Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

for

A Highly Selective NIR-Emitting Zinc Sensor by Using Schiff Base Binding to Turn-On Excited-State Intramolecular Proton Transfer

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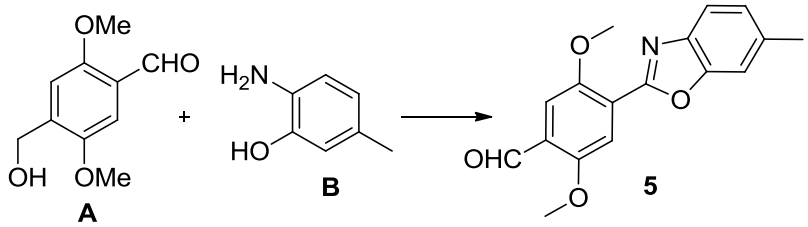
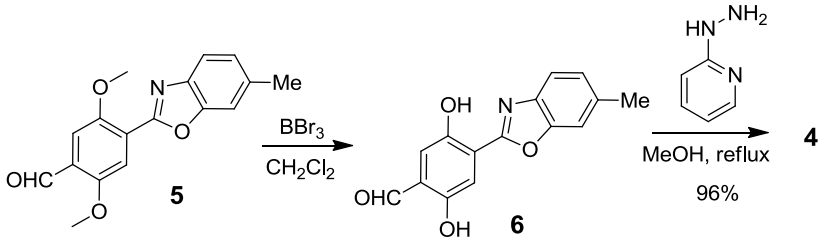
Synthesis of 5



Scheme S1. Synthesis of intermediate **5**

Intermediates **A**[1] (600 mg, 3.06 mmol) and **B** (394 mg, 3.2 mmol) were dissolved in EtOH (50 mL), and the mixture was refluxed overnight. The crude product mixture was concentrated under vacuum and dried in a vacuum oven overnight. The resulting mixture was then re-dissolved in 80 mL anhydrous DCM (Solution I). PCC (1.5 g) and silica gel (4.5 g) were mixed in 80 mL DCM and stirred at room temperature for one hour (Solution II). The PCC solution II was added to the solution I and the mixture was stirred overnight and then filtered through a short pad of silica and washed by EtOAc. The organic phase was collected, concentrated in vacuum, and purified on a silica gel column to give the desirable product **5** in 53% yield as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): 10.46 (1H, s), 7.74 (1H, s), 7.67 (1H, d, J = 8.4 Hz), 7.47 (1H, s), 7.38 (1H, s), 7.15 (1H, d, J = 8.4 Hz), 3.98 (6H, s), 2.48 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$): 189.0, 160.0, 155.7, 152.5, 151.0, 139.9, 136.4, 126.8, 126.2, 122.6, 119.9, 114.5, 111.1, 110.9, 56.9, 56.5, 22.0.



Scheme S2. Synthesis of sensor **4**

Synthesis of 6

Compound **5** (445 mg, 1.5 mmol) was dissolved in 4.0 mL anhydrous DCM, and the solution was cooled to -78℃. Then BBr_3 (495 mg, 2 mmol) was added slowly, and the resulting mixture was warmed slowly to room temperature and stirred overnight. Then the reaction mixture was quenched by addition of water, and the DCM layer was separated on a silica gel column to give compound **6** in 88% yield as yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): 11.02 (1H, s), 11.35 (1H, s), 9.92 (1H, s), 7.65 (1H, d, *J* = 7.8 Hz), 7.65 (1H, s), 7.46 (1H, s), 7.31 (1H, s), 7.25 (1H, d, *J* = 7.8 Hz), 2.54 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$): 196.1, 160.7, 153.3, 150.9, 149.7, 137.7, 137.4, 126.8, 123.1, 120.6, 119.2, 117.9, 114.9, 111.1, 21.9.

Synthesis of sensor 4

Compound **6** (200 mg, 0.74 mmol) and 2-hydrazinylpyridine (109 mg, 1.0 mmol) were refluxed in 8.0 mL MeOH for 2 hours, and **4** was precipitated out as a yellow solid. The resulting mixture was cooled down to 0°C and compound **4** was collected by simple filtration in 96% yield. Melting point 305-306 °C. ^{1}H NMR (300 MHz, $CDCl_3$): 11.09 (1H, s), 10.56 (1H, s), 10.01 (1H, s), 8.27 (1H, s), 8.12 (1H, d, *J* = 4.2 Hz), 7.66 (3H, m), 7.44 (1H, s), 7.38 (1H, s), 7.24 (1H, d, *J* = 8.1 Hz), 7.18 (1H, d, *J* = 8.4 Hz), 6.79 (1H, dd, *J* = 5.4 Hz, *J* = 6.6 Hz), 2.46 (3H, s). ^{13}C NMR (75 MHz, $CDCl_3$): 161.9, 156.9, 150.9, 149.5, 148.9, 148.3, 138.5, 137.9, 136.5, 135.7, 127.4, 126.9, 118.9, 115.9, 113.3, 113.3, 111.4, 110.7, 107.0, 21.7. HRMS (m/z): calcd for $C_{20}H_{16}N_4O_3$, [**4** - e]$^+$, 360.1222, found, 360.1317; HRMS (m/z): calcd for $C_{20}H_{14}N_4O_3Zn$, [**4**+**Zn**$^{2+}$-**2H**$^+$-e]$^+$, 422.0357, found, 422.0496.

Cell Culture

Human hepatocellular carcinoma cells (HepG2) were cultured in RPMI 1640 medium supplemented with 10% FBS, 100 μg/ml penicillin and 100 μg/ml streptomycin. Human umbilical vein endothelial cells (HUVECs) were cultured in endothelial basal medium-2 supplement with single quots and 10% fetal bovine serum (FBS). Cells were incubated at 37°C under 5% CO_2. For the control group, cells were treated with 10 μM dye **4** in PBS buffer for 30 minutes, then the cells were washed with PBS for 3 times and fixed by 4% paraformaldehyde for imaging. For the zinc sensing, cells were first incubated with 30 μM Zn^{2+} in cell culture medium, then the cells were washed 3 times with PBS, and the cells were further incubated with 10 μM dye **4** in PBS for another 30 mins. Then the cells were washed with PBS 3 times and fixed by 4% paraformaldehyde for imaging. Fluorescence images of Zn^{2+} detection was carried out on an Olympus IX81 Fluorescent Microscopy. The samples was excited by using light that passes through an exciter filter BP460-495, and the images were collected in the bright field and green channel with a barrier filter BA510-550 respectively.

Spectroscopic Measurements. NMR spectra were collected on a Varian 300 Gemini spectrometer. Mass spectrometric data were obtained on a HP1100LC/MSD mass spectrometry. HRMS data were performed on an ESI-TOF MS system(Waters, Milford, MA). UV-Vis spectra were acquired on a Hewlett-Packard 8453 diode-array spectrometer. Fluorescence spectra were obtained on a HORIBA Jobin Yvon NanoLog spectrometer.

