Cysteamine-based cell permeable Zn^{2+}-specific molecular bioimaging materials: from animal to plant cells

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Supporting Information's

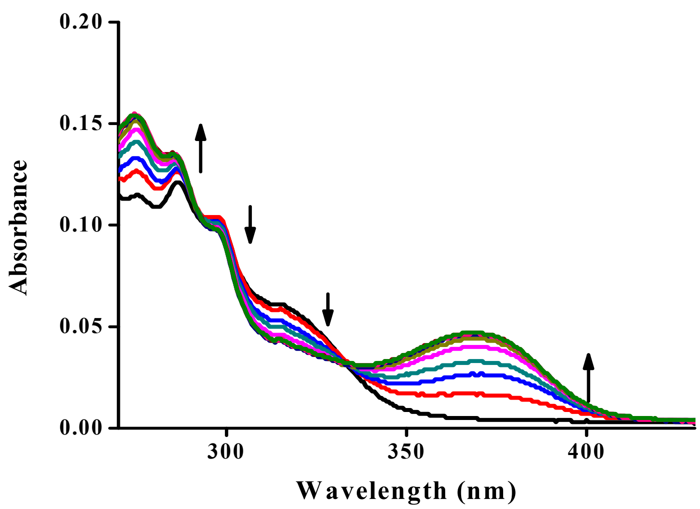

Figure S1. UV-vis spectra of **L1** (10 μM) in the presence of increasing amount of Zn^{2+} (0 – 20 μM) in DMF.

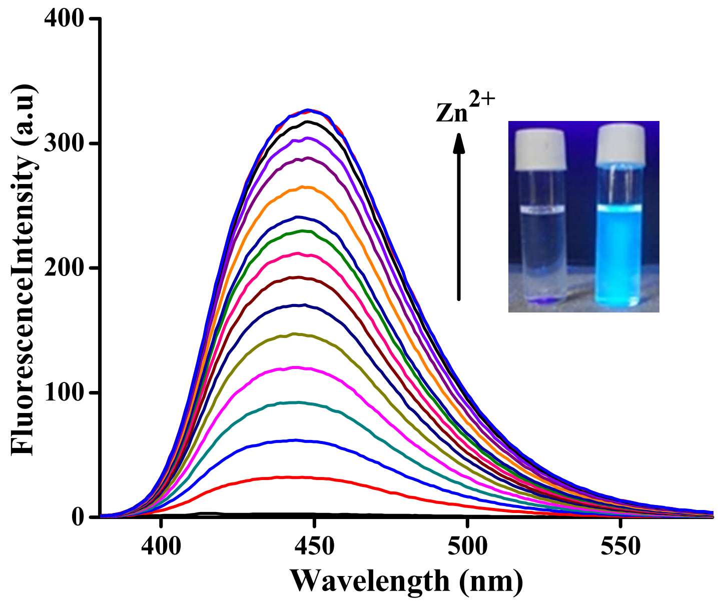

Figure S2. Emission spectra of **L1** (10 μM) in DMF in the presence of increasing amount of Zn^{2+} (0 - 20 μM) in filtered milliQ water. Inset: Colour of **L1** (10 μM) before and after addition of Zn^{2+} (20 μM) under 365nm UV-lamp.

