Supporting Information

A new ICT and CHEF based visible light excitable fluorescent probe easily detects "in vivo" Zn^{2+}

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CONTENTS

1. Determination of detection limit:

The detection limit was calculated based on the absorption and fluorescence titration. To determine the S/N ratio, the emission intensity of BQ without Zn^{2+} was measured by 10 times and the standard deviation of blank measurements was determined. The detection limit is then calculated with the following equation:

$DL = K^* Sb_1/S$

Where K = 2 or 3 (we take 3 in this case); Sb_1 is the standard deviation of the blank solution; S is the slope of the calibration curve.

For Zn^{2+}:

From the graph we get slope = 1.138×10^{11}, and Sb_1 value is 892.32. Thus using the formula we get the Detection Limit = 2.35×10^{-8} M i.e. BQ can detect Zn^{2+} in this minimum concentration by fluorescence techniques.

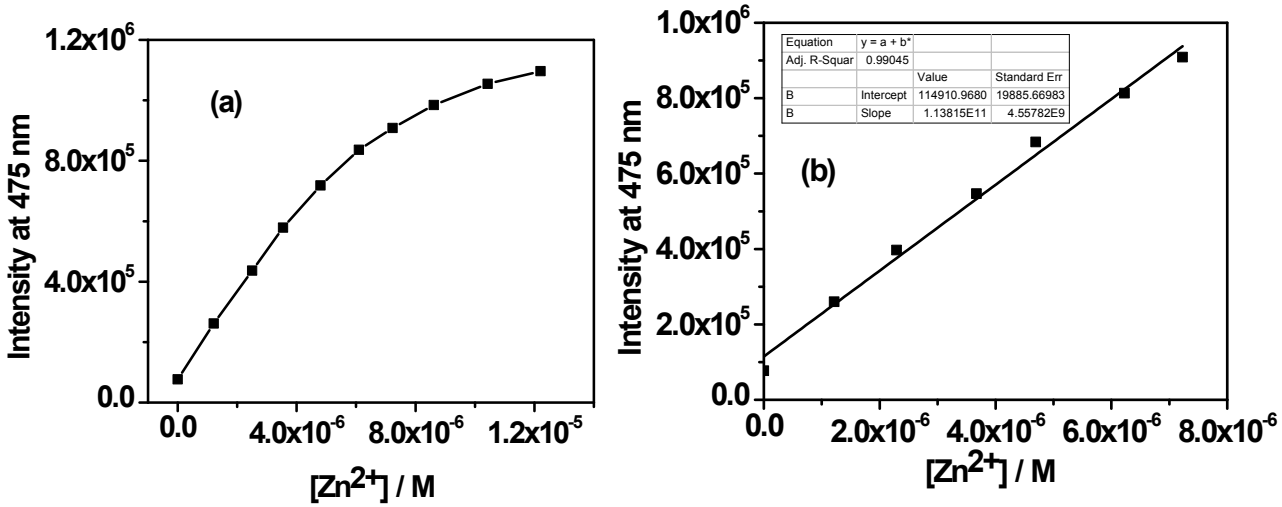

Figure S1: Emission of BQ at 475 nm depending on the concentration of Zn^{2+}

From the graph we get slope = 28469.227, and Sb_1 value is 0.00504, Thus using the formula we get the Detection Limit = 5.31×10^{-7} M i.e. BQ can detect Zn^{2+} in this minimum concentration by UV/vis techniques.