Reference

1. D. T. Witiak, J. T. Loper, S. Ananthan, A. M. Almerico, V. L. Verhoef and J. A. Filppi, *J. Med. Chem.* 1989, **32**, 1636-1642.

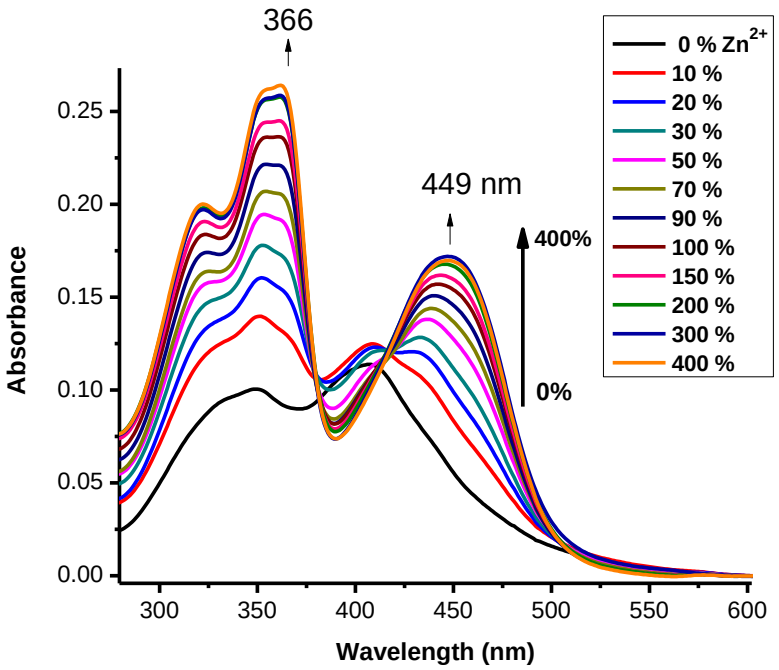


Fig. S1 Absorbance spectra of **4** (10 µM) in EtOH:H_2O = 1:1 upon addition of increasing concentrations of Zn^{2+} cation.

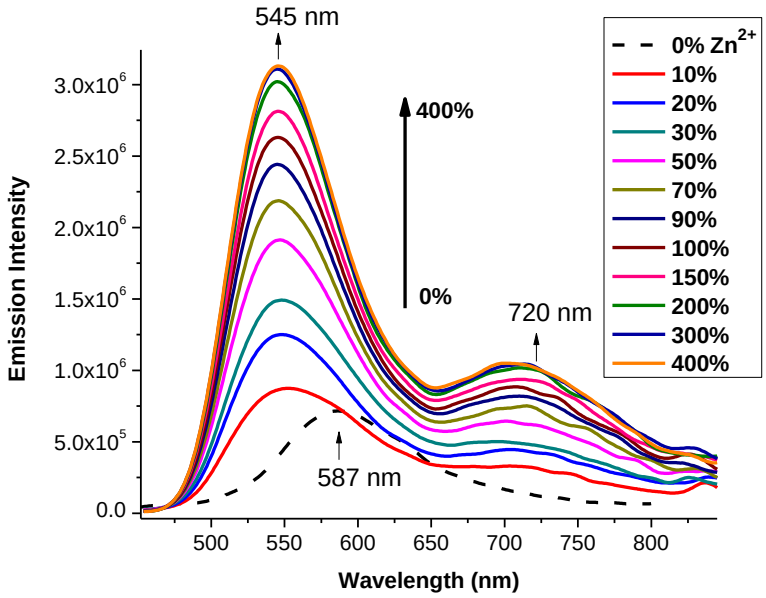


Fig. S2 Fluorescence spectra of **4** (10 µM) in EtOH:H2O = 1:1 upon addition of different concentrations of Zn^{2+} cation.

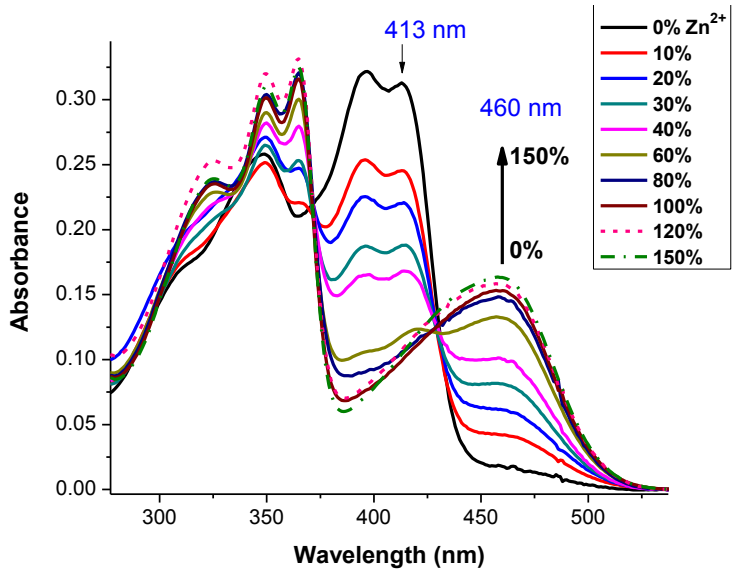


Fig. **S3**. Absorbance spectra of **4** (10 μM) in MeOH upon addition of increasing concentrations of Zn^{2+}.

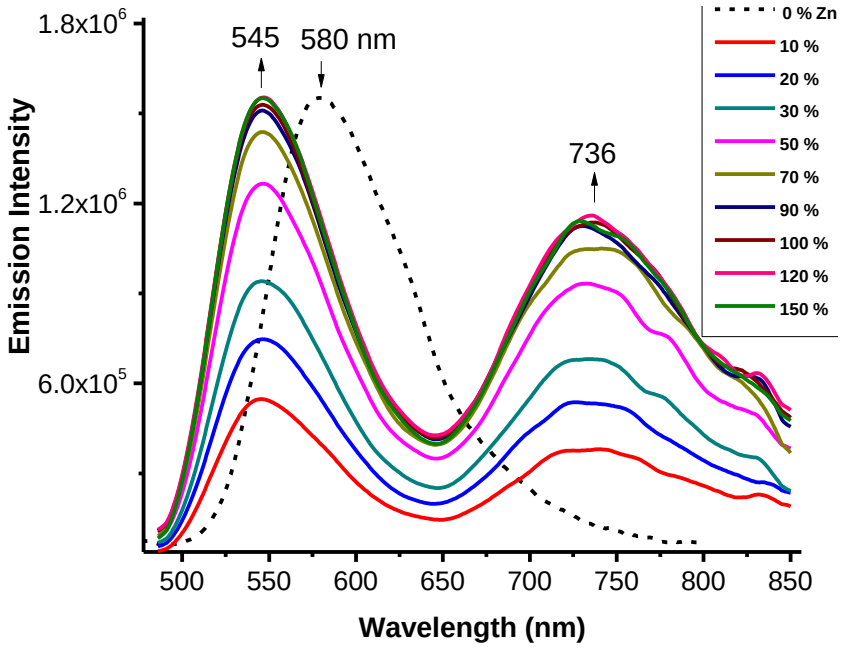


Fig. S4 Normalized fluorescence spectra of ligand **4** (10 μM) in MeOH upon addition of Zn^{2+} when excited at 460 nm. The signal intensity of the ligand was increased to a comparable level with the curve of its zinc complex **4**-Zn for comparison.

