Supporting Information

A simple Schiff base as dual-responsive fluorescent sensor for bioimaging recognition Zn^{2+} and Al^{3+} in living cells

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1. Materials and general methods

Methyl picolinate (98%), hydrazinium hydrate (80%), 4-(diethylamino)salicylaldehyde (99%), dimercapto propanol (98%), and metal chlorides were purchased from Sigma-Aldrich Chemical and used without further purification. All the solvents were bought from commercial sources and used with no further treament. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Ultrashield TM 400 PLUS spectrometer with tetramethylsilane as an internal standard. Fluorescence spectral measurements were recorded on a Jobin Yvon FluoroLog-3-TCSPC spectrofluorometer. ESI-MS measurements were performed on a Waters Q-TOF premier Mass Spectrometer. UV-vis spectra measurements were recorded on Cary 4000 spectrophotometer. Absorption of MTT experiments were performed using Tecan Infinite M1000 Pro reader. Cell images were taken on Olympus FV1000 Inverted Confocal IX81 Microscope.

2. ^{1}H NMR and ^{13}C NMR spectra of Picolinohydrazide

^{1}H NMR spectrum of picolinohydrazide

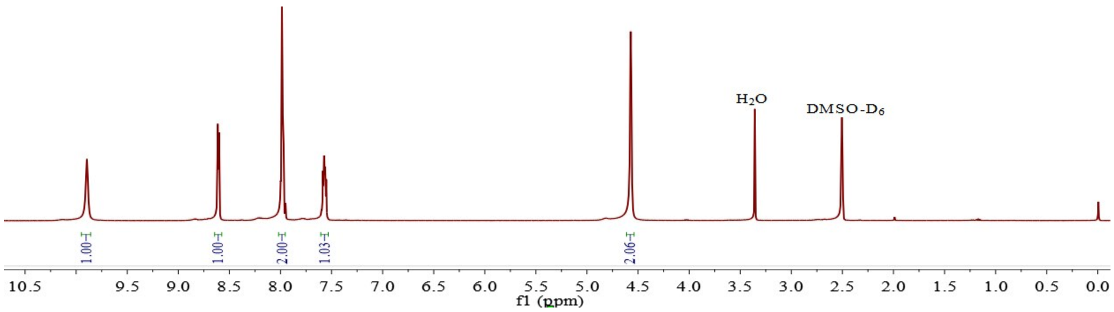

Figure S1. ^{1}H NMR (400 MHz, DMSO-d_6) d (ppm): 9.89 (s, 1 H), 8.61 (d, J = 8.0 Hz, 1 H), 7.98 (m, 2 H), 7.57 (m, 1 H), 4.57 (s, 2H).

^{13}C NMR spectrum of picolinohydrazide

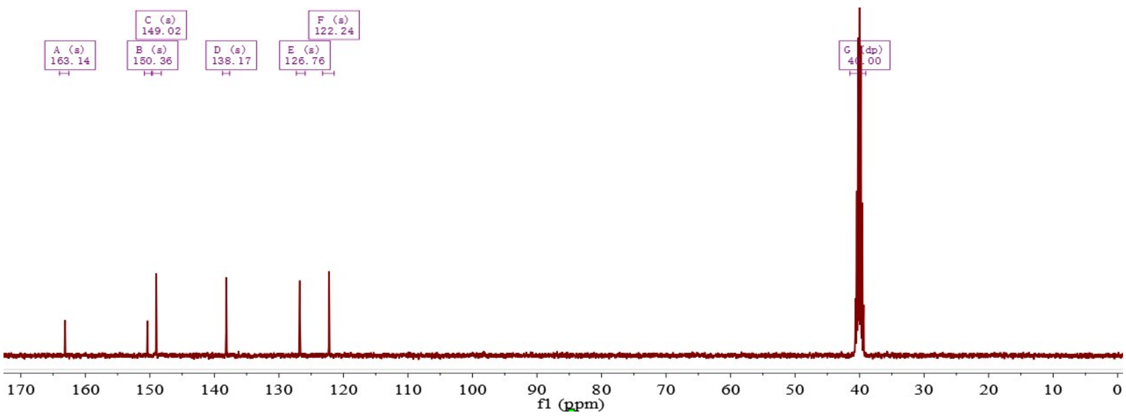

Figure S2. ^{13}C NMR (100 MHz, DMSO-d_6) d (ppm): 163.14, 150.36, 149.02, 138.17, 126.76, 122.24.