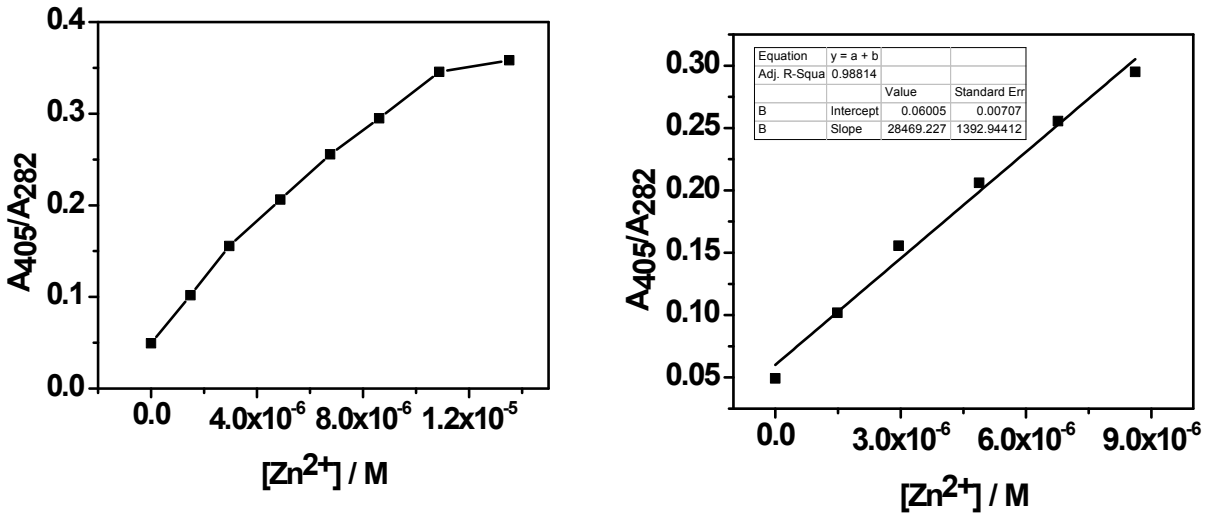

Figure S2: Absorbance ratio of BQ at (A_{405}/A_{282}) depending on the concentration of Zn^{2+}

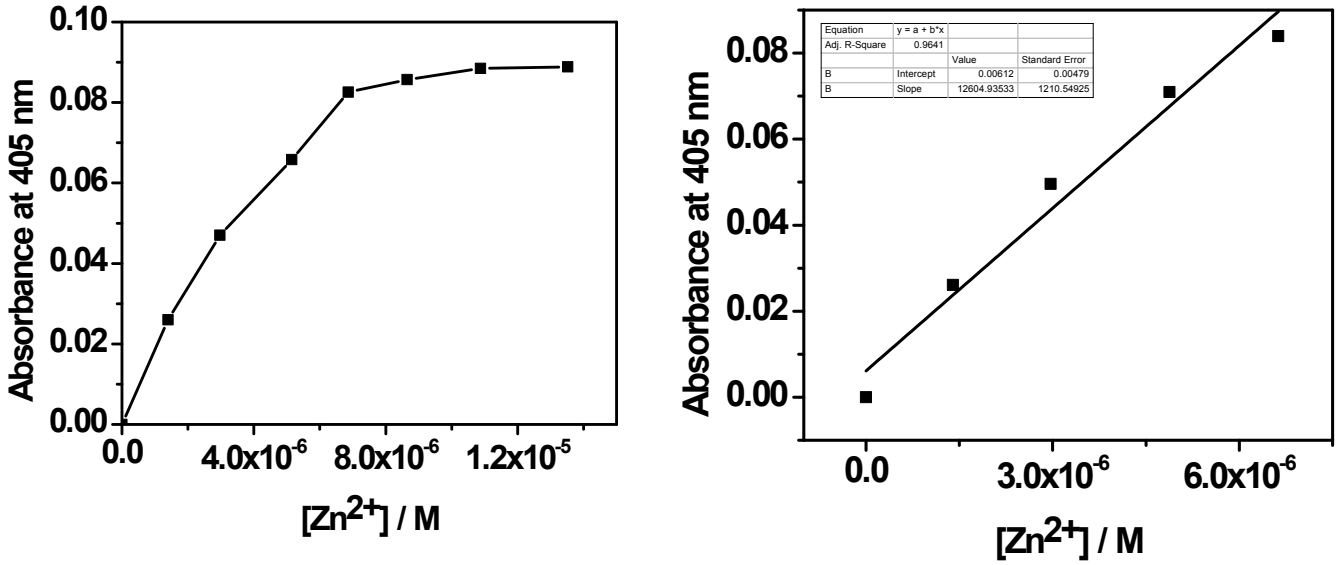

Figure S3: Absorbance BQ at (A_{405}) depending on the concentration of Zn^{2+}

2. Determination of Association Constant (K_a):

By UV-vis method:

Association constant was calculated according to the Benesi-Hildebrand equation. K_a was calculated following the equation stated below.

$$1/(A\text{-}A_o) = 1/\{K(A_{max}–A_o)\,[M^{x+}]^n\} + 1/[A_{max}\text{-}A_o]$$

Here A_o is the absorbance of receptor in the absence of guest, A is the absorbance recorded in the presence of added guest, A_{max} is absorbance in presence of added $[M^{x+}]_{max}$ and K_a is the association constant, where $[M^{X+}]$ is $[Zn^{2+}]$. The association constant (K_a) could be determined from the slope of the straight line of the plot of $1/(A\text{-}A_o)$ against $1/[Zn^{2+}]$ and is found to be 5.77×10^5 M^{-1}.