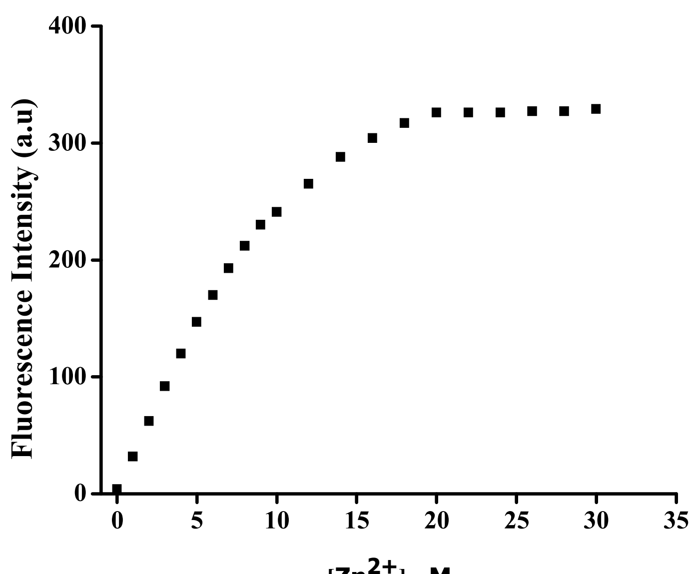

Figure S3. Changes in fluorescence intensity value of **L1** as a function of Zn^{2+} concentration.

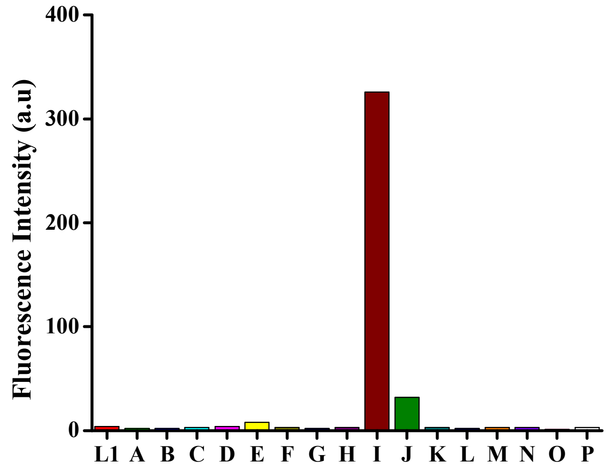

Figure S4. Emission spectra of **L1** (10 μM) in DMF in the presence of 100 μM of biologically and environmentally important metals in filtered milliQ water. A, B, C, D, E, F, G, H, I, J, K, L, M, N,O, P corresponds to Li^{+}, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Ba^{2+}, Sr^{2+}, Zn^{2+}(30 μM), Cd^{2+}, Pb^{2+}, Hg^{2+}, Cu^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+} respectively.

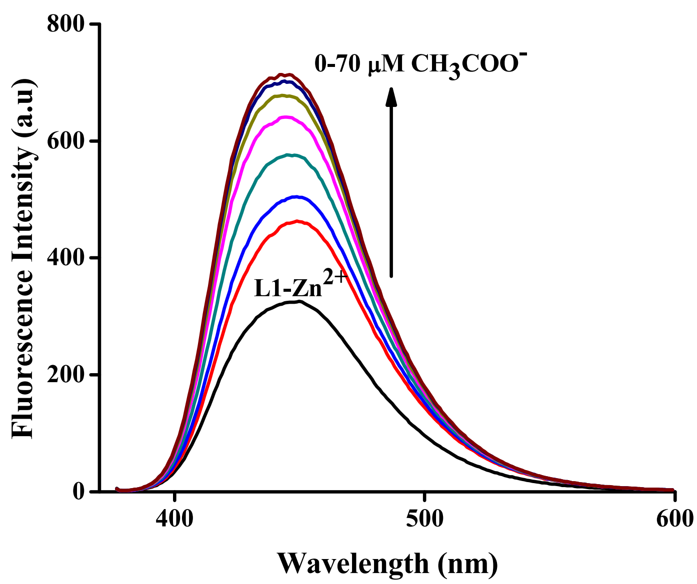

Figure S5a. Fluorescence spectra of **L1**-Zn^{2+},triggerd by acetate ion.