3. ^{1}H NMR, ^{13}C NMR and MS spectra of BDNOL

^{1}H NMR spectrum of BDNOL

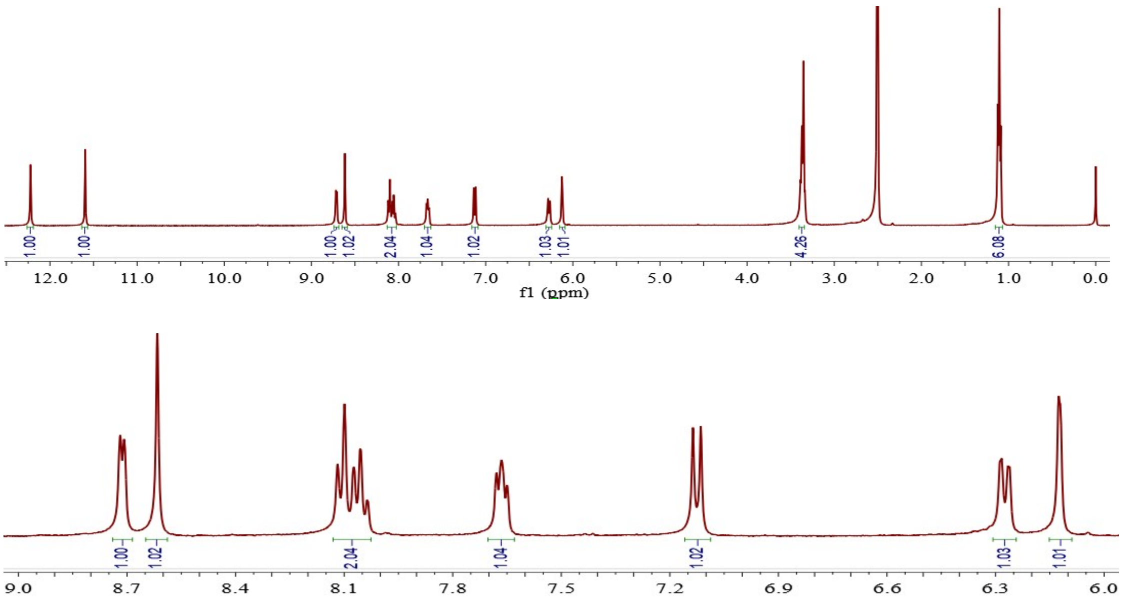

Figure S3. ^{1}H NMR (400 MHz, DMSO-d_6) d (ppm): 12.22 (s, 1H), 11.59 (s, 1H), 8.71 (d, J = 7.6 Hz, 1H), 8.58 (d, 1H), 8.08 (m, 2H), 7.66 (t, J = 3.8 Hz, 1H), 7.13 (d, J = 8.4 Hz, 1H), 6.27 (d, J = 8.4 Hz, 1H), 6.13 (s, 1H), 3.36 (q, J = 7.0 Hz, 4H), 1.11 (t, J = 7.0 Hz, 6H). The above is the whole spectrum of ^{1}H NMR and the below is the partial spectrum.