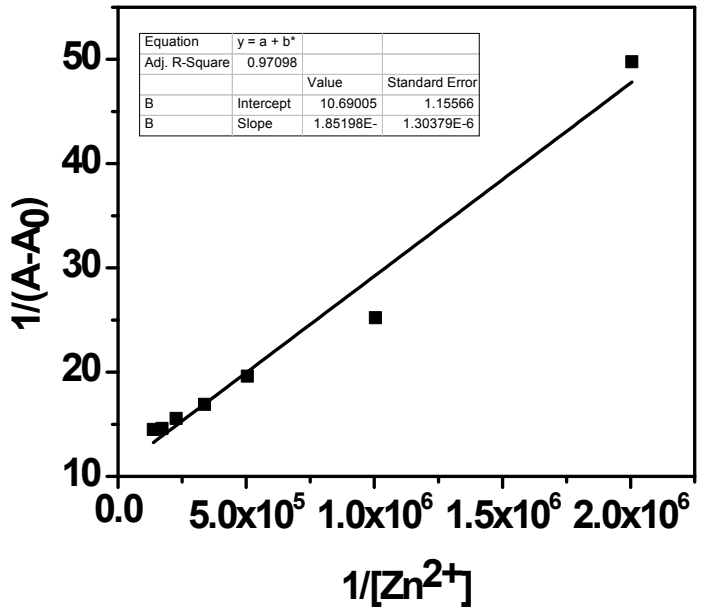

Figure S4: Benesi–Hildebrand plot from UV/vis titration data of receptor (10 μM) with Zn^{2+} concentration

By fluorescence method:

The binding constant value of Zn^{2+} with receptor has been determined from the emission intensity data following the modified Benesi–Hildebrand equation, $1/\Delta I = 1/\Delta I_{max} + (1/K_a[C])(1/\Delta I_{max})$. Here $\Delta I = I–I_{min}$ and $\Delta I_{max} = I_{max}–I_{min}$, where I_{min}, I, and I_{max} are the emission intensities of receptor considered in the absence of Zn^{2+}, at an intermediate Zn^{2+}

concentration, and at a concentration of complete saturation where K is the binding constant and [C] is the Zn^{2+}concentration respectively. From the plot of $[1 / (I - I_{min})]$ against $[C]^{-1}$ for receptor, the value of K has been determined from the slope. The association constant (K_a) as determined by fluorescence titration method for the receptor with Zn^{2+} is found to be 2.48×10^5 M^{-1}.

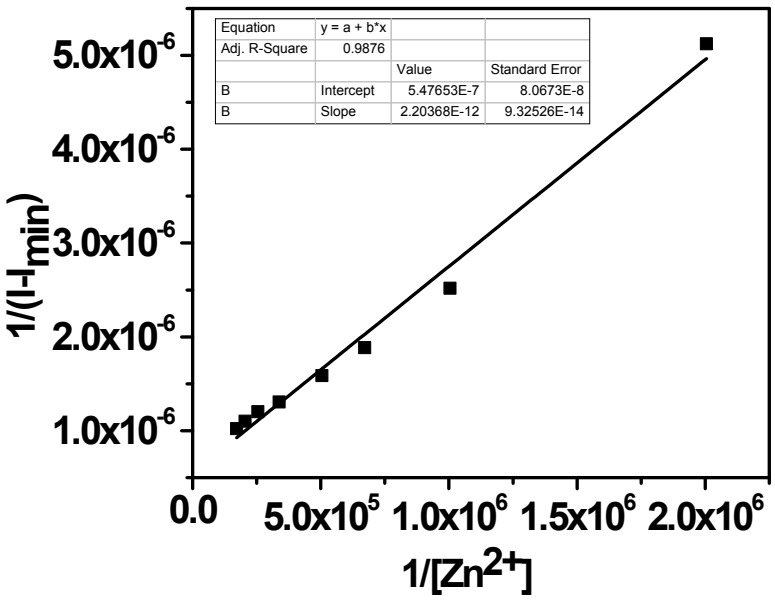

Figure S5: Benesi–Hildebrand plot from fluorescence titration data of receptor (10 μM) with Zn^{2+}.

3. General procedure for drawing Job's plot by fluorescence method:

Stock solution of same concentration of sensor and Zn^{2+} was prepared in the order of 10 μM in [CH_3OH/ H_2O, 1/9, v/v] (at 25 °C) at pH 7.4 in HEPES buffer. The absorbance spectrum in each case with different *host–guest* ratio but equal in volume was recorded. Job's plots were drawn by plotting $\Delta I.X_{host}$ *vs* X_{host} (ΔI = change of absorbance of the absorption spectrum at 405 nm during titration and X_{host} is the mole fraction of the host in each case, respectively).