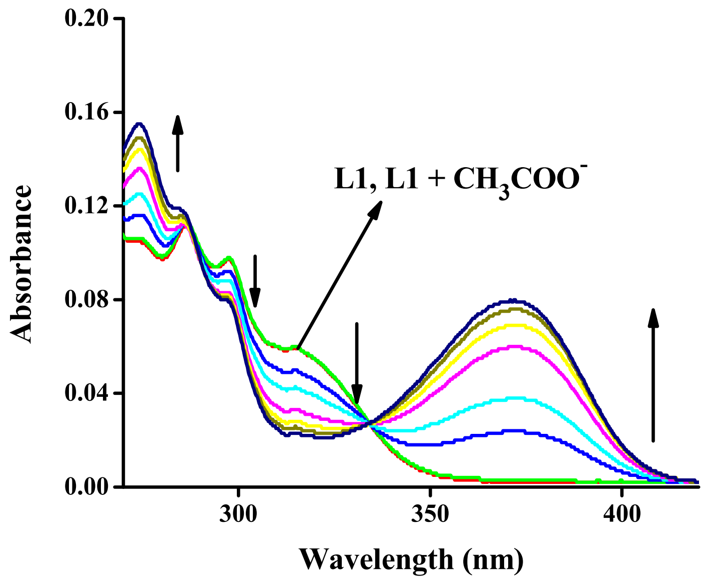

Figure S5b. UV-vis absorption spectra of **L1**-Zn^{2+}, triggerd by acetate ion.

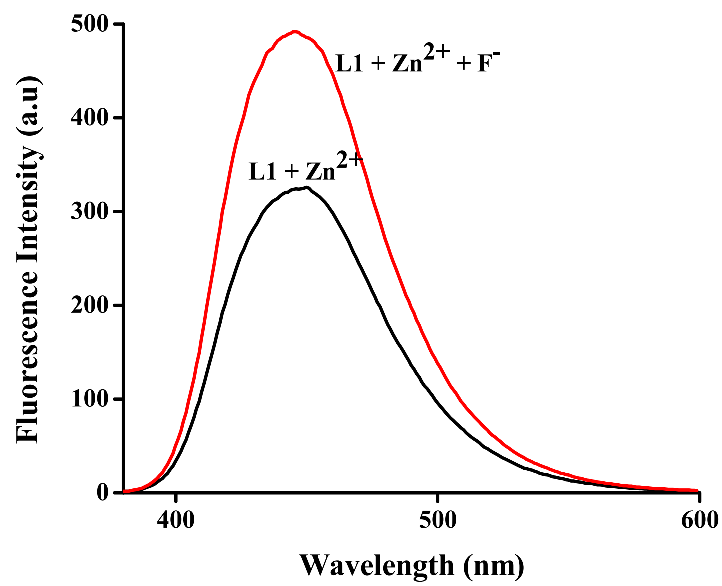

Figure S6. Fluorescence spectra of **L1**-Zn^{2+},triggerd by fluoride ion.

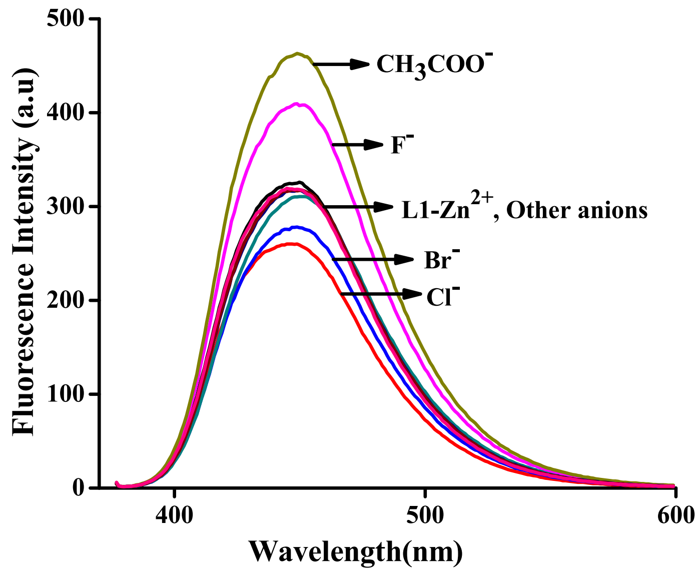

Figure S7. Effect of Cl^-, Br^-, I^-, F^-, CH_3COO^-, NO_3^-, ClO_4^-, CN^- (20 μM) on the emission spectrum of **L1**-Zn^{2+}.