^{13}C NMR spectrum of BDNOL

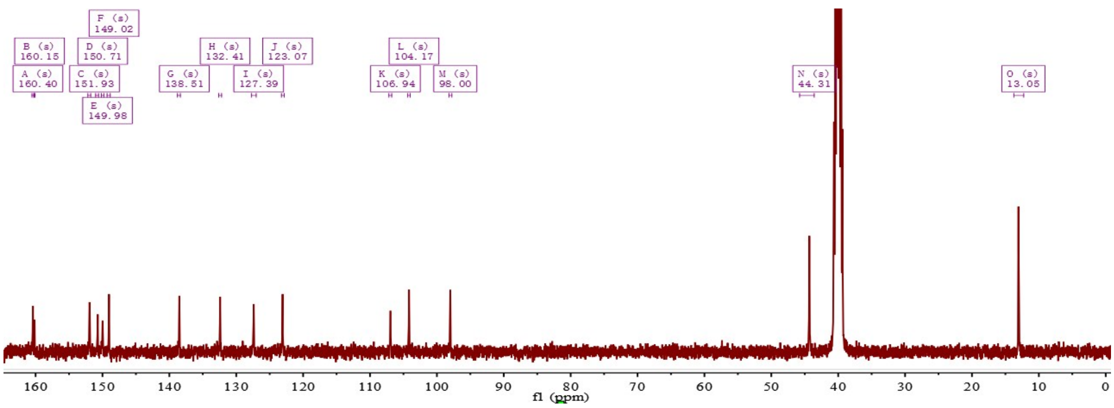

Figure S4. ^{13}C NMR (100 MHz, DMSO-d_6) d (ppm): 160.40, 160.15, 151.93, 150.71, 149.98, 149.02, 138.51, 132.41, 127.39, 123.07, 106.94, 104.17, 98.00, 44.31, 13.05.

MS spectrum of BDNOL

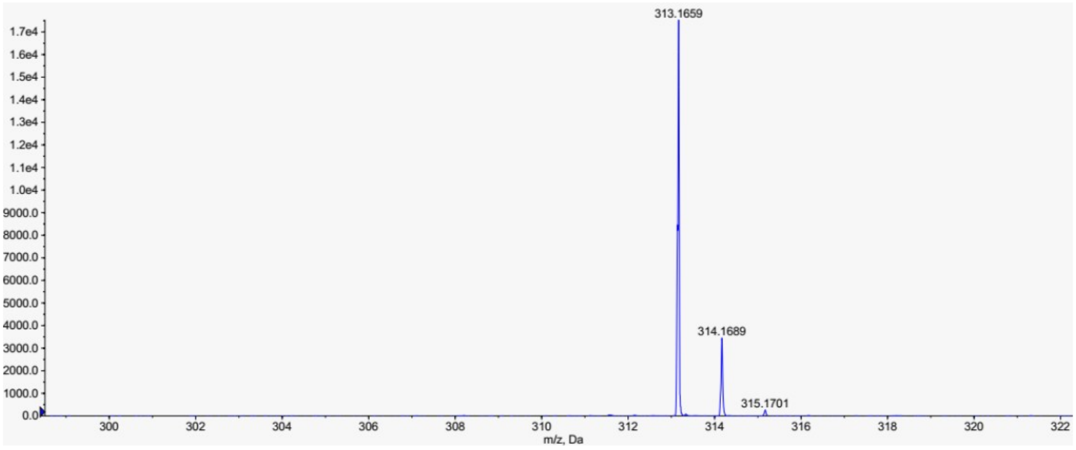

Figure S5. ESI mass spectra of BDNOL. HRMS calcd for $C_{17}H_{21}N_4O_2$ [BDNOL+H]$^+$: 313.1665, found: 313.1659.

4. The selectivity of QLSA

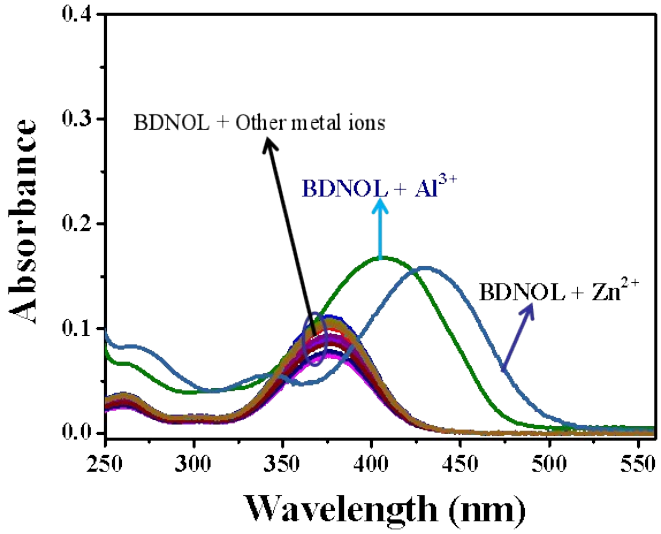

Figure S6. Absorption spectra obtained for BDNOL (10 μM) in CH_3OH/HEPES buffer (1/4, v/v, pH 7.2) after the addition of 5.0 equiv. of Na^+, K^+, Mg^{2+}, Ca^{2+}, Co^{2+}, Cu^{2+}, Ni^{2+}, Mn^{2+}, Fe^{2+}, Fe^{3+}, Cr^{3+}, Cd^{2+}, Hg^{2+}, Pb^{2+}, Ag^+, Al^{3+} and Zn^{2+} (λ_{ex}: 390 nm).