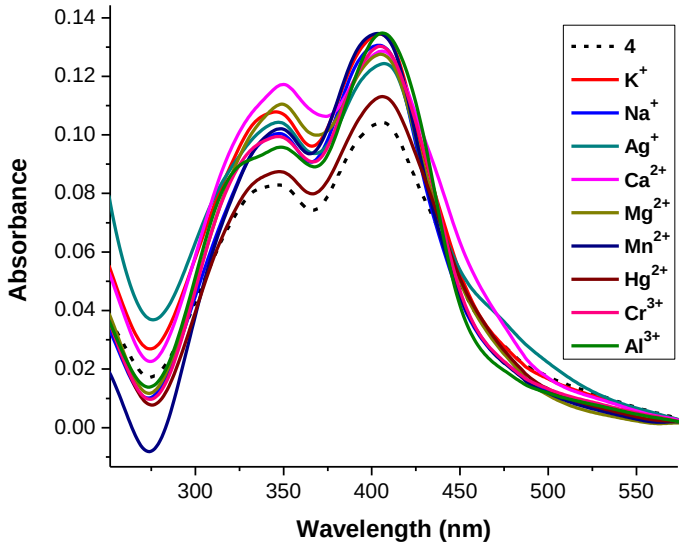


Fig. S5 Absorbance spectra of **4** (10 μM) in EtOH : H_2O = 1:1 upon addition of 5.0 equiv. of different ions.

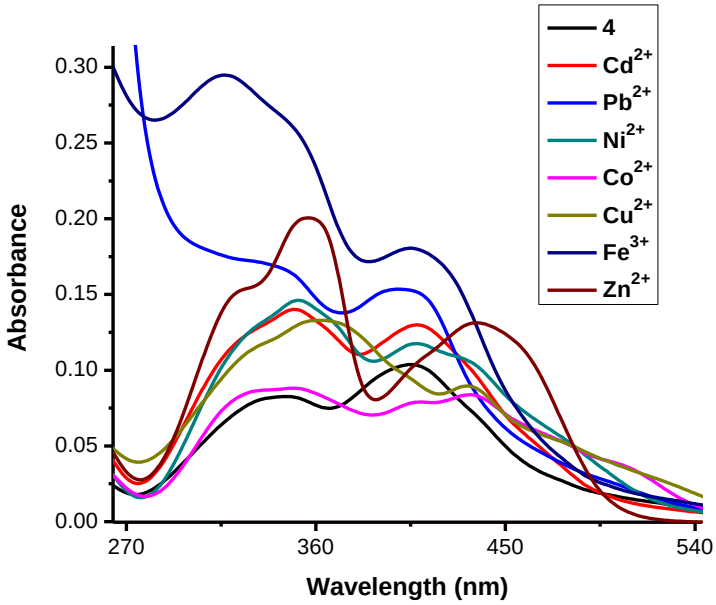


Fig. S6 Absorbance spectra of **4** (10 μM) in EtOH : H_2O = 1:1 upon addition of 5.0 equiv. of different ions.

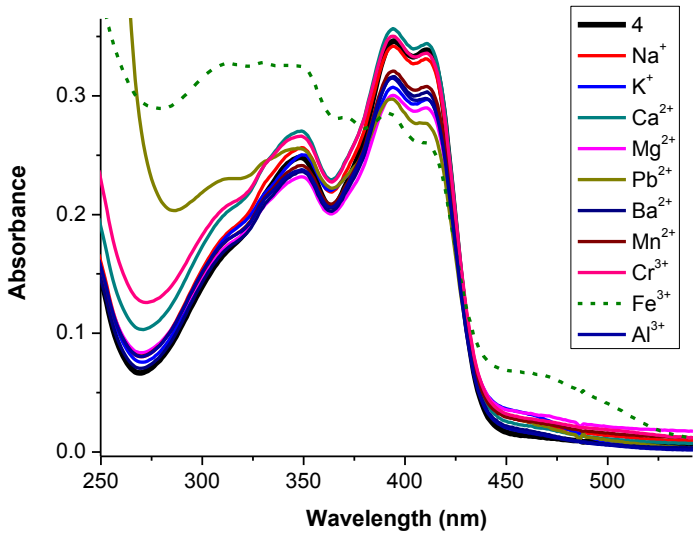


Fig. S7 Absorbance spectra of **4** (10 μM) in EtOH : HEPES(10 mM) = 1:1 upon addition of 5.0 equiv. of different ions.

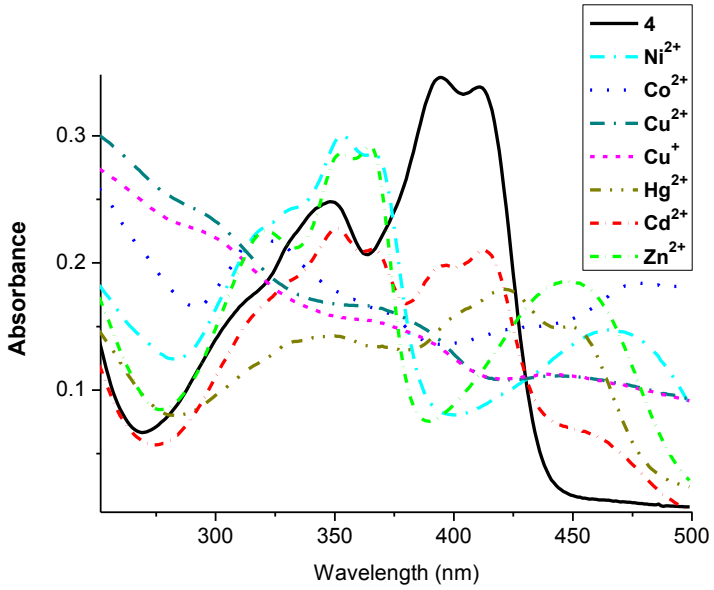


Fig. S8 Absorbance spectra of **4** (10 μM) in EtOH : HEPES(10 mM) = 1:1 upon addition of 5.0 equiv. of different ions.