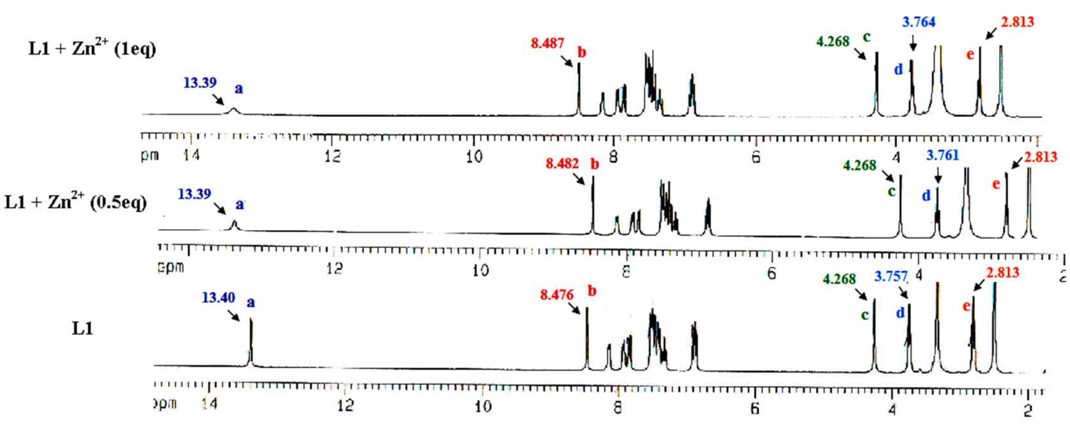

Figure S8. ^1H NMR titration of **L1** in the presence of different concentration of Zn^{2+}

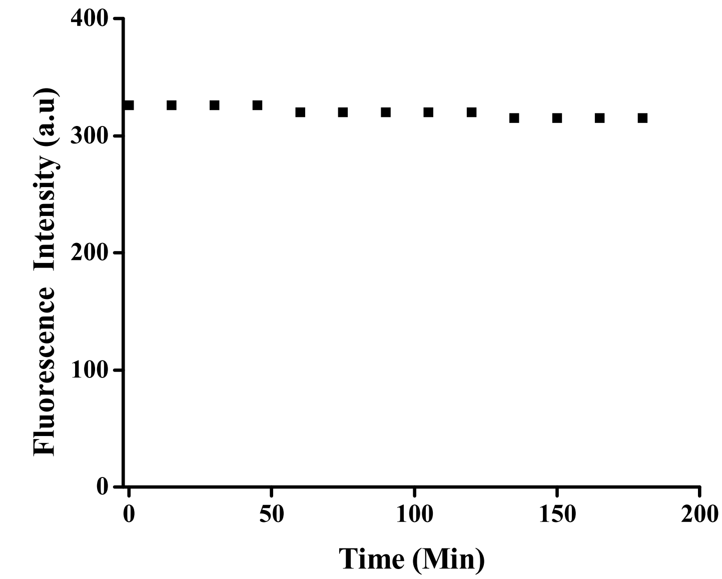

Figure S9. Effect of time on the emission spectrum of **L1-**Zn^{2+}.