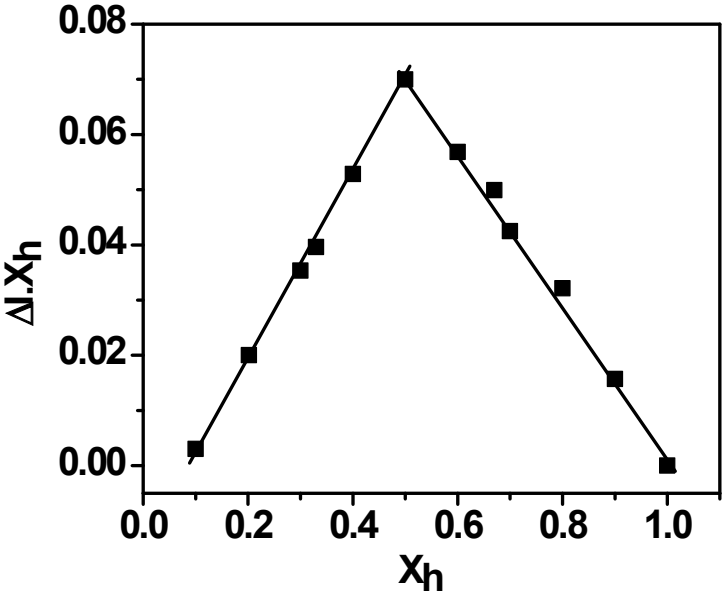

Figure S6: Job's plot diagram of receptor for Zn^{2+} (where X_h is the mole fraction of the host and ΔI indicates the change of absorbance at 405 nm).

4. Competition study

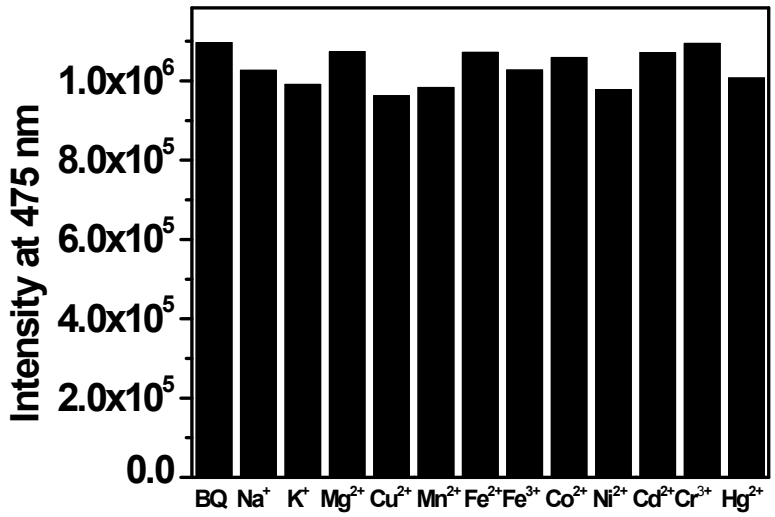

Figure S7: Competition study using Fluorescence method, after addition of different analytes (30 μM) in the solution of BQ (10 μM) in presence of Zn^{2+} (20 μM).

5. Determination of fluorescence Quantum Yields (Φ) of BQ and its complex with Zn^{2+} ion:

For measurement of the quantum yields of BQ and its complex with Zn^{2+}, we recorded the absorbance of the compounds in methanol solution. The emission spectra were recorded using the maximal excitation wavelengths, and the integrated areas of the fluorescence-corrected spectra were measured. The quantum yields were then calculated by comparison with fluorescein (Φs = 0.97 in basic ethanol) as reference using the following equation:

$$\Phi X = \Phi S \times \left(\frac{Ix}{Is}\right) \times \left(\frac{As}{Ax}\right) \times \left(\frac{nx}{ns}\right)^2$$

Where, x & s indicate the unknown and standard solution respectively, Φ is the quantum yield, *I* is the integrated area under the fluorescence spectra, *A* is the absorbance and *n* is the refractive index of the solvent.

We calculated the quantum yield of BQ and BQ-Zn^{2+} using the above equation and the value is 0.02 and 0.22 respectively.