5. The response time of BDNOL towards Al^{3+}/Zn^{2+}

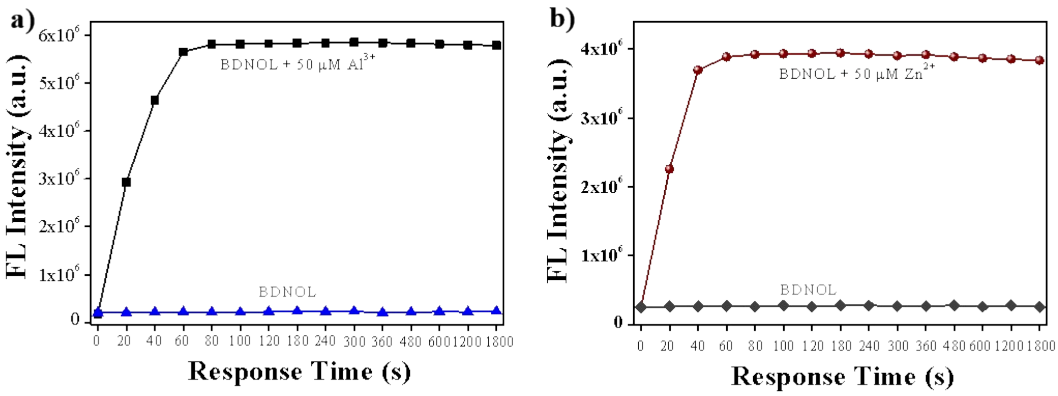

Figure S7. The fluorescence response time of BDNOL (10 μM) towards (a) Al^{3+} (λ_{em} 504 nm), and (b) Zn^{2+} (λ_{em} 575 nm) in CH_3OH/HEPES buffer (1/4, v/v, pH 7.2) (λ_{ex}: 390 nm).

6. Fluorescence testing in tap water

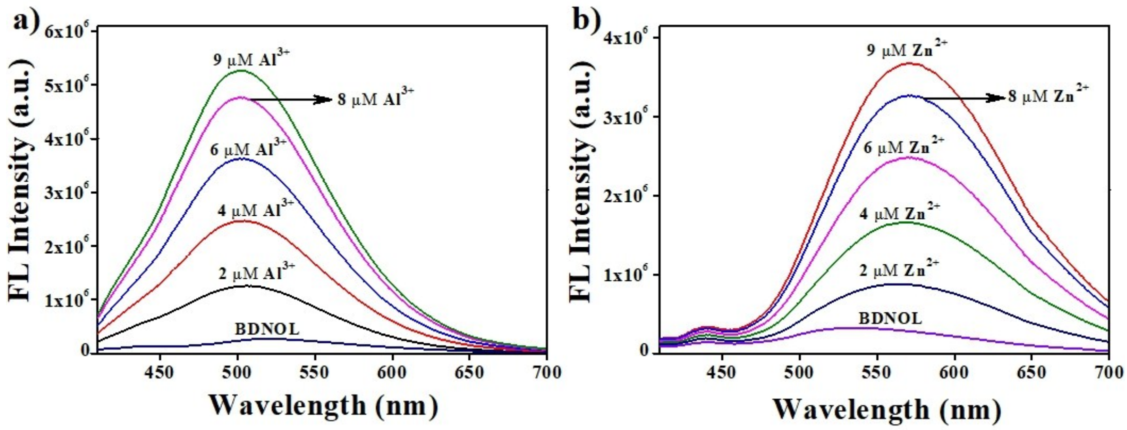

Figure S8. Variation of fluorescence emission recorded for BDNOL (10 μM) upon addition of (a) Al^{3+} and (b) Zn^{2+}, λ_{ex}: 390 nm. The solvent is tap water-methanol (4/1, v/v).

