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Ratiometric and Absolute Water Soluble Fluorescent Tripodal Zinc Sensor and its Application in Killing Human Lung Cancer Cells

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1. General method of UV-vis and fluorescence titrations:

By UV-vis method:

For UV-vis titrations, stock solution of the receptor was prepared in 10μM HEPES buffer solution (at 25°C) for the titration of cations. The solution of the guest cation zinc using its perchlorate salts in the order of 2 x 10^{-4}M were also prepared in deionized water in HEPES buffer at pH = 7.4. Solutions of various concentrations containing sensor and increasing concentrations of cation were prepared separately. The spectra of these solutions were recorded by means of UV-vis methods.

General procedure for drawing Job plot by Fluorescence method:

Stock solution of same concentration of sensor and Zn^{2+} were prepared in the order of ≈ 1.0 x 10^{-5} by using HEPES buffer solution. The intensity in each case with different *host–guest* ratio but equal in volume was recorded. Job plots were drawn by plotting $\Delta I.X_{host}$ *vs* X_{host} (ΔI = change of intensity of the fluorescent spectrum during titration and X_{host} is the mole fraction of the host).

By fluorescence method:

For fluorescence titrations, stock solution of the sensor was prepared in 10 μM in for the titration of cations in HEPES buffer at pH = 7.4 (at 25°C). The solution of the guest cations using their perchlorate in the order of 200 μM were also prepared in deionized water. Solutions of various concentrations containing sensor and increasing concentrations of cations were prepared separately. The spectra of these solutions were recorded by means of fluorescence methods.

2. Association constant determination:

The binding constant value of cation Zn^{2+} with the sensor has been determined from the emission intensity data following the modified Benesi–Hildebrand equation, $1/\Delta I = 1/\Delta I$ max + (1/K[C])(1/ΔI max). Here ΔI = I-Imin and ΔI max = Imax-Imin, where Imin, I, and Imax are the emission intensities of sensor considered in the absence of guest , at an intermediate concentration and at a concentration of complete saturation of guest where K is the binding constant and [C] is the guest concentration respectively. From the plot of (Imax-Imin)/(I-Imin) against $[C]^{-1}$ for sensor, the value of K has been determined from the slope. The association constant (K_a) as determined by fluorescence titration method for sensor with $Zn^{2+}$is found to be 4 x 10^4 M^{-1} (error < 10%).

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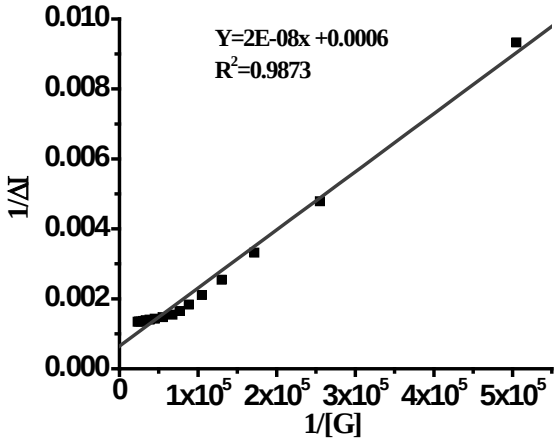

Fig. S1: Benesi–Hildebrand plot from fluorescence titration data of receptor (10µM) with Zn^{2+}.

3. Determination of fluorescence quantum yield:

Here, the quantum yield φ was measured by using the following equation,

$\varphi_x = \varphi_s (F_x / F_s)(A_s / A_x)(n_x^2 / n_s^2)$

Where,

X & S indicate the unknown and standard solution respectively, φ = quantum yield,

F = area under the emission curve, A = absorbance at the excitation wave length,

n = index of refraction of the solvent. Here φ measurements were performed using anthracene in ethanol as standard [φ = 0.27] (error ~ 10%)

4. Calculation of the detection limit:

The detection limit DL of **TAQ** for Zn^{2+} was determined from the following equation[1]:

DL = K* Sb1/S

Where K = 2 or 3 (we take 3 in this case); Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve.

From the graph Fig.S2, we get slope = 4E+07, and Sb1 value is 42.67365.

Thus using the formula we get the Detection Limit for Zn^{2+} = 3.2 µM.