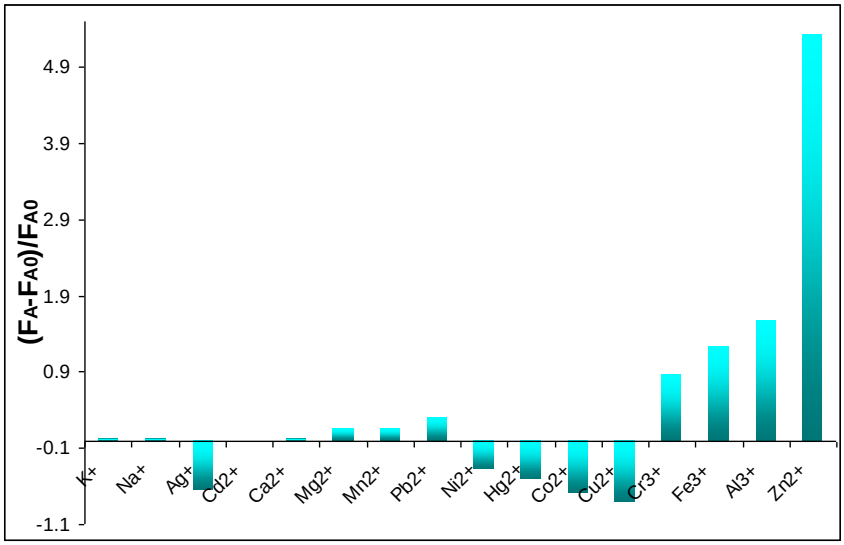


Fig. S9 Variation of the fluorescence of **4** in the presence of 5.0 equiv. of various cations in EtOH:H_2O = 1:1 (**F_A**: Fluorescence area of **4** with 5 equiv. of various cations**; F_{A0}**: Fluorescence area of **4**).

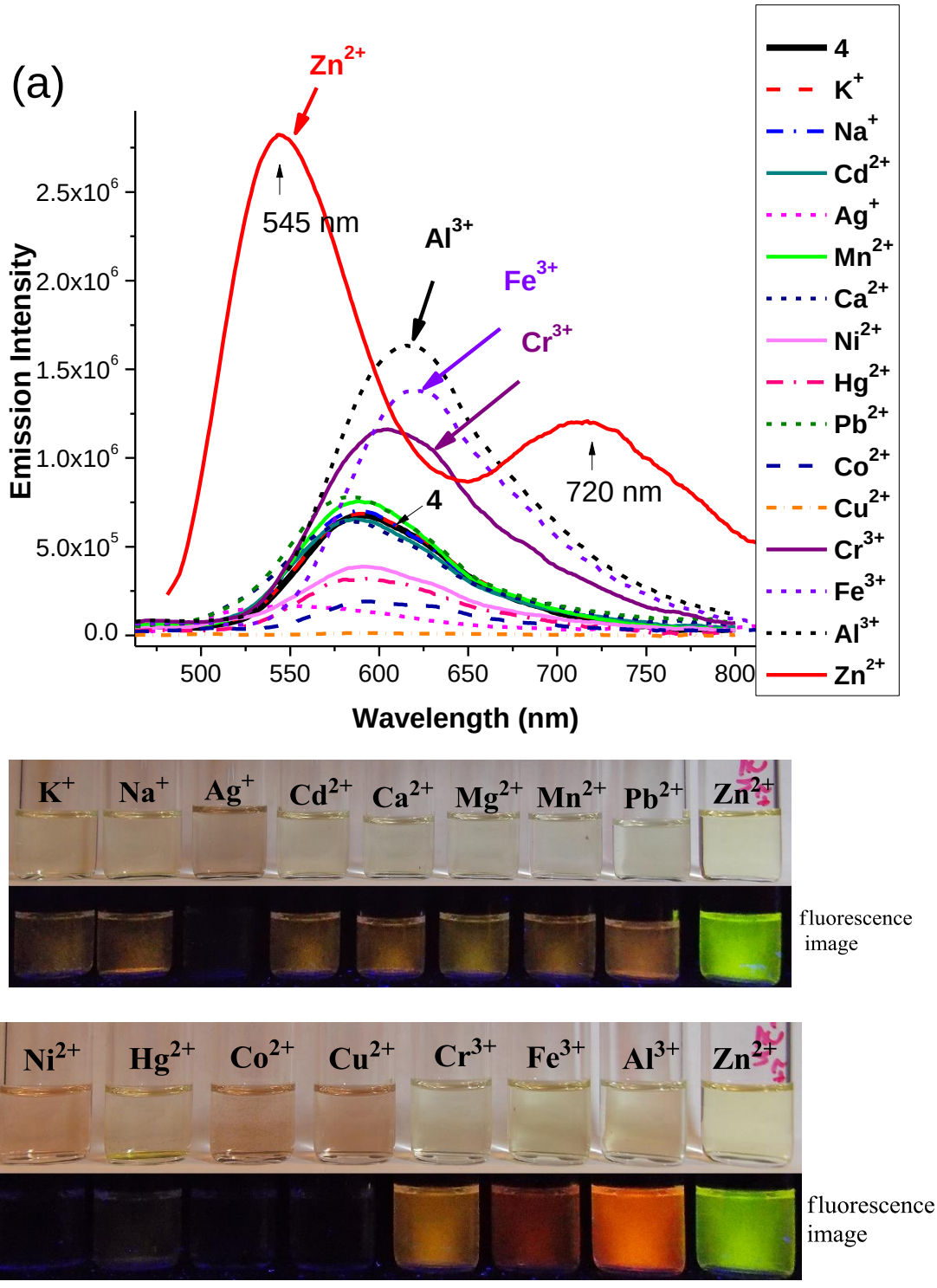


Fig. S10 Fluorescence spectra of dye **4** (10 μM) in the presence of 5.0 equiv. of different metal ions in EtOH:H_2O = 1:1 and their respective fluorescence images under UV-lamp irradiation (365 nm).

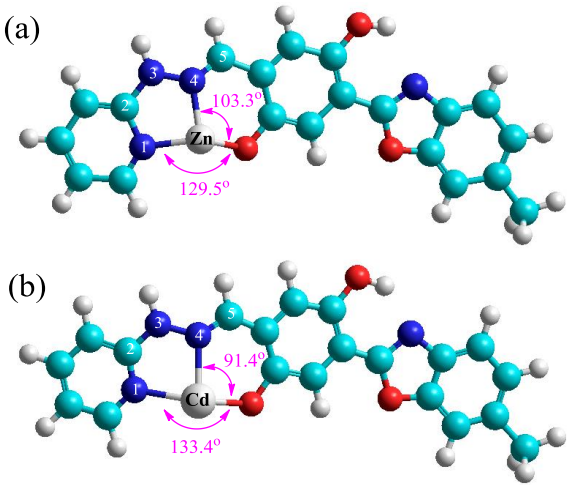


Figure S11. Molecular modeling of complexes **4-Zn** (a) and **4-Cd** (b) by using HyperChem (optimized with AM1 setting). The nitrogen, carbon and oxygen atoms are in blue, cyan and red colors, respectively.