Table S1. Detection of Al^{3+} and Zn^{2+} in tap water samples.

Sample	Al^{3+}/Zn^{2+}added (μM)	Found (μM)				Mean	Recovery	R.S.D.
		1	2	3	4			
Al^{3+}	2.0	1.85	2.03	1.92	1.97	1.94	97.0%	3.82%
	4.0	3.94	3.98	3.87	4.08	3.97	99.3%	2.19%
	6.0	5.97	6.04	5.91	6.17	6.02	100.3%	1.86%
	8.0	7.78	8.07	7.93	8.16	7.99	99.9%	2.08%
	9.0	9.06	8.87	8.91	9.12	8.99	100.0%	1.32%
Zn^{2+}	2.0	1.91	2.12	2.09	2.04	2.04	102.0%	4.64%
	4.0	3.89	4.15	4.11	4.09	4.06	101.5%	2.90%
	6.0	6.03	6.14	6.27	6.08	6.13	102.2%	1.73%
	8.0	7.90	8.21	8.15	8.27	8.13	101.6%	2.03%
	9.0	9.06	9.19	9.24	9.17	9.17	101.9%	0.84%

7. Job's plot

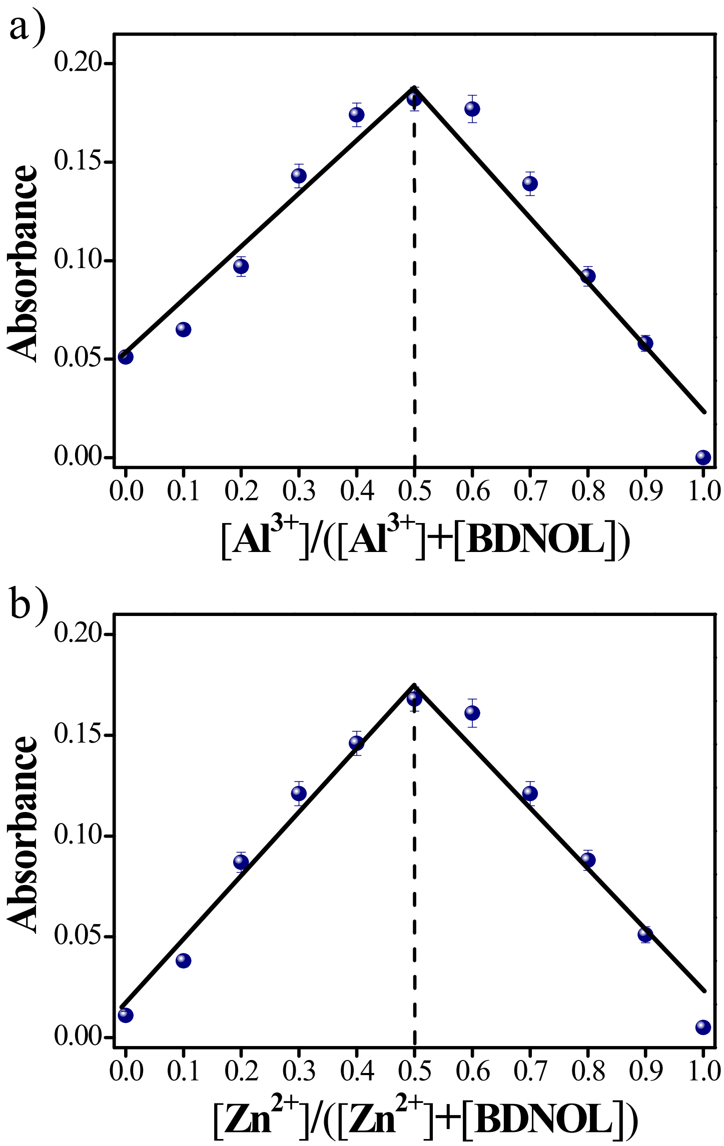

Figure S9. Job's plot obtained for BDNOL towards (a) Al^{3+} and (b) Zn^{2+} ions. The total concentration of BDNOL and Al^{3+}/Zn^{2+} was fixed at 20 μM.