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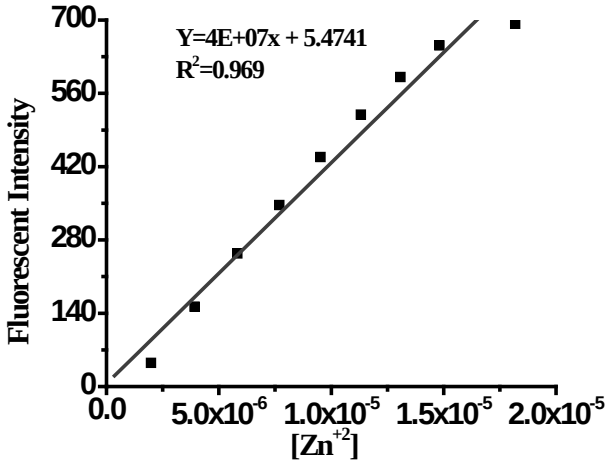
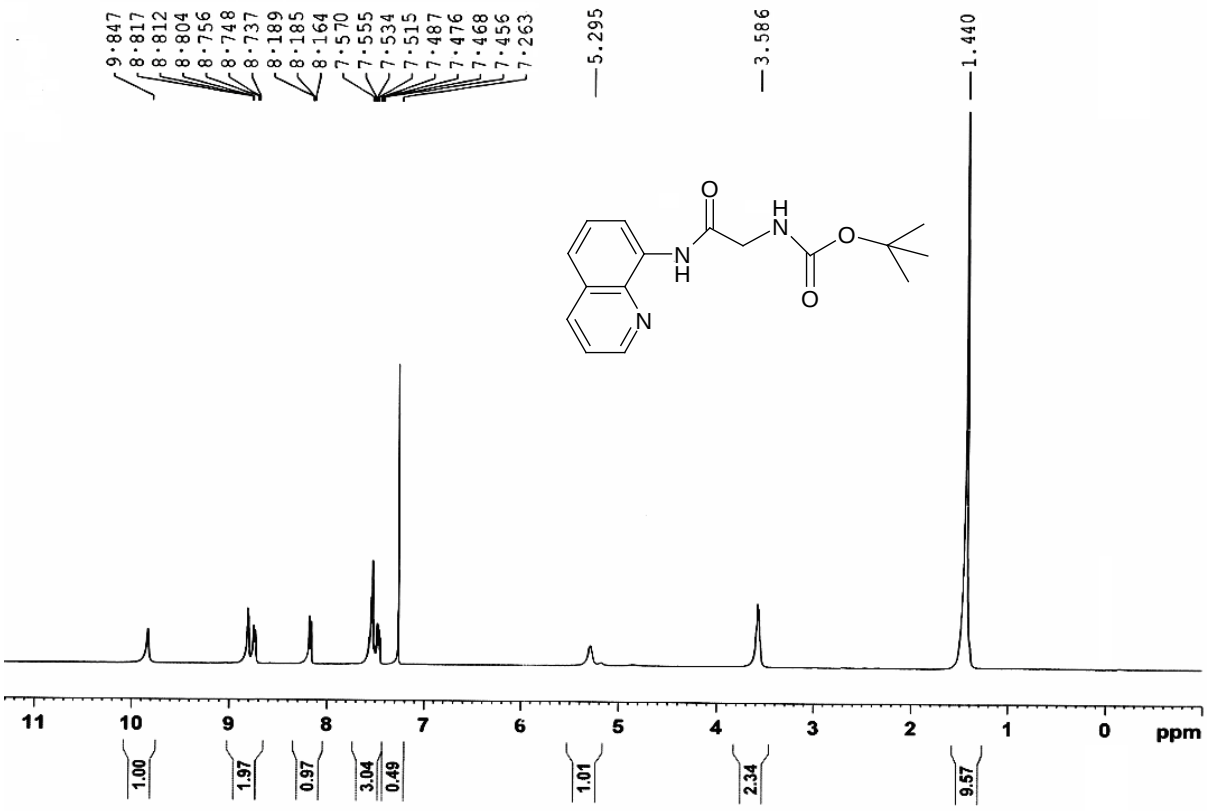
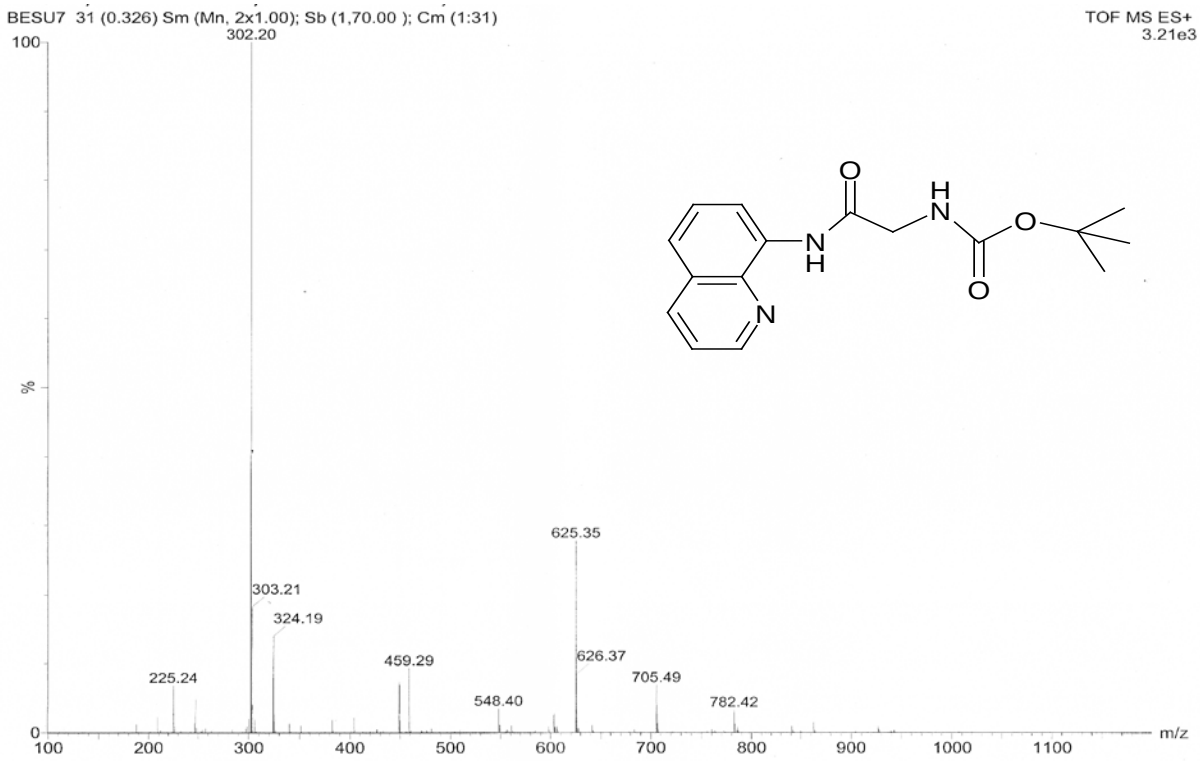