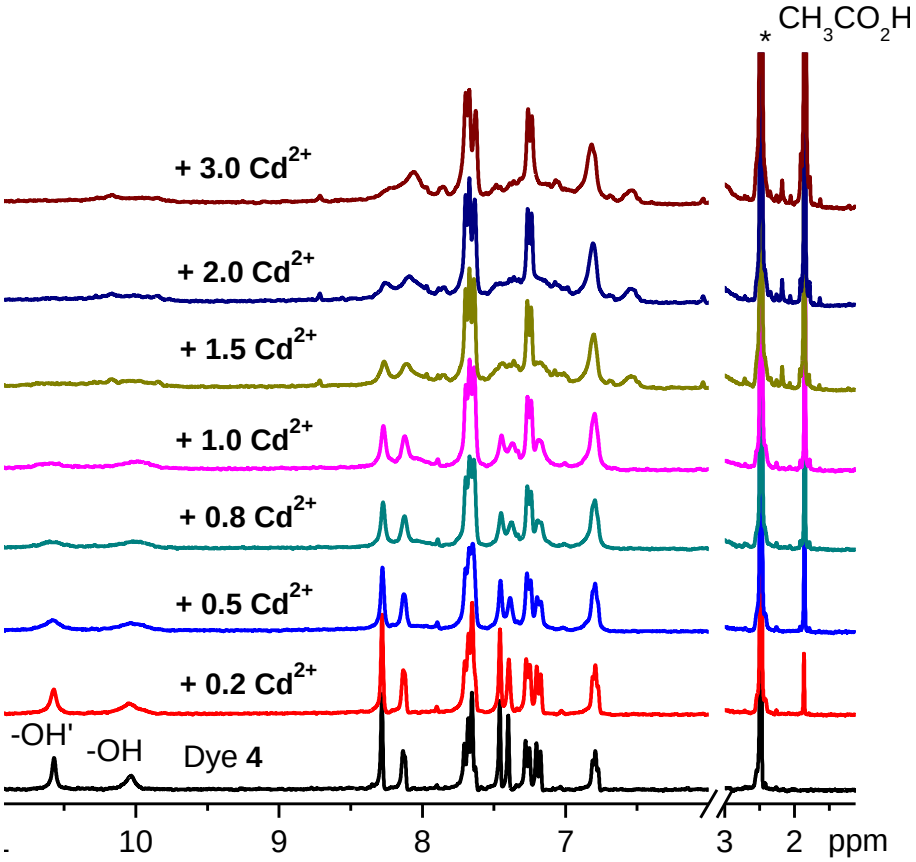


Figure S12. ^{1}H NMR titration of **4** with $Cd(OAc)_2.2H_2O$ in DMSO-d_6. The starred signal at ~2.50 ppm was attributed to the solvent DMSO (the water signal at ~3.3 ppm was not shown). The signal of acetic acid at 1.85 ppm confirmed the presence of Cd^{2+} cation.

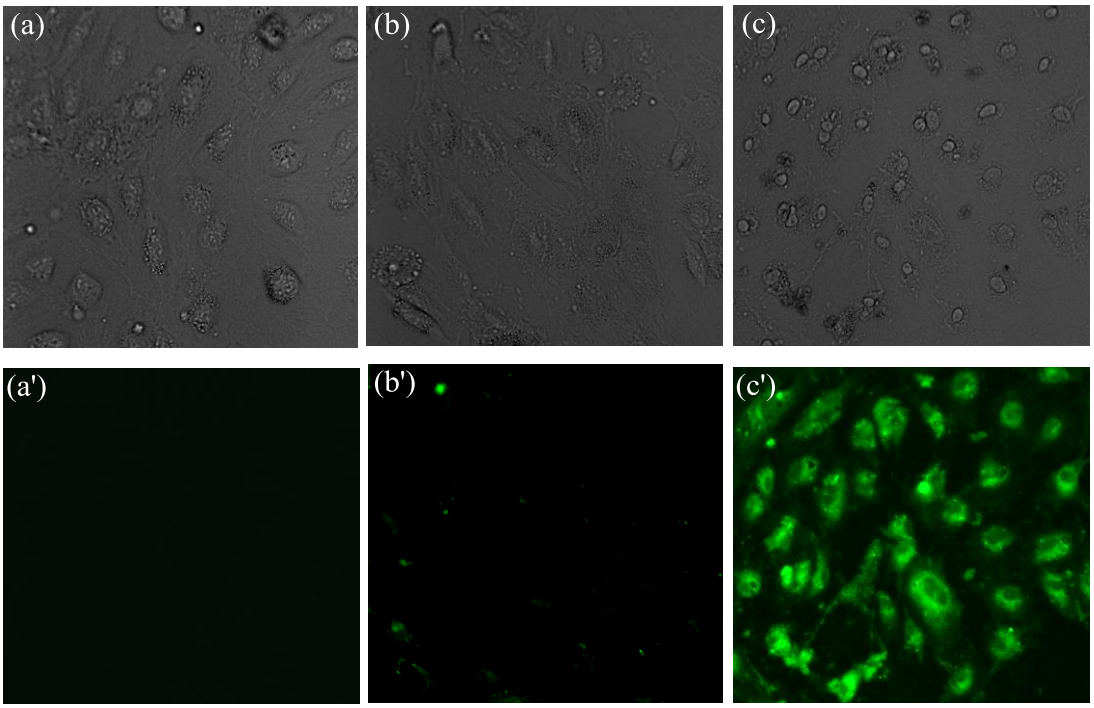


Figure S13. Fluorescence images of Zn^{2+} detection in live Human umbilical vein endothelial cells: 1. cells without any treatment (a); 2. cells incubated with 10 μM dye 4 for 30 mins (b); 3. cells exposed to 30 μM Zn^{2+} for 30 mins, washed with PBS 3 times and then incubated with 10 μM dye 4 in PBS for another 30 mins (c). The images (a), (b), and (c) were collected at bright field, while the images (a'), (b'), and (c') were collected on a fluorescence microscope (with excitation light passing through a blue filter).