8. ESI-MS data for BDNOL-Al^{3+} and BDNOL-Zn^{2+}

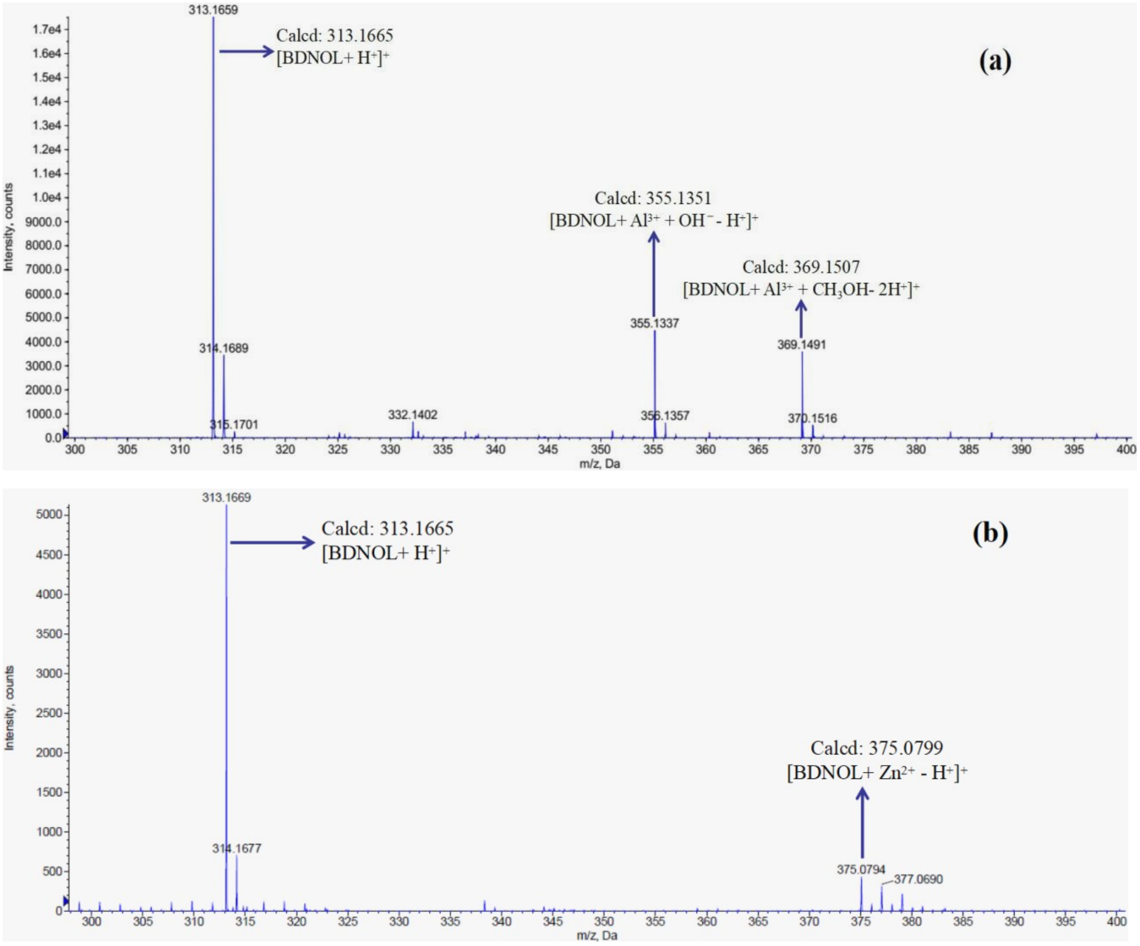

Figure S10. (a) ESI mass spectra of BDNOL in the presence of Al^{3+} (5.0 equiv.), m/z 355.1351 (calcd = 355.1337) corresponding to [BDNOL + Al^{3+} + OH^-—H^+]$^+$ and m/z 369.1507 (calcd = 369.1491) corresponding to [BDNOL + Al^{3+} + CH_3OH—$2H^+$]$^+$, indicating the formation of a 1:1 QLSA-Al^{3+} complex. (b) ESI mass spectra of BDNOL in the presence of Zn^{2+} (5.0 equiv.), m/z 375.0799 (calcd = 375.0794) corresponding to [BDNOL + Zn^{2+}—H^+]$^+$, indicating the formation of a 1:1 QLSA-Zn^{2+} complex.

