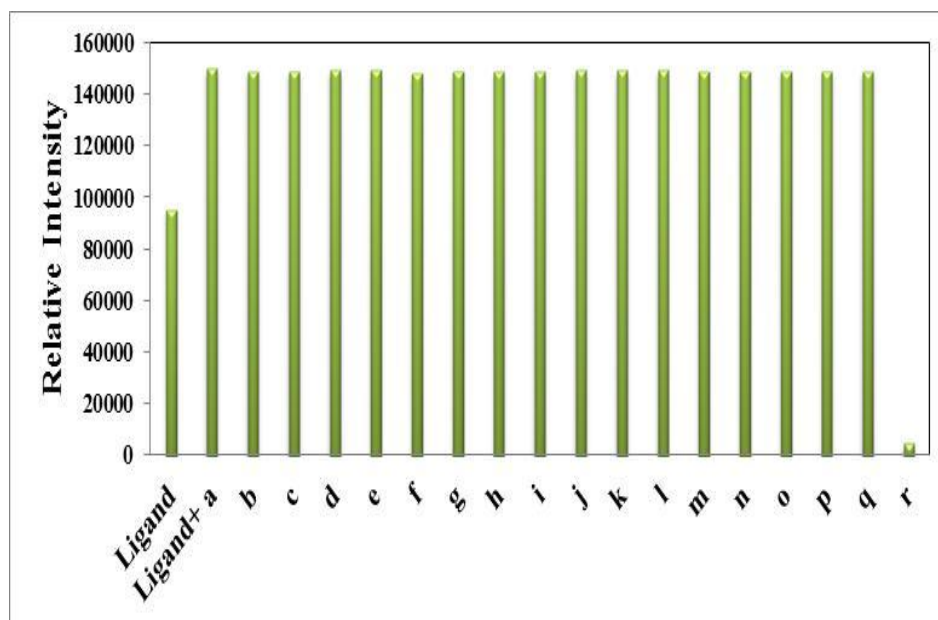
**Fig.S9** Job's plot obtained from the absorption titration of TEQC with  $\text{Sr}^{2+}$