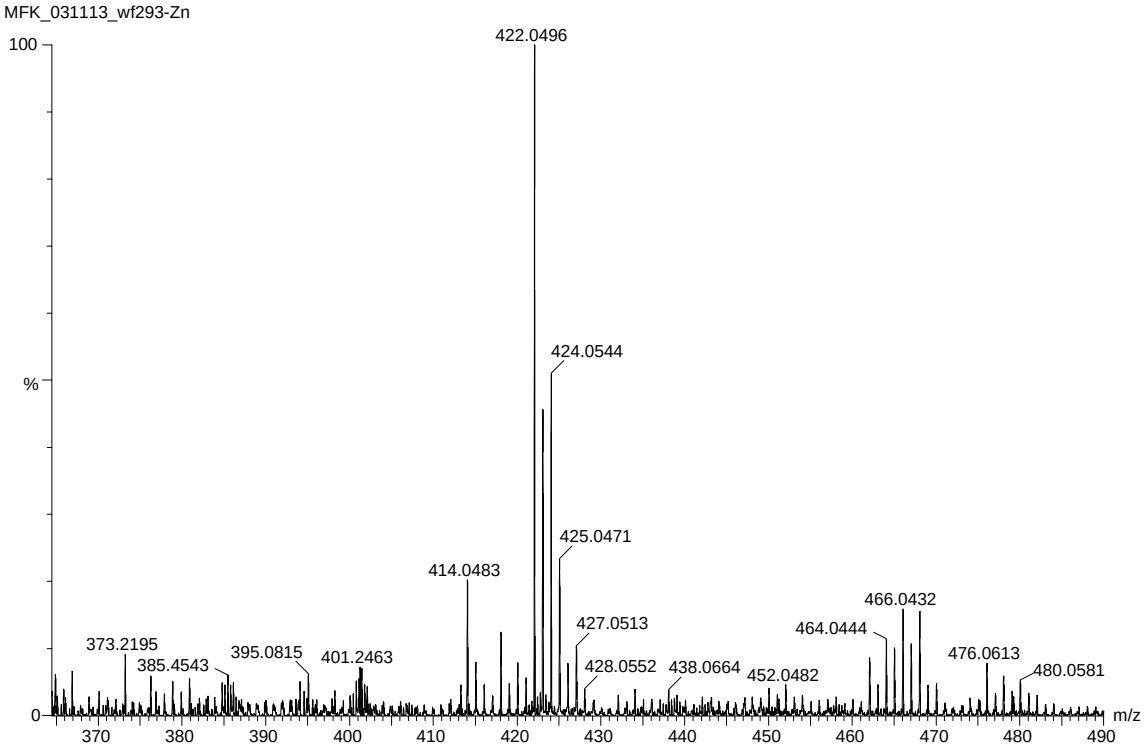


Figure S14. HRMS spectrum of **4-Zn**; calcd for [**4**+Zn^{2+}-$2H^{+}$-e]$^{+}$ $C_{20}H_{14}N_4O_3Zn$: 422.0357, found 422.0496.

Determination of Binding Constant

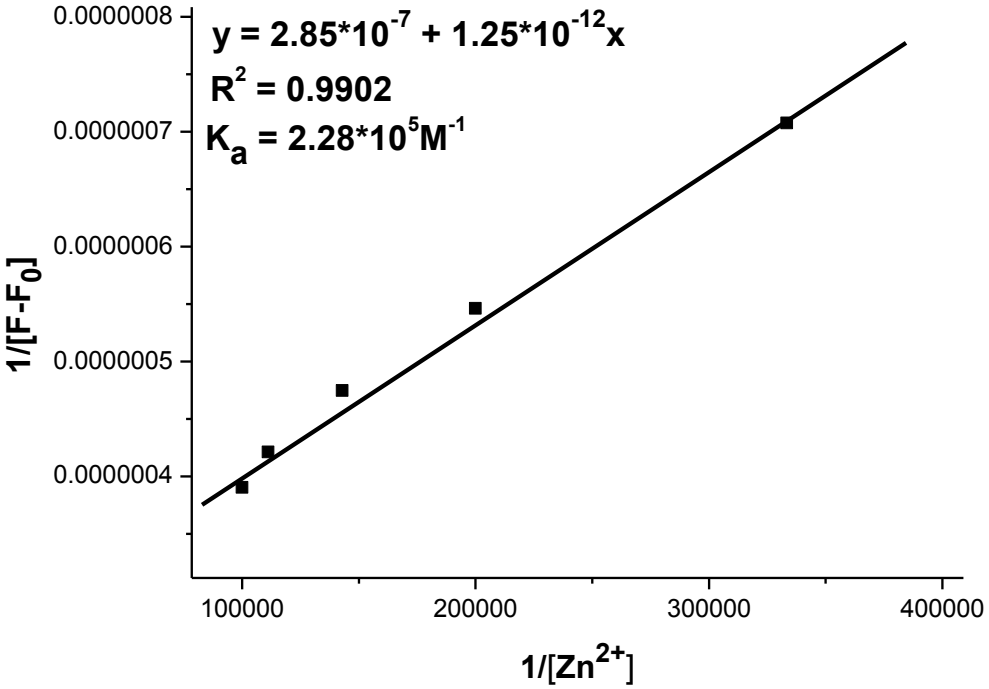
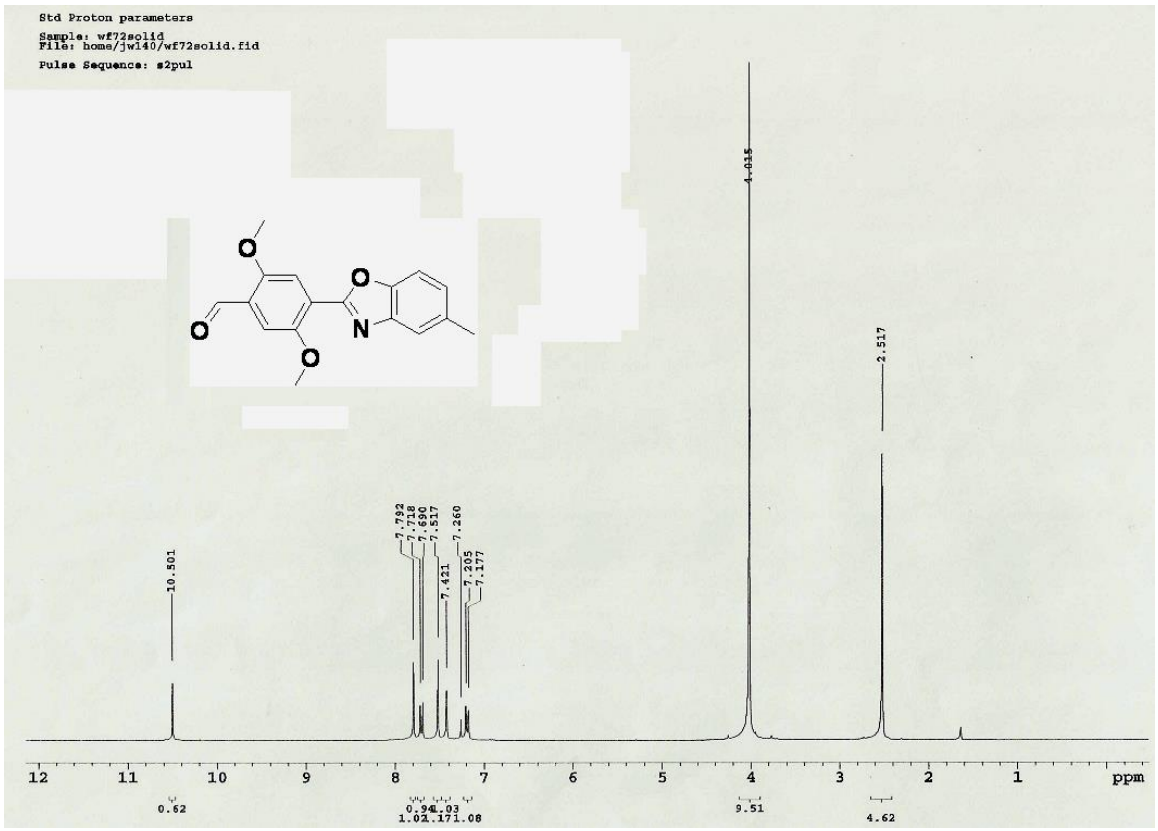


Fig. S15. Benesi–Hildebrand plot of Dye **4** with Zn^{2+} in EtOH:H2O (v/v 1:1). The excitation wavelength was 435 nm and the observed wavelength was 545 nm. The binding constant was 2.28×10^{-5} M^{-1} for Zn^{2+} binding.