9. ^{1}H NMR analysis of BDNOL-Al^{3+}/Zn^{2+}

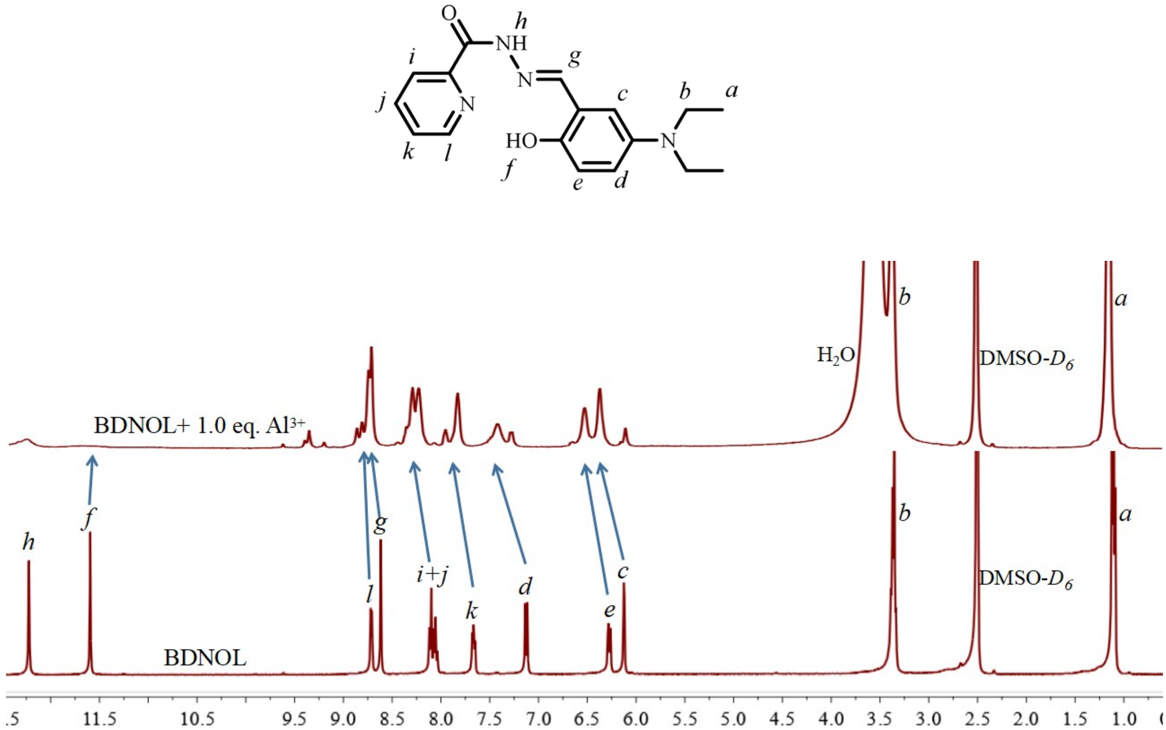

Figure S11. ^{1}H NMR analysis of BDNOL and BDNOL-Al^{3+} in DMSO-d_6.