**Fig. S10** Shows the effect of fluorescence intensities of TEQC with  $\text{Cd}^{2+}$  complex by varying pH.

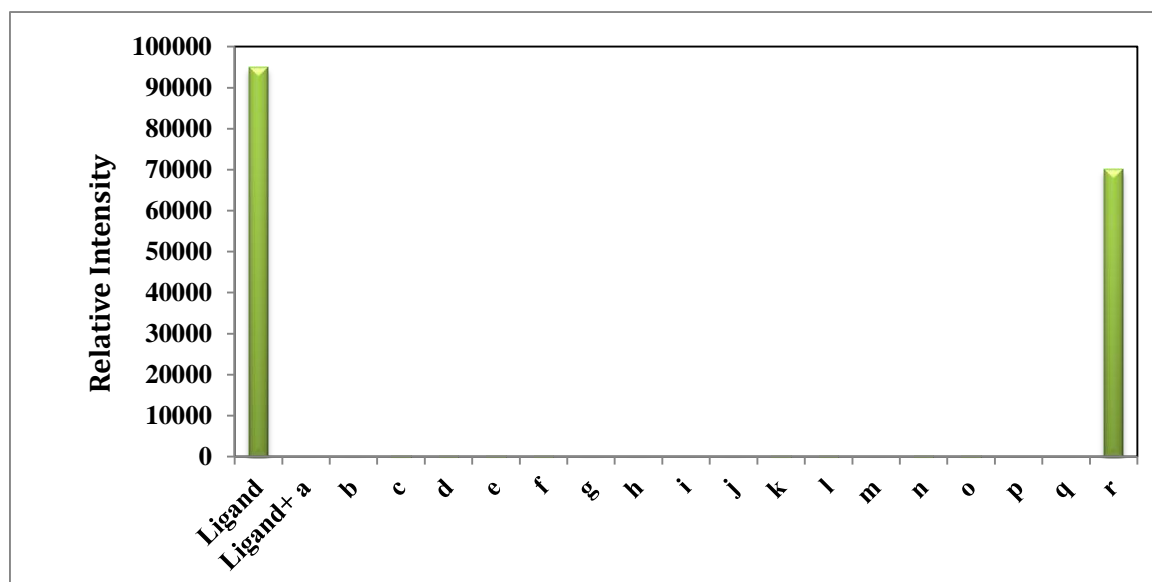


**Fig. S11** Shows the effect of fluorescence intensities of TEQC with  $\text{Sr}^{2+}$  complex by varying pH.

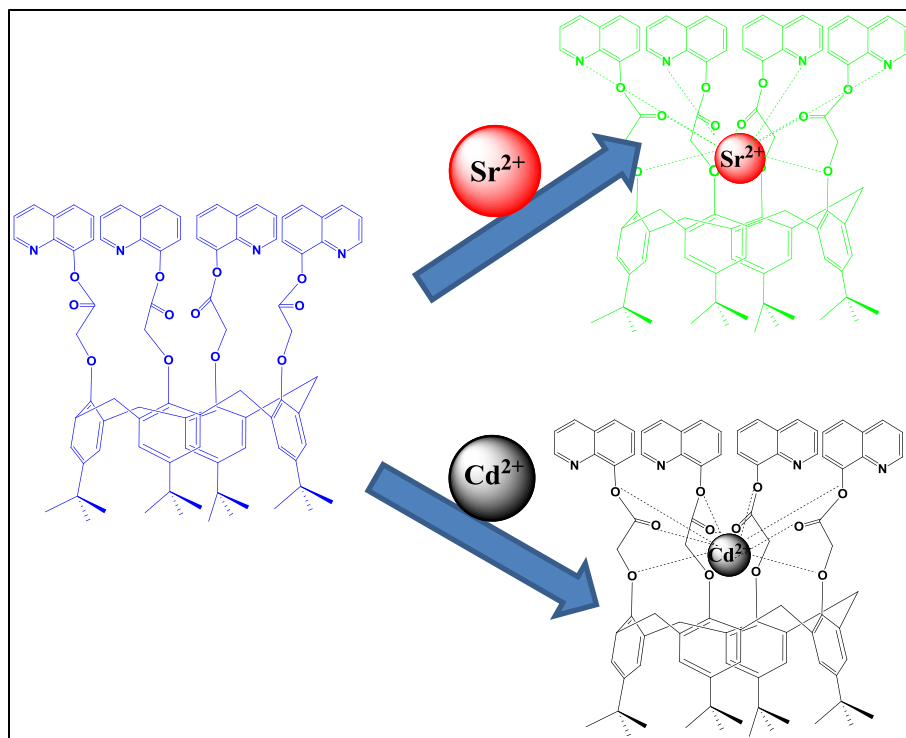


**Fig. S12** Competitive emission spectra of TEQC ( $1 \times 10^{-6}$  M) with  $\text{Cd}^{2+}$  in presence of other cations (a = Ligand +  $\text{Sr}^{2+}$ , b = a +  $\text{Zn}^{2+}$ , c = a +  $\text{Cd}^{2+}$ , d = a +  $\text{Fe}^{2+}$ , e = a +  $\text{Hg}^{2+}$ , f = a +  $\text{Na}^+$ , G