6. pH study

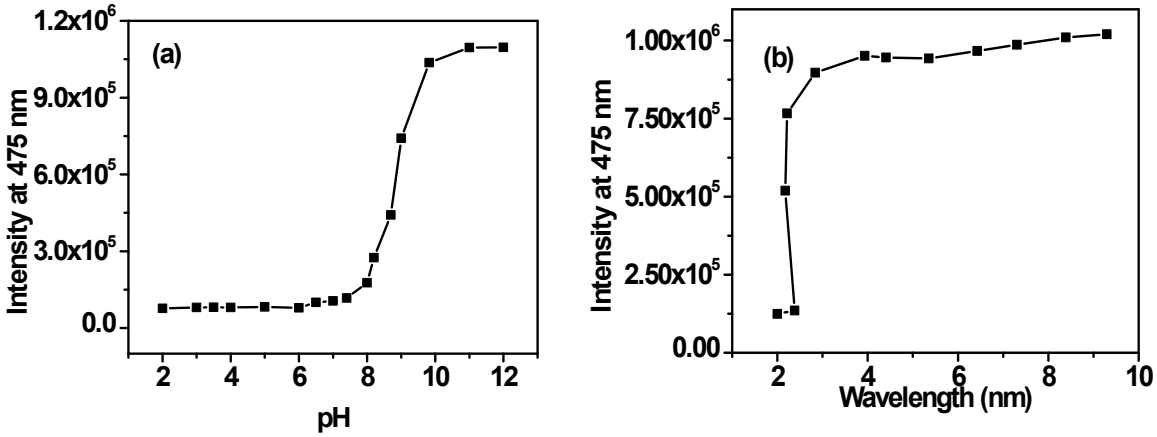

Figure S8: Fluorescence response of (a) BQ and (b) BQ-Zn^{2+} at 475 nm (10 μM) as a function of pH in CH_3OH/ H_2O (1/ 9, *v/v*), pH is adjusted by using aqueous solutions of 1 M HCl or 1 M NaOH

Comparison of present probe with the existing probes:

Table S1: The comparison of the present probe with recently reported probes for Zn^{2+} have been outlined in this table.

Fluorophore used	Type of response	Detection limit	Living cell imaging	Reference
Quinoline-pyridine	Colorimetric, fluorometric	6.6×10^{-8} M	No	*Dalton Trans*., 2013, 42, 15514
Quinoline-pyridine	Colorimetric, fluorometric	4.9×10^{-8} M	Yes	*Dalton Trans*., 2014, 43, 706–713
Pentaquinone	Colorimetric, Fluorometric	3.5×10^{-9} Ml^{-1}	No	*Dalton Trans.,* 2013, 42, 975
Pyridine-hydrazone	Colorimetric, fluorometric	69 ppb	Yes	*Org. Biomol. Chem.,* 2014, 12, 4975.
benzothiazole	Colorimetric, fluorometric	67 μM	Yes	*Dalton Trans*., 2015, 44, 2097–2102
benzoxazole	Colorimetric, fluorometric	1.63×10^{-8} M	Yes	*Chem.Commun*., 2014, 50, 7514.
8-aminoquinoline	Colorimetric, fluorometric	10^{-7} M	No	*New J. Chem*., 2014, 38, 1802.
benzaldehyde	Colorimetric, fluorometric	76 μM	Yes	*Dalton Trans.,* 2013, 42, 16569.
Quinoline	Colorimetric, fluorometric	2.35×10^{-8} M	Yes	*Present work*