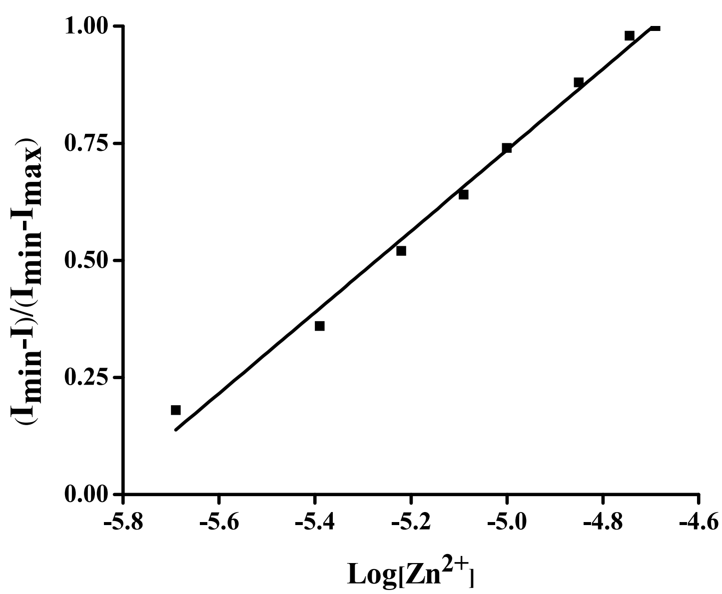

Figure S10. Calculation of limit of detection[1] of Zn^{2+} by **L1**.

Detection limit: 1.41 µM

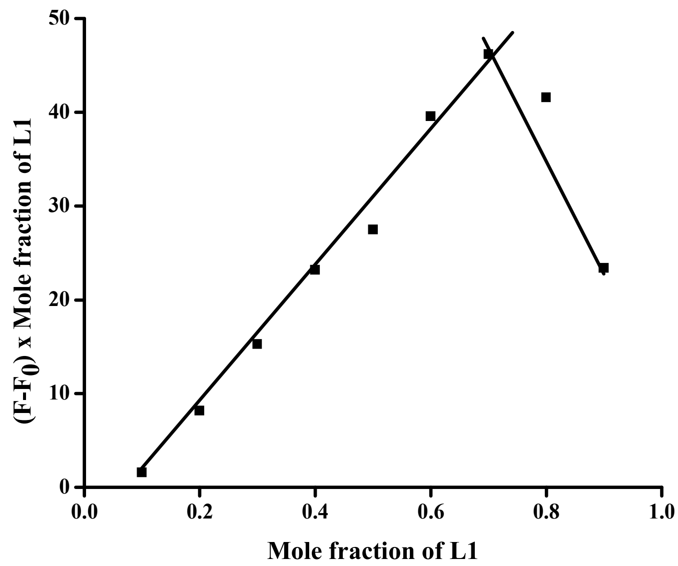

Figure S11. Job's plot[2] for the identification of **L1**-Zn^{2+} complex stoichiometry.