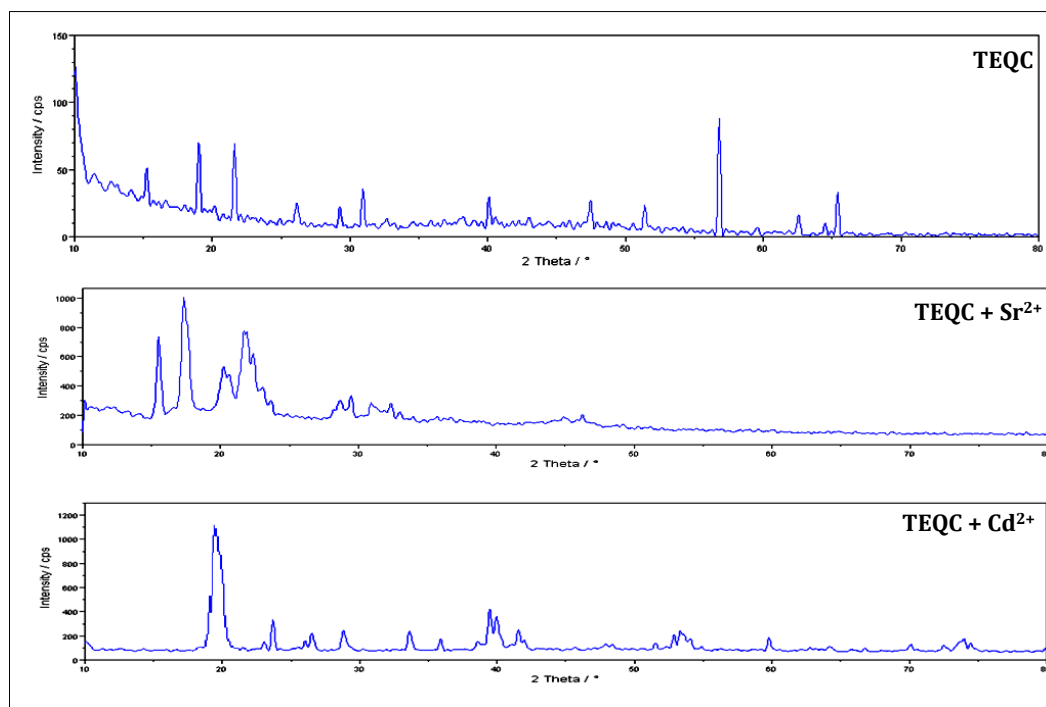
= a + K<sup>+</sup>, h = a + Cu<sup>2+</sup>, i = a + Ni<sup>2+</sup>, j = a + Mn<sup>2+</sup>, k = a + Cr<sup>3+</sup>, l = a + Pb<sup>2+</sup>, m = a + Ba<sup>2+</sup>, n = a + Ce<sup>3+</sup>, o = a + Co<sup>2+</sup>, p = a + Ca<sup>2+</sup>, q = a + Mg<sup>2+</sup> and r = a + Cd<sup>2+</sup>)



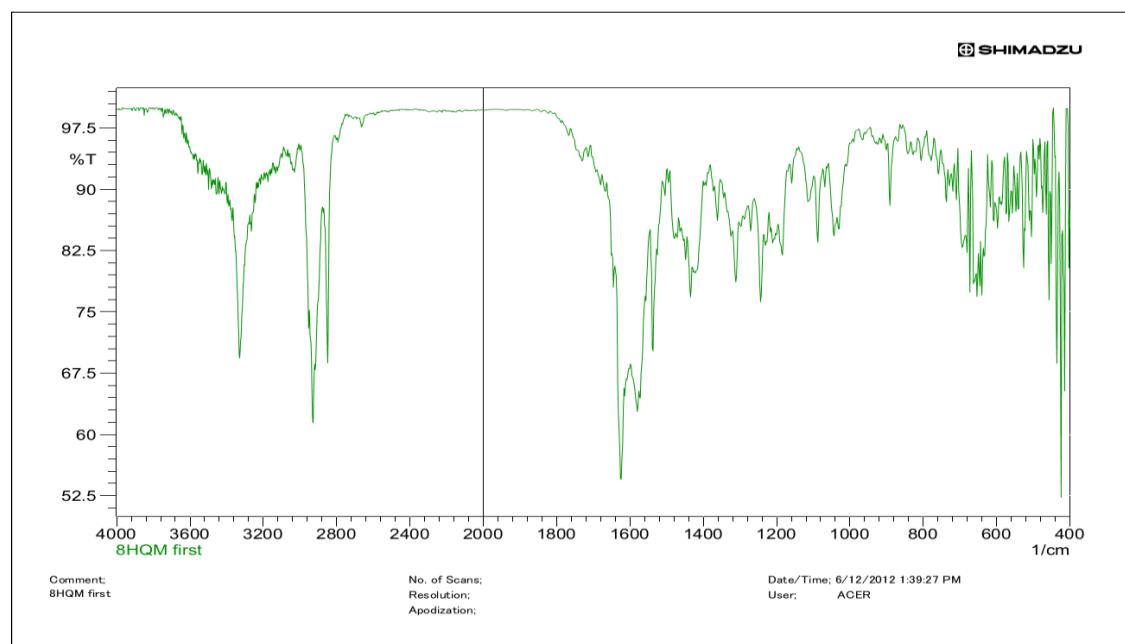
**Fig. S13** Competitive emission spectra of TEQC ( $1 \times 10^{-6}$  M) with Sr<sup>2+</sup> in presence of other cations (a = Ligand + Cd<sup>2+</sup>, b = a + Zn<sup>2+</sup>, c = a + Cd<sup>2+</sup>, d = a + Fe<sup>2+</sup>, e = a + Hg<sup>2+</sup>, f = a + Na<sup>+</sup>, G = a + K<sup>+</sup>, h = a + Cu<sup>2+</sup>, i = a + Ni<sup>2+</sup>, j = a + Mn<sup>2+</sup>, k = a + Cr<sup>3+</sup>, l = a + Pb<sup>2+</sup>, m = a + Ba<sup>2+</sup>, n = a + Ce<sup>3+</sup>, o = a + Co<sup>2+</sup>, p = a + Ca<sup>2+</sup>, q = a + Mg<sup>2+</sup> and r = a + Sr<sup>2+</sup>)