The fluorescence intensity at 545 nm was plotted against the molar fraction of dye **4** under a constant total concentration (10 μM). The association constant (Ka) of dye **4**–Zn^{2+} complexes was determined by the consequent equation (1):

$$1/(I - I_0) = 1/\{\mathrm{Ka} \times (I_{max} - I_0) \times [Zn^{2+}]\} + 1/(I_{max} - I_0)$$

The association constant Ka was evaluated graphically by plotting $1/(I - I_0)$ against $1/[Zn^{2+}]$. The data were linearly fit and the Ka value was obtained from the slope and intercept of the line. The association constant (Ka) of Zn^{2+} binding with dye **4** was found to be 2.28×10^{5} M^{-1}.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7.205
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11
10
9
8
7
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1.08
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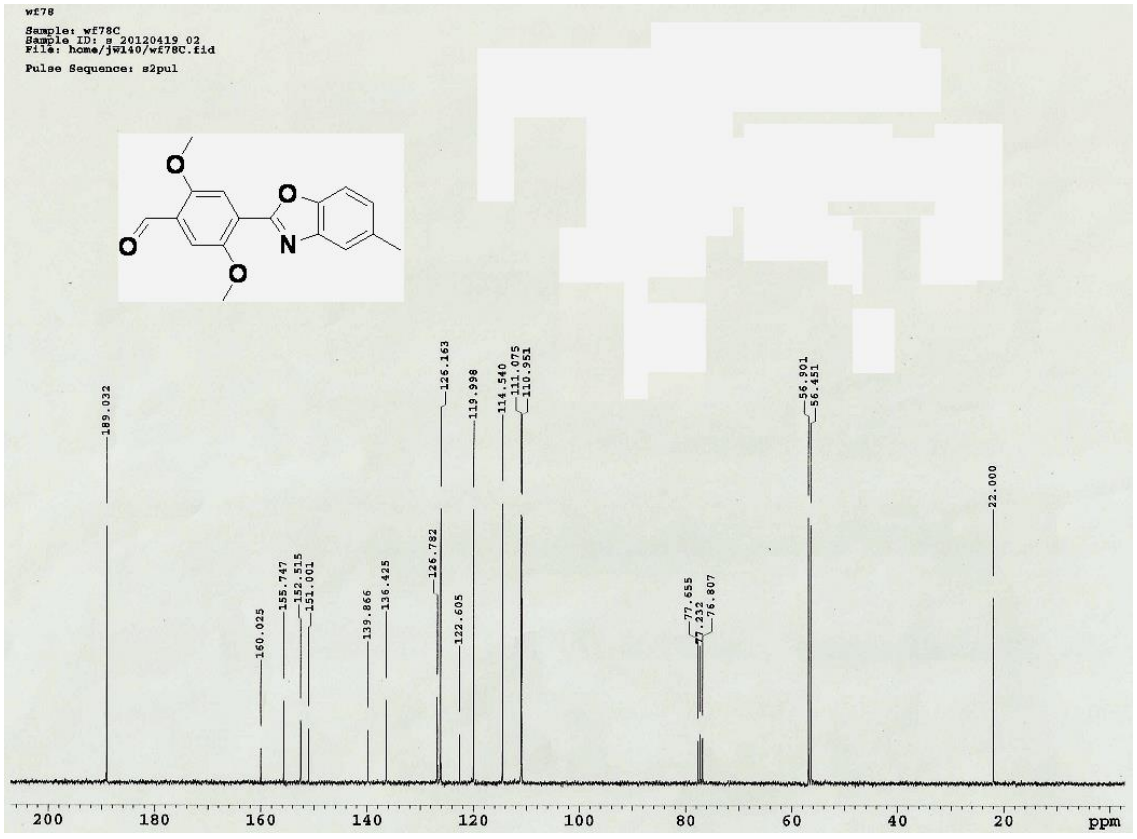
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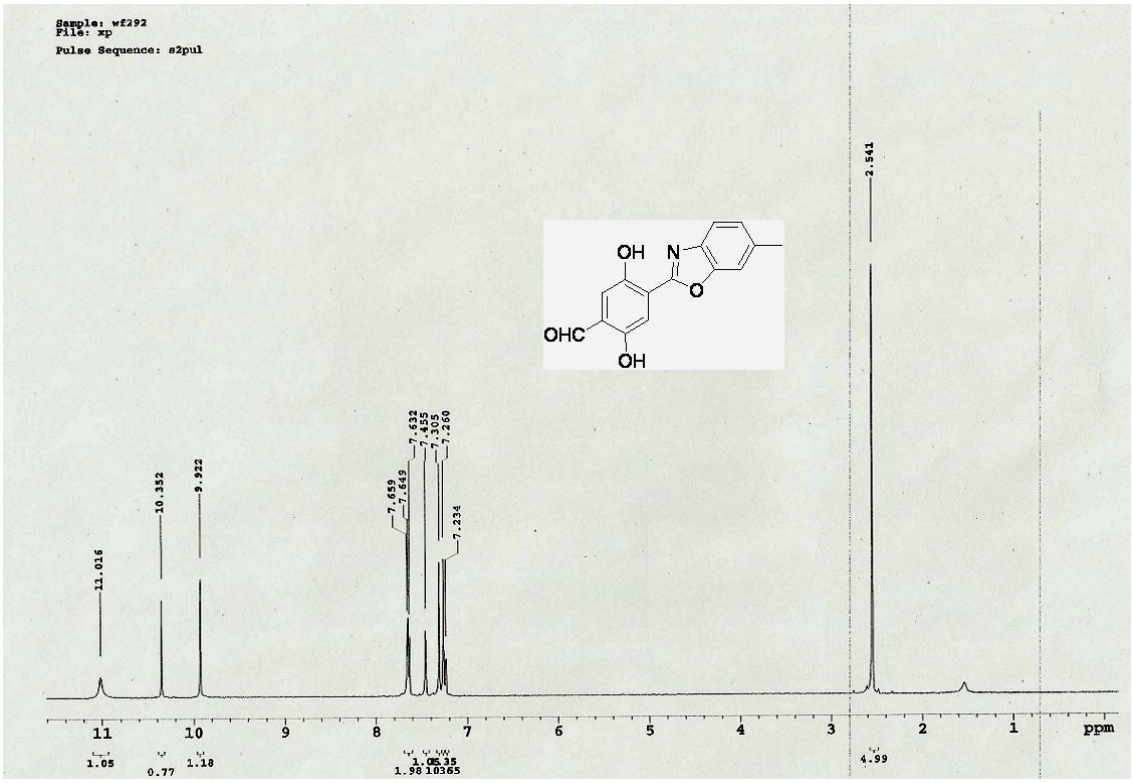
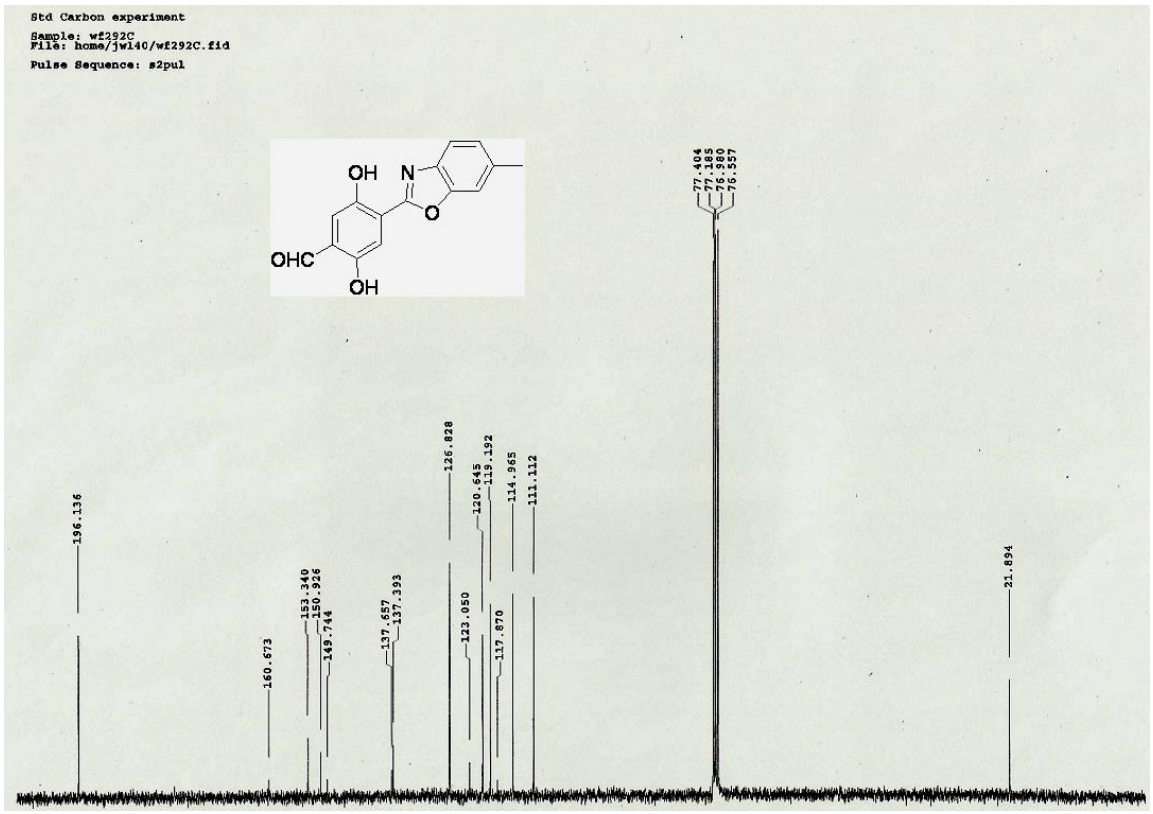
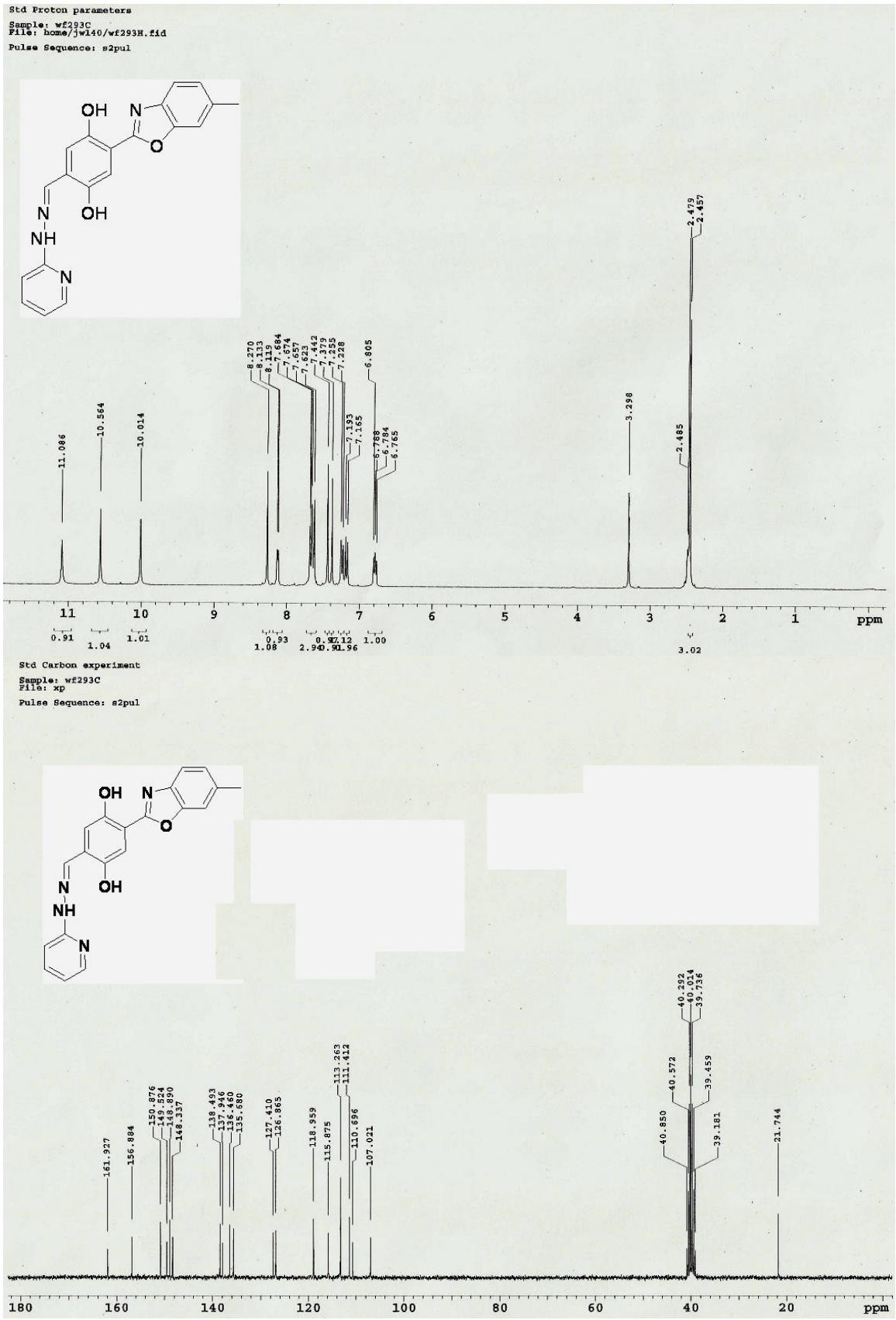


Note: the signals at 11.01 and 9.92 ppm are attributed to phenolic protons.

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