7. ^{1}H NMR spectrum of BQ

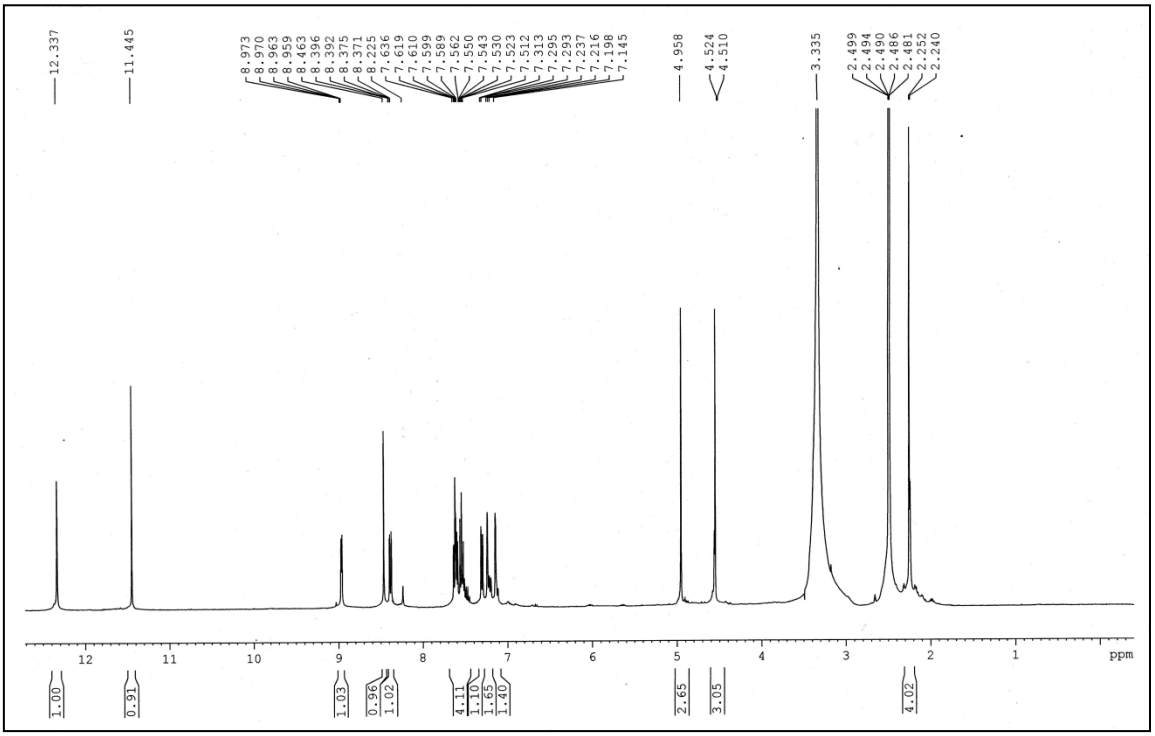

Figure S9: ^{1}H NMR (400 MHz) spectrum of BQ in DMSO-d_6.

8. ^{13}C NMR of BQ

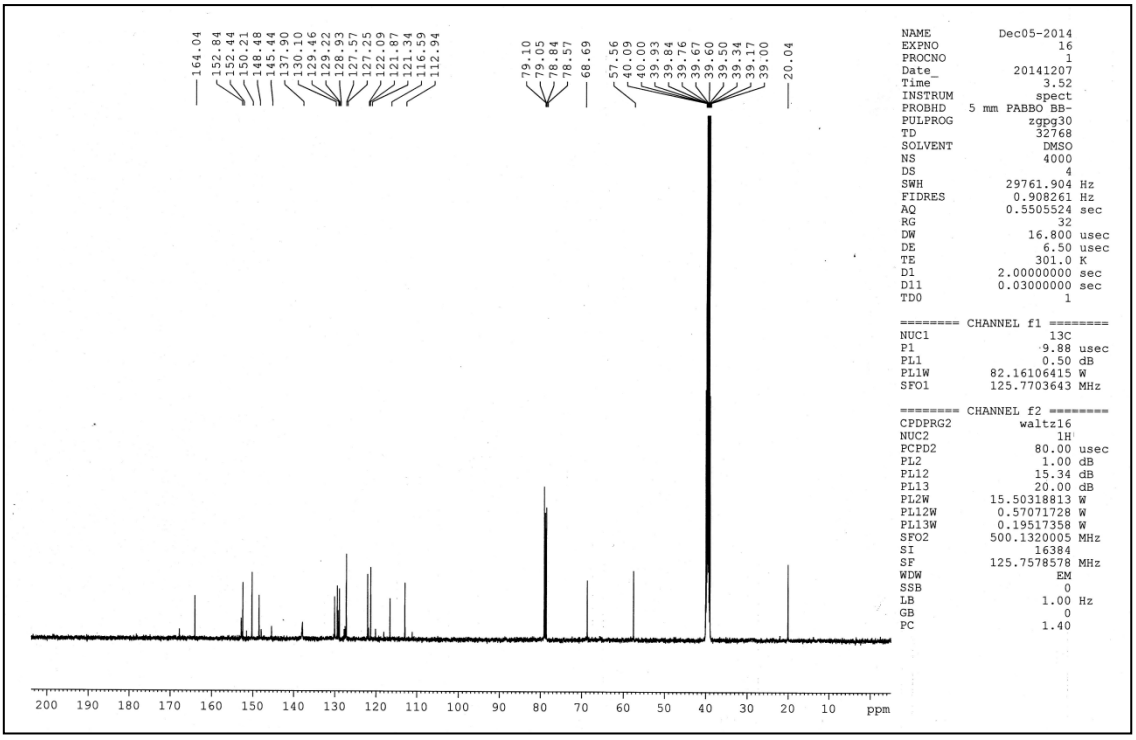

Figure S10: ^{13}C NMR (125 Hz) spectrum of BQ in DMSO-d_6.