**Fig. S14** Proposed mechanism of TEQC ligand with  $\text{Cd}^{2+}$  and  $\text{Sr}^{2+}$ .



**Fig S15** Comparative powder X-ray diffraction pattern of TEQC, TEQC +  $\text{Sr}^{2+}$  and TEQC +  $\text{Cd}^{2+}$



**Fig. S16** FT-IR spectra of TEQC ligand showing absence of  $\text{-OH}$  group in TEQC ligand.

<b>Sample</b>	<b>Added (nM)</b>	<b>Found by AAS (nM)</b>	<b>Found by proposed sensor (nM)</b>	<b>Recovery (%)</b>
Water sample 1	0	2.8	2.5	-----
Water sample 2	10	13.6	13.2	105.60 ± 4
Water sample 3	20	24.8	24.2	107.55 ± 4
Water sample 4	30	33.9	33.7	103.69 ± 2
Water sample 5	100	105.6	105.2	102.63 ± 1

**Table S1:** Results of the determination of Cd<sup>2+</sup> in industrial waste water samples.

<b>Sample</b>	<b>Added Sr<sup>2+</sup> (nM)</b>	<b>Found by AAS</b>	<b>Found by proposed sensor (nM)</b>	<b>Recovery (%)</b>
Water sample 1	0	8.9	8.5	-----
Water sample 2	10	15.6	15.2	115.67 ± 4
Water sample 3	20	26.8	26.4	107.01 ± 4
Water sample 4	30	37.7	37.2	104.41 ± 2
Water sample 5	100	110.6	110.4	101.75 ± 1

**Table S2:** Results of the determination of Sr<sup>2+</sup> in industrial waste water samples.



Method	Recognized cation	Linear range	Limit of detection	Reference
Graphite Furnace Atomic Absorption Spectrometry (GF AAS)	Cd <sup>2+</sup>	2–20 ng L <sup>-1</sup>	0.6 ng L <sup>-1</sup>	3
Flame atomic absorption spectrometry	Cd <sup>2+</sup>	0.34–0.68 µg g <sup>-1</sup>	5.0 µg g <sup>-1</sup>	4
Flow injection inductively coupled plasma atomic emission spectrometric methods	Cd <sup>2+</sup>	5 - 100 ng ml <sup>-1</sup>	1.1 ng ml <sup>-1</sup>	5
Multi-Syringe Flow Injection Analysis system (MSFIA)	Cd <sup>2+</sup>	Measure frequency (14) scans per hour	0.79 µg g <sup>-1</sup>	6
High-performance chelation ion chromatography	Sr <sup>2+</sup>	5 to 50 mg L <sup>-1</sup>	3.23±0.15 mg L <sup>-1</sup>	7
Ion-selective electrode	Sr <sup>2+</sup>	1.0 × 10 <sup>-2</sup> - 1.0 × 10 <sup>-7</sup> M	2.7 × 10 <sup>-8</sup> M	8
Ion-selective electrode	Sr <sup>2+</sup>	3.98 × 10 <sup>-6</sup> - 1.0 × 10 <sup>-1</sup> M	2.82 × 10 <sup>-6</sup> M	9
<b>Present method</b>	<b>Cd<sup>2+</sup> and Sr<sup>2+</sup></b>	<b>0-160 nM and 0-100 nM</b>	<b>0.94 pM and 1.04 pM</b>	<b>-----</b>

**Table S3:** Comparison of proposed TEQC fluorescence sensor with various previously reported Cd<sup>2+</sup> and Sr<sup>2+</sup> determination methods.

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