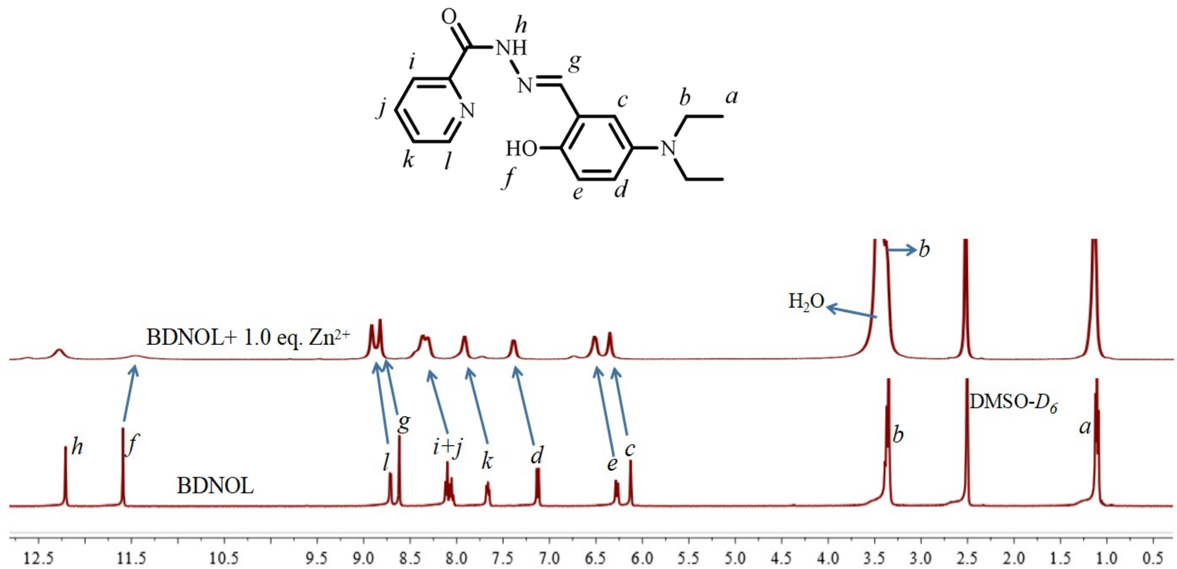

Figure S12. ^{1}H NMR analysis of BDNOL and BDNOL-Zn^{2+} in DMSO-d_6.

10. MTT analysis

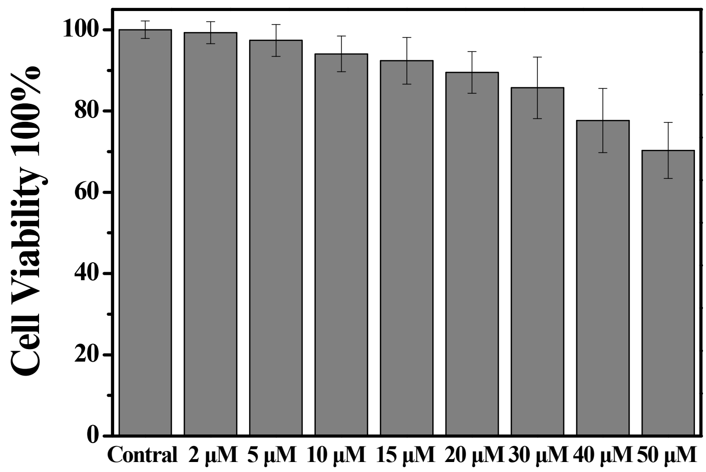

Figure S13. MTT assay of BDNOL on Hela cells for 12 h.

11. The association constant K_a

The association constant (K_a) of BDNOL-Al^{3+}/Zn^{2+} was obtained from nonlinear curve fitting of the fluorescence titration data according to Benesi-Hildebrand equation (Eq. 1) [1-3], where F_0, F, and F_{max} are the fluorescence intensity of BDNOL in the absence of Al^{3+}/Zn^{2+}, at a certain concentration of Al^{3+}/Zn^{2+} ions and a complete-interaction concentration of Al^{3+}/Zn^{2+}, [M] is the metal ion concentration, n is the binding stoichiometry, and K_a is the association constant.

$$\frac{1}{F-F_0} = \frac{1}{K_a \bullet (F_{\max} - F_0) \bullet [\mathrm{M}]^{n}} + \frac{1}{F_{\max} - F_0} \qquad \textbf{(Eq. S1)}$$

12. References

[1] A. K. Mandal, M. Suresh, P. Das, E. Suresh, M. Baidya, S. K. Ghosh, A. Das, *Org. Lett.*, 2012, 14, 2980–2983.

[2] H.A. Benesi, J. H. Hildebrand, *J. Am. Chem. Soc.*, 1949, 71, 2703–2707.

[3] K. C. Sham, C. S. Lee, K. Y. Chan, S. M. Yiu, W. T. Wong, H. L. Kwong, *Polyhedron*, 2011, 30, 1149–1156.