9. HRMS of BQ

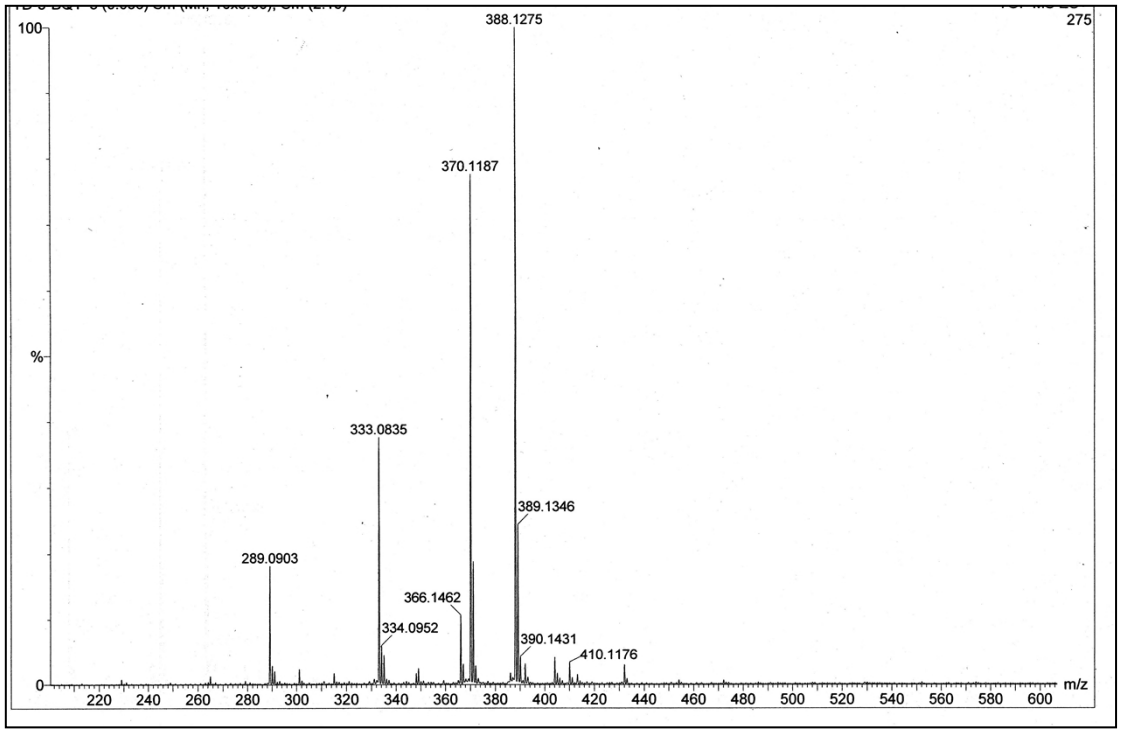

Figure S11: HRMS of BQ.