Fig. S2: Changes of Fluorescence Intensity of **TAQ** as a function of $[Zn^{2+}]$ at 506 nm.

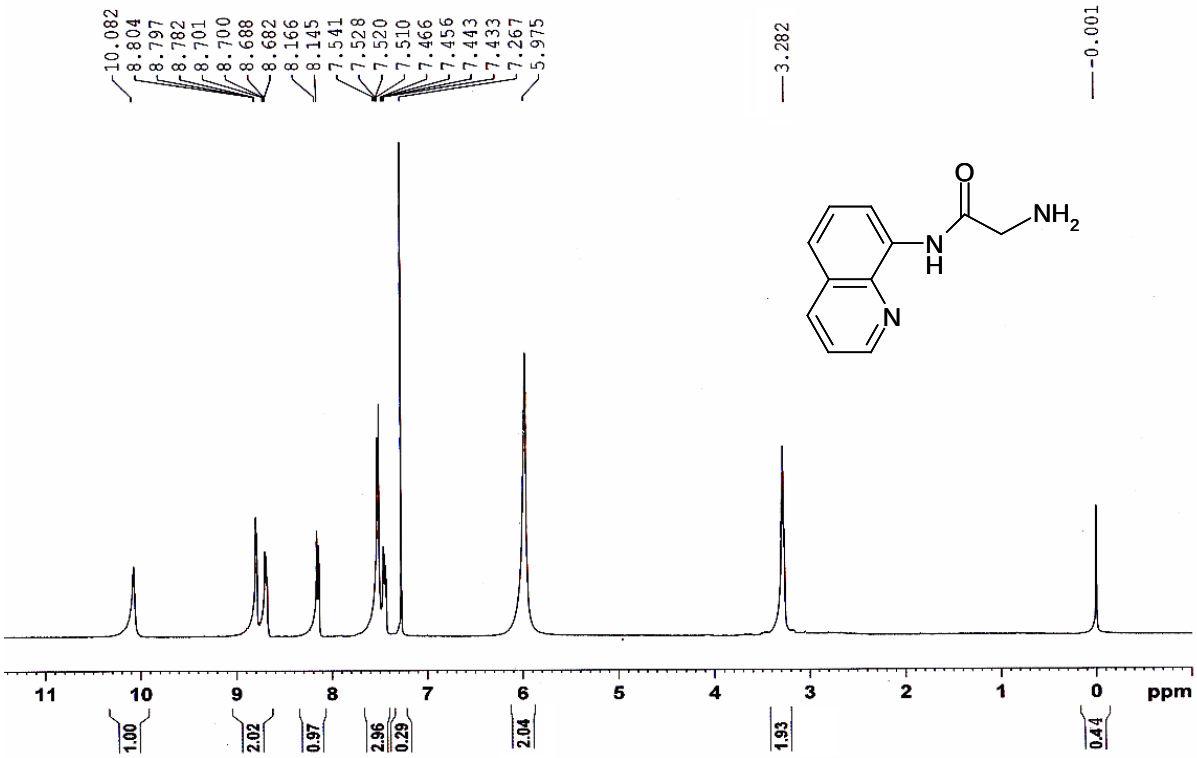
5. 1H NMR spectrum (S3) of Compound 1:



6. Mass spectrum (S4) of Compound 1:



7. ^{1}H NMR spectrum (S5) of Compound 2:



8. Mass spectrum (S6) of Compound 2:

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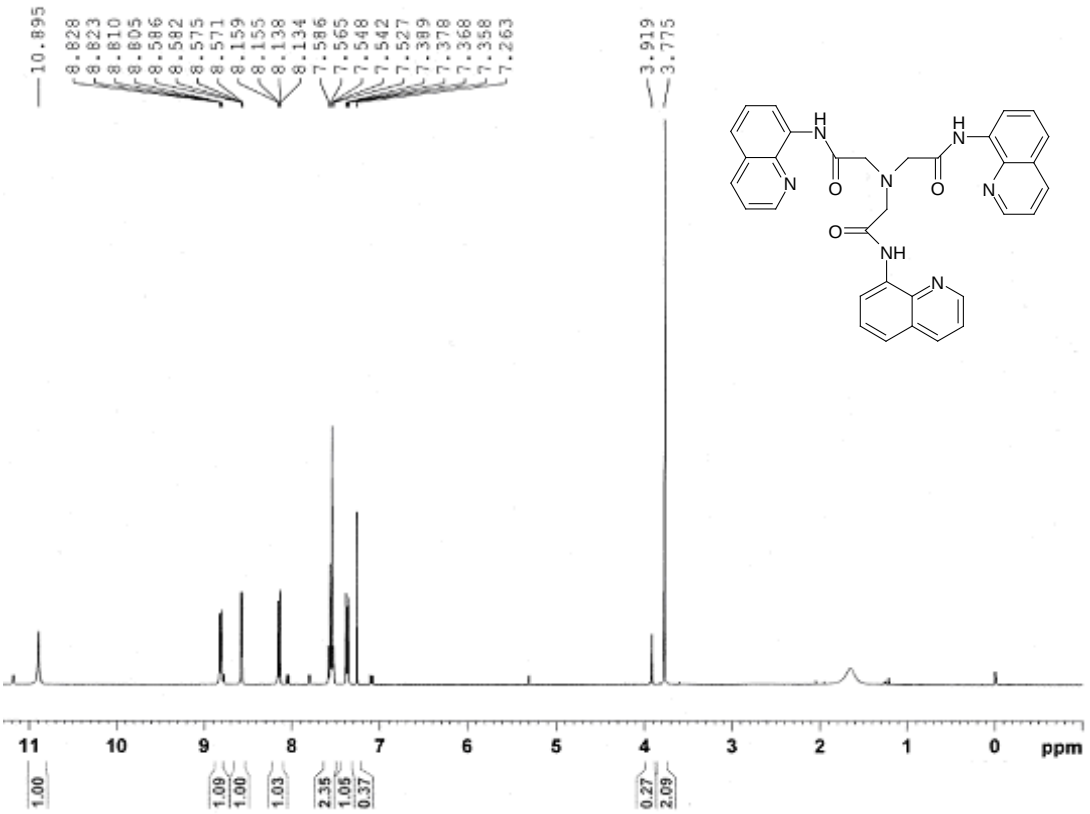
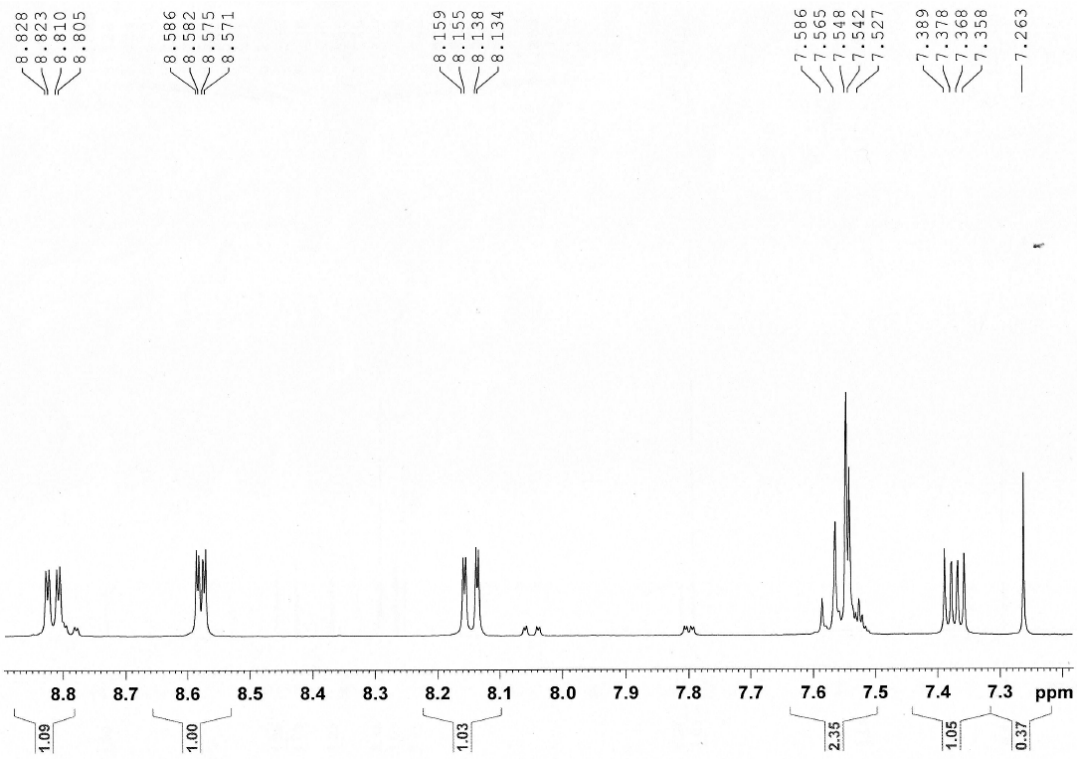
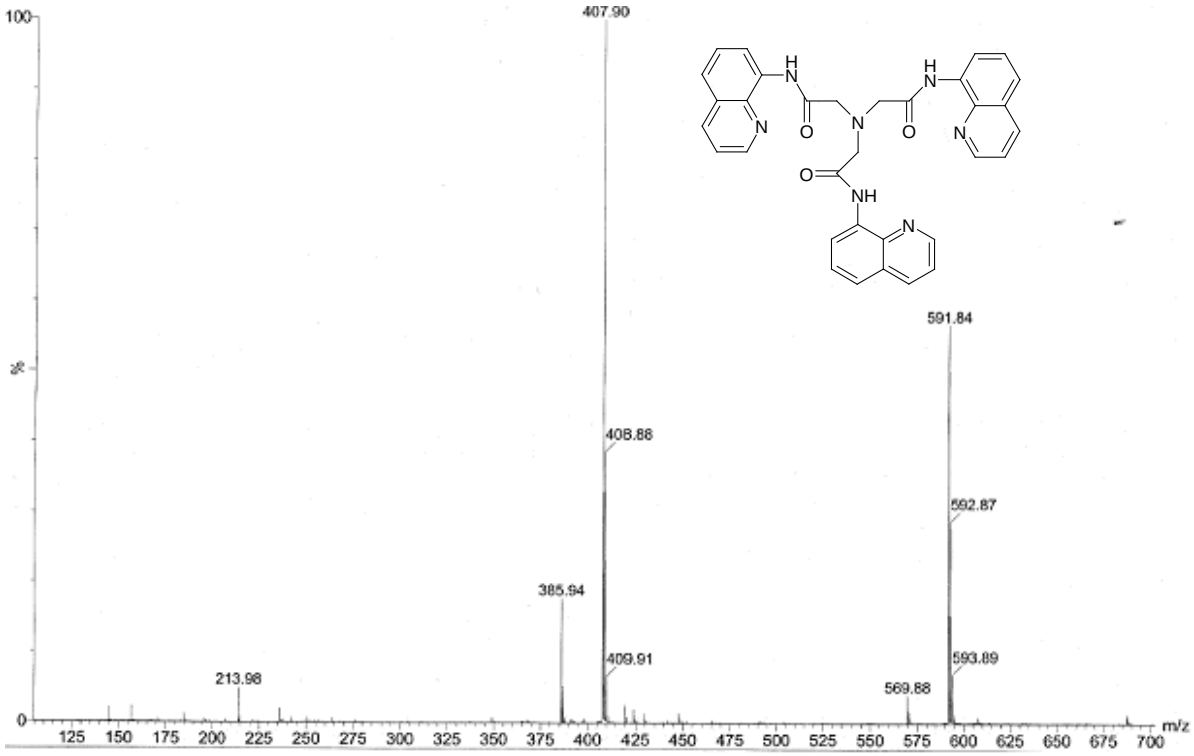
9. ^{1}H NMR spectrum (S7) of Compound TAQ:



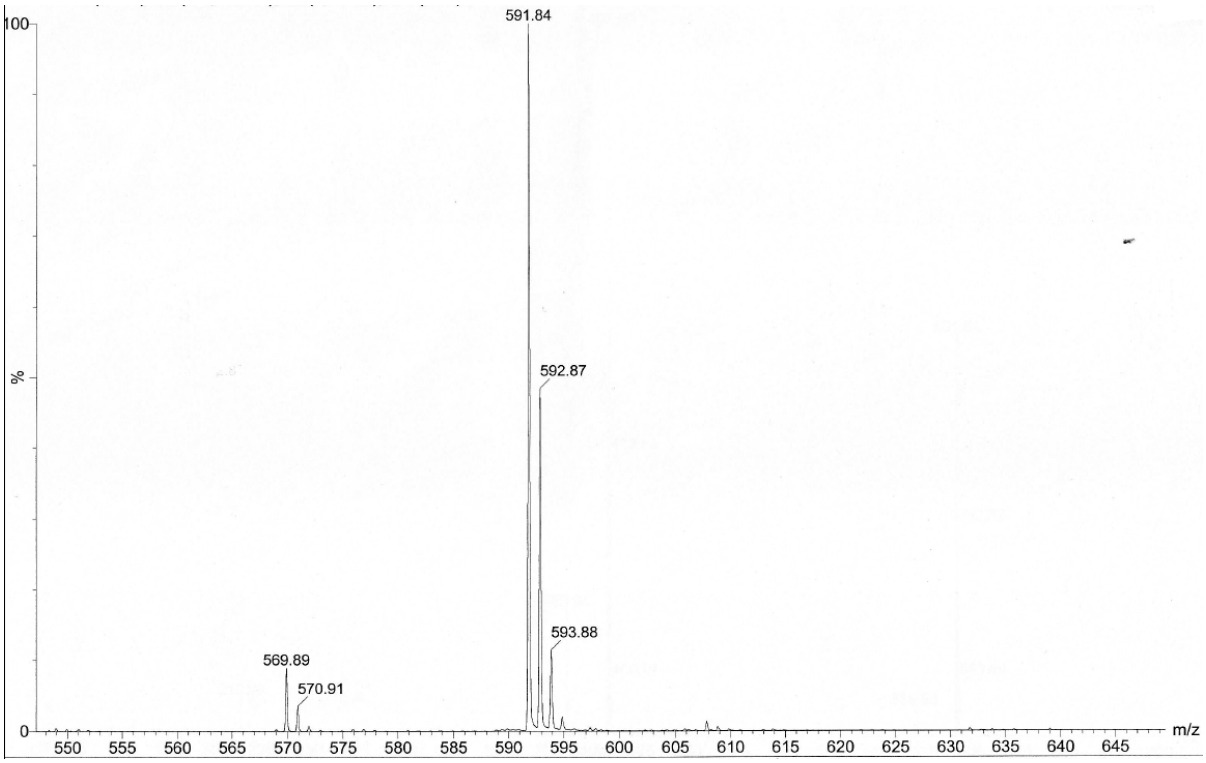
10. ^{1}H NMR spectrum (S8) of Compound TAQ (expansion mode):