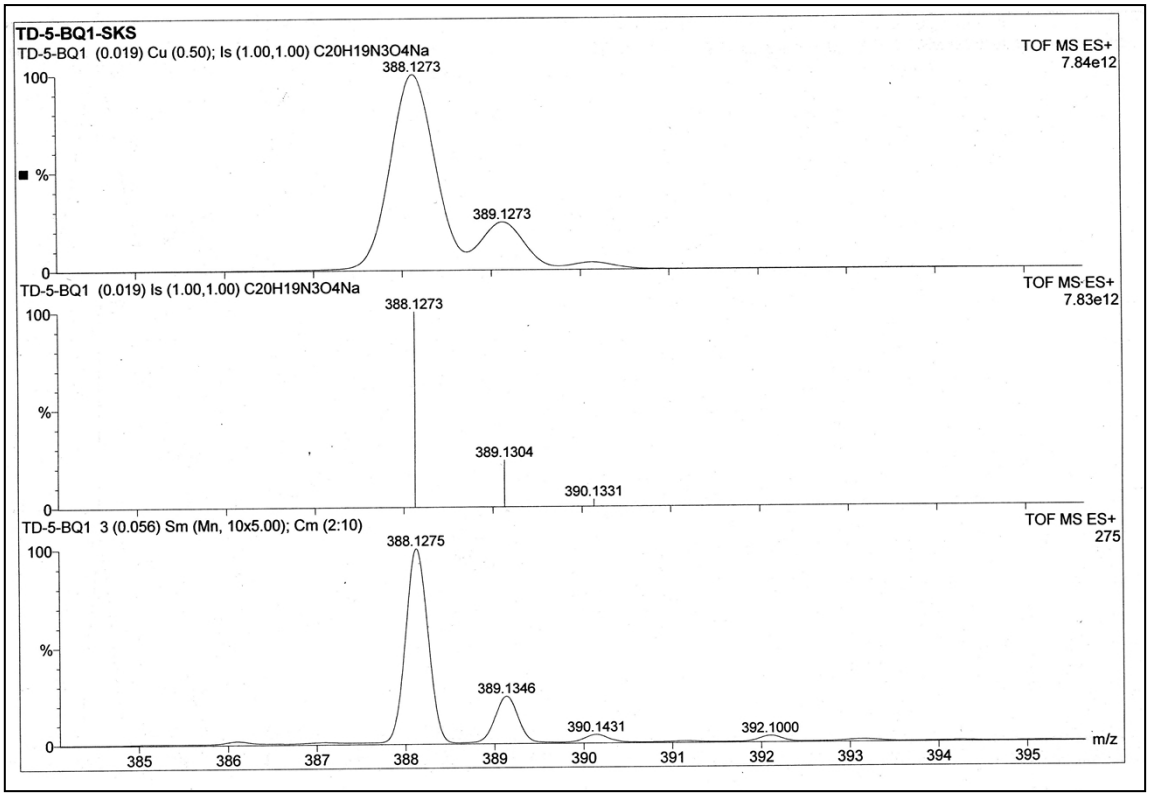

Figure S11a: HRMS (expansion) of BQ.

10. **HRMS of BQ-Zn^{2+} complex**:

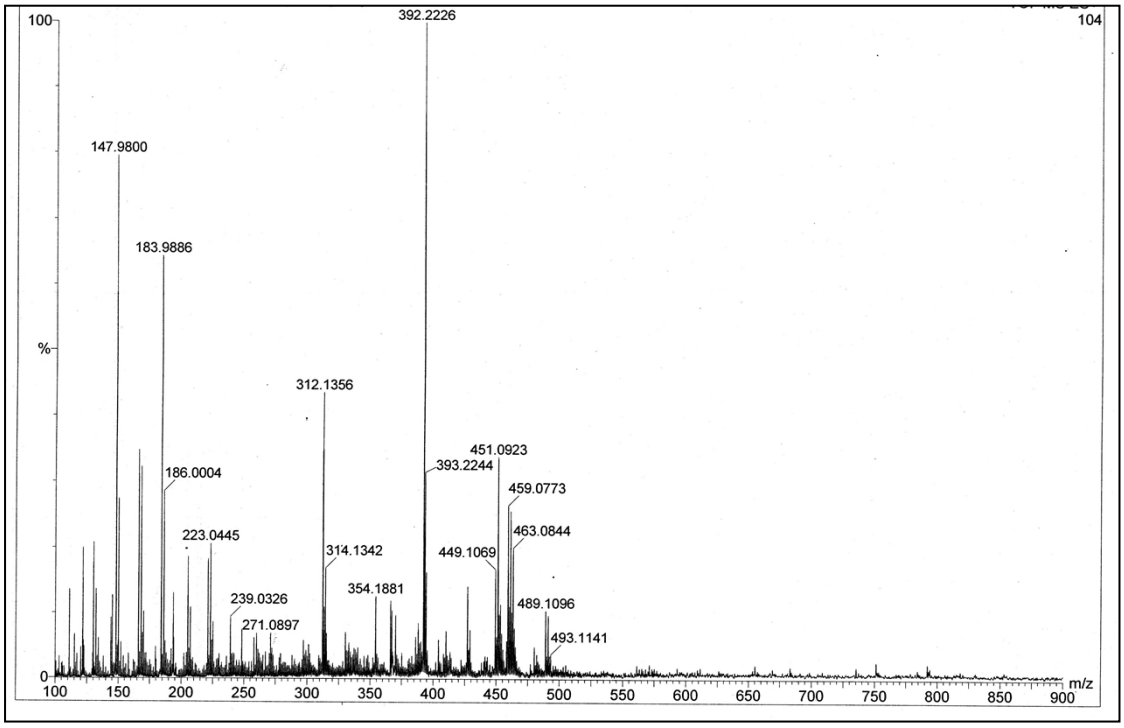

Figure 12: HRMS of BQ-Zn^{2+} complex