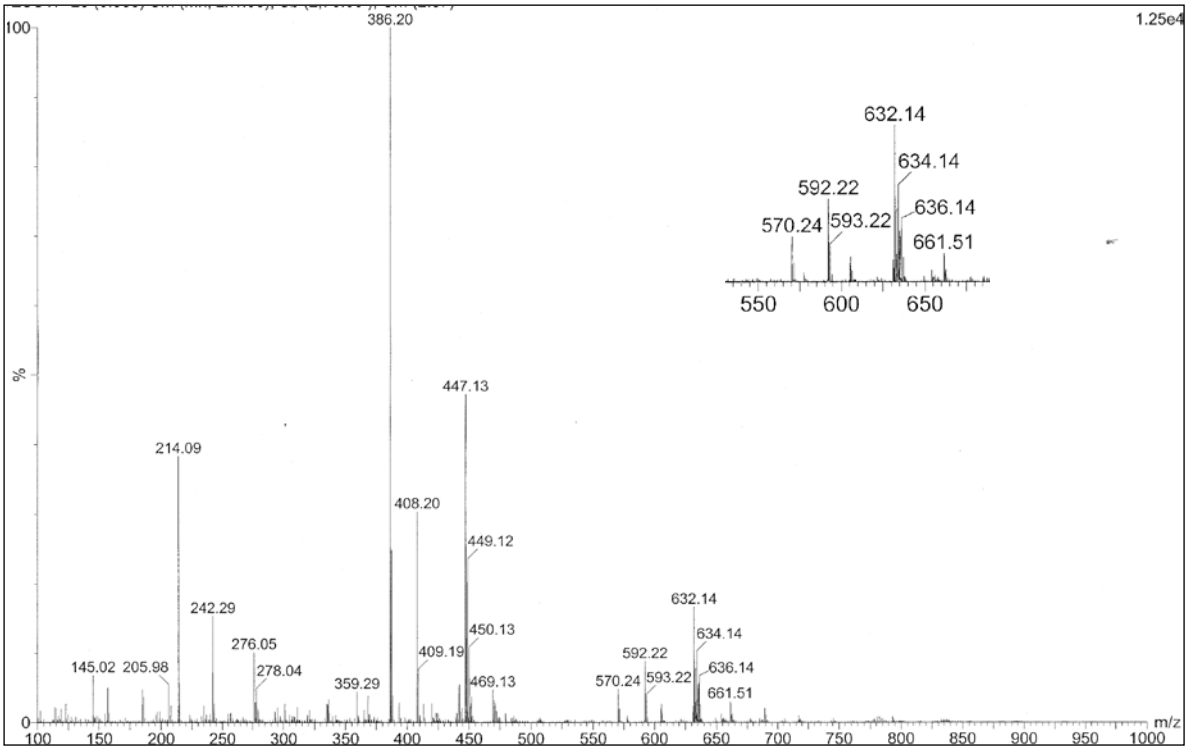
11. Mass spectrum (S9) of Sensor TAQ:



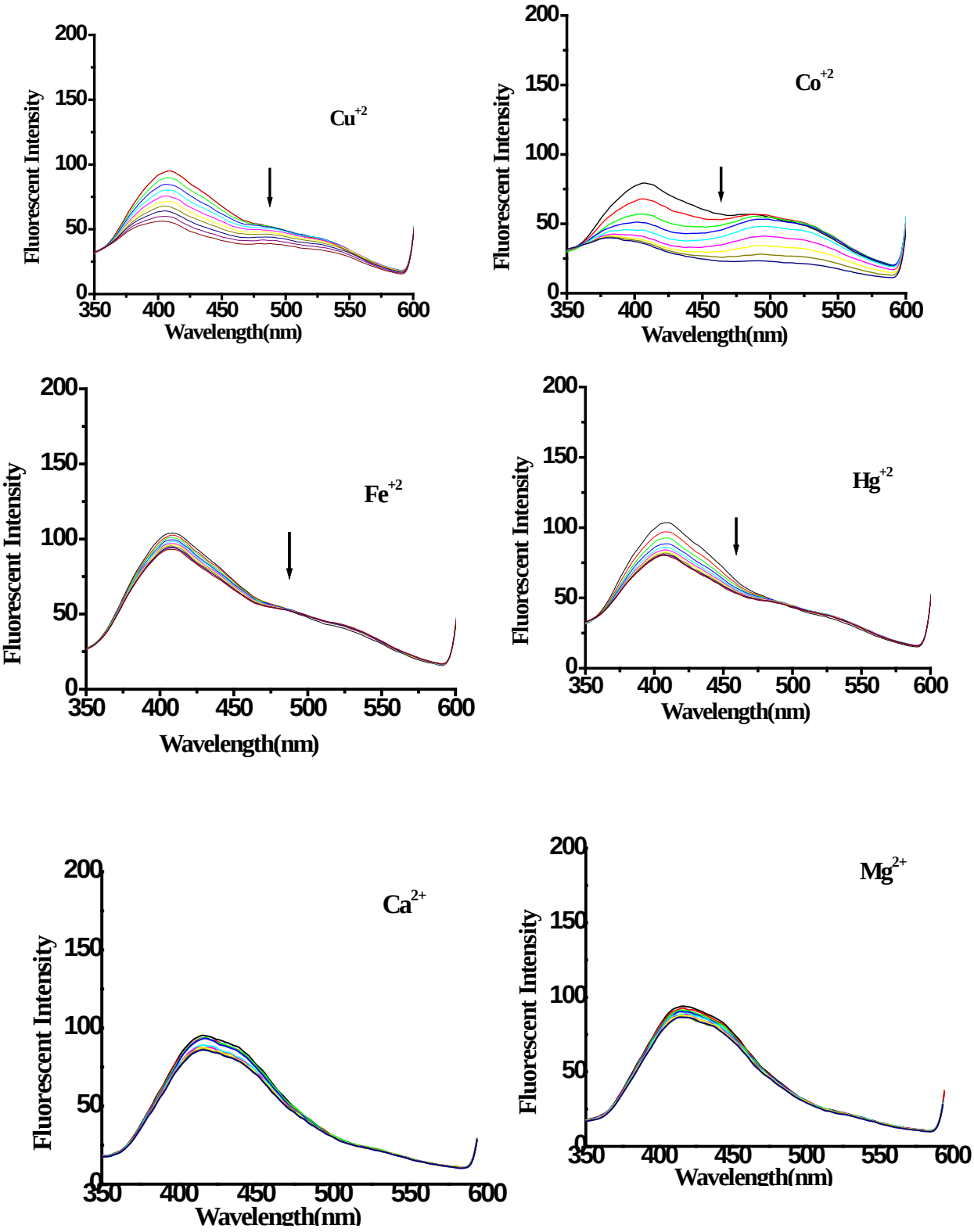
12. Mass spectrum (S10) of Sensor TAQ (Expansion Mode):



13. Mass spectrum (S11) of Zn^{2+}–complex:



14. Fluorescence titration spectra of receptor (Fig.S12) ($1x10^{-5}$ M) with different guest cations($2x10^{-4}$M) in aqueous HEPES buffer solution at pH-7.4:



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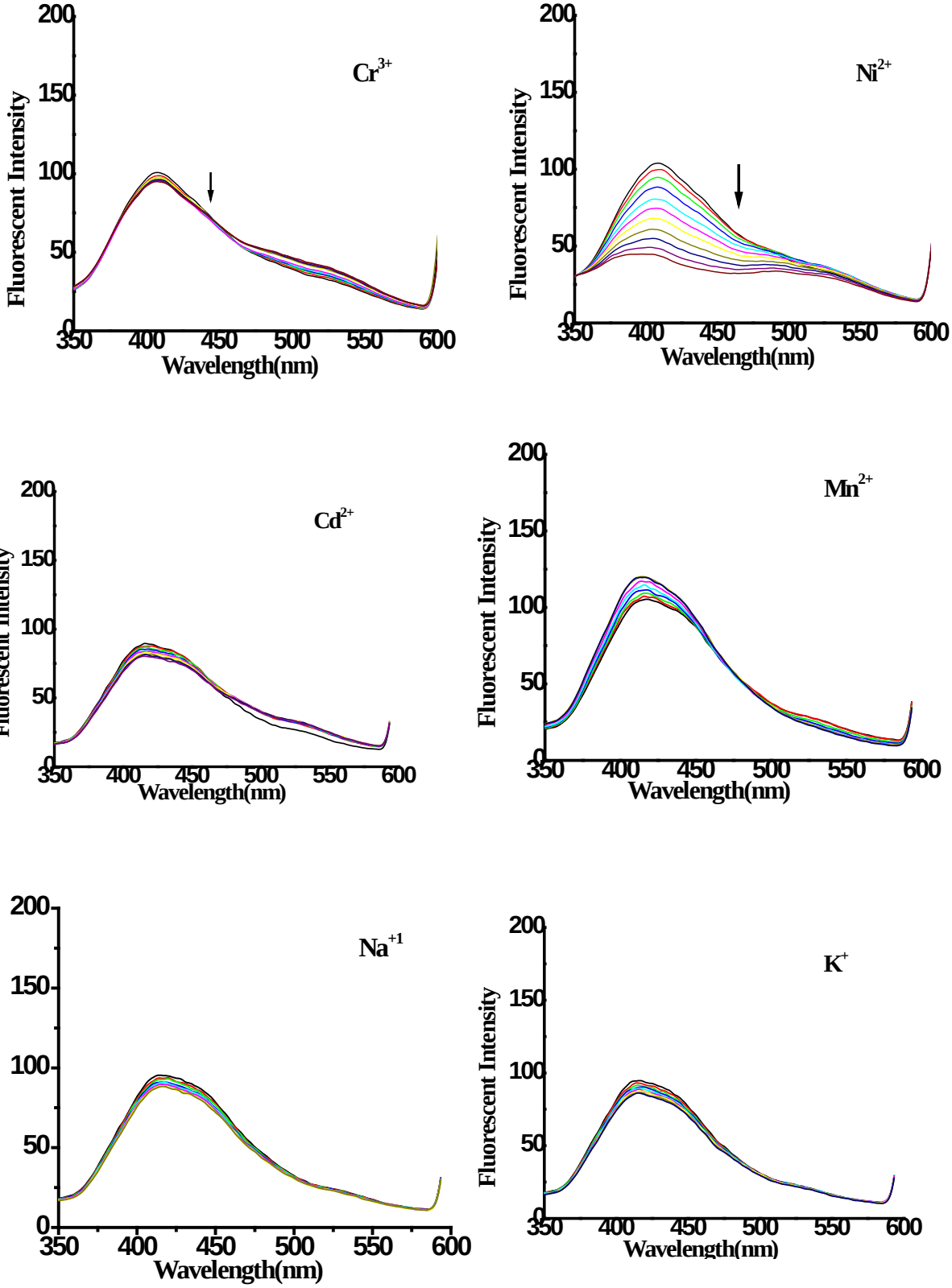

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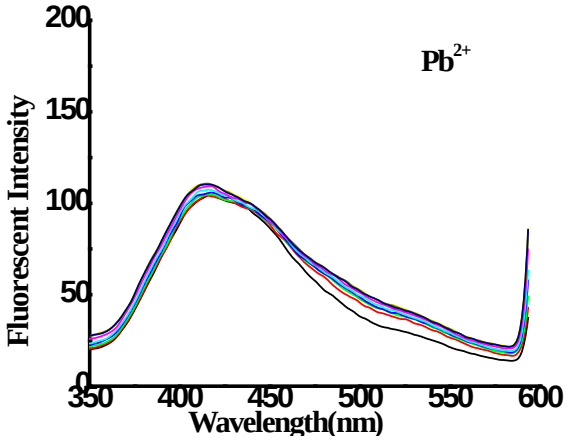
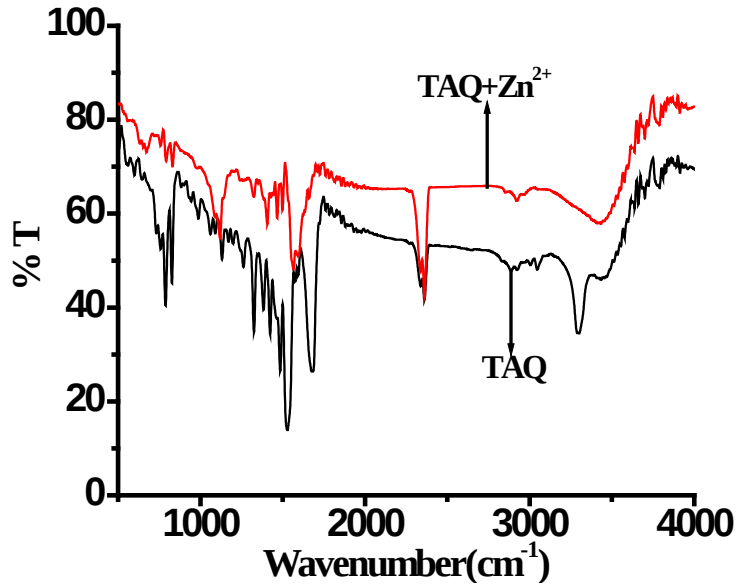


15. **IR spectral analysis (Fig.S13) of Zn^{2+} complex with TAQ :**



1. Zhu, M.; Yuan, M.; Liu, X.; Xu, J.; Lv, J.; Huang, C.; Liu, H.; Li, Y.; Wang, S.; Zhu, D. *Org. Lett.* 2008**, 10**, 1481-1484.