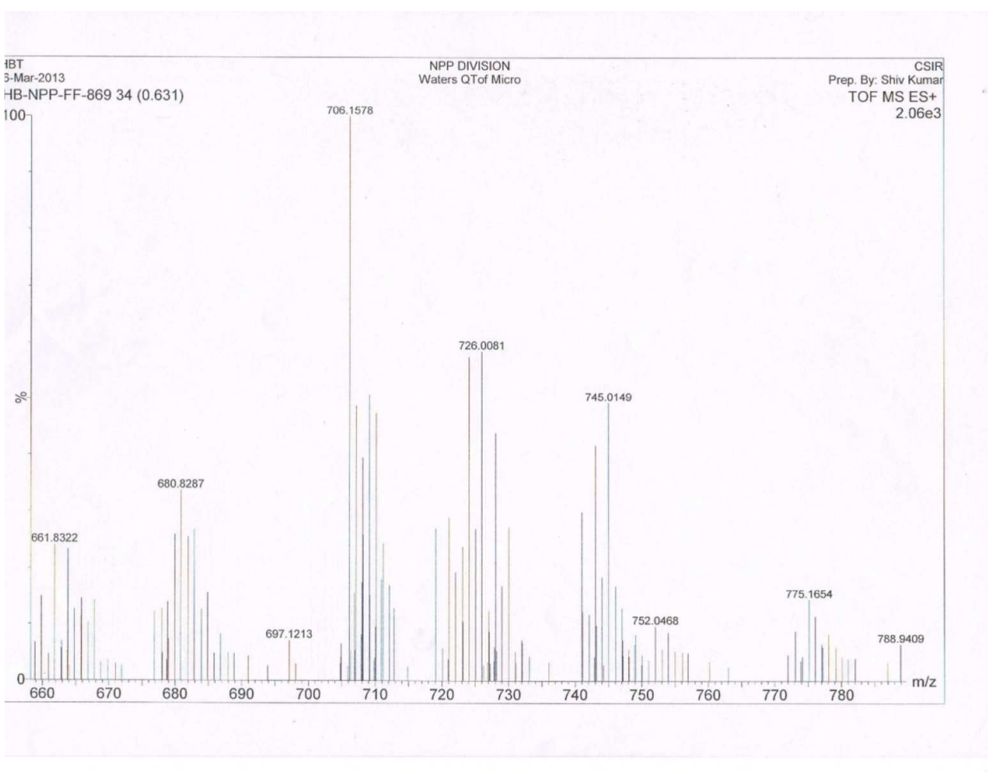

Figure S12. ESI mass spectra of **L1**-Zn^{2+}

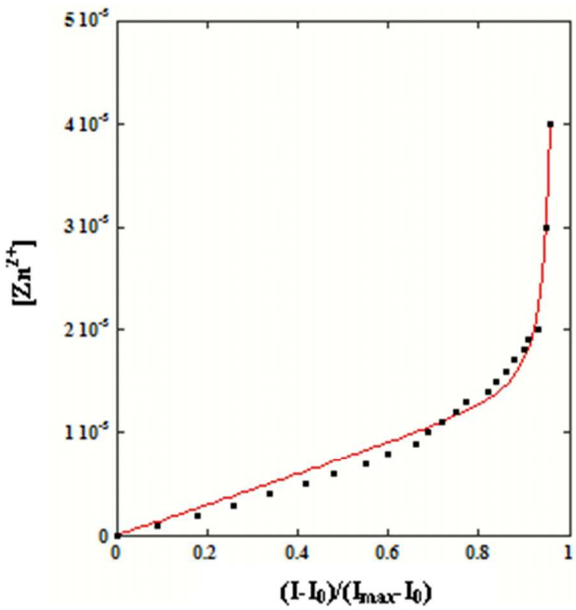

Figure S13. Binding constant calculation[3,4] for the **L1**-Zn^{2+} complex.

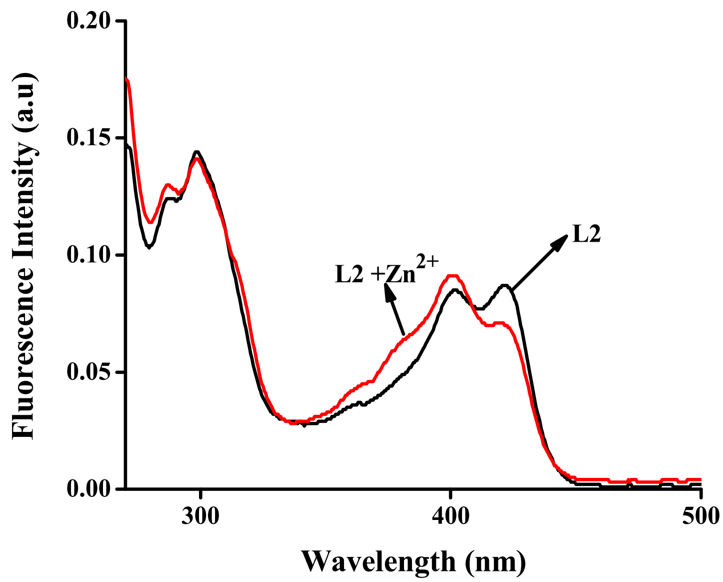

Figure S14. UV-vis absorption spectra of **L2** (10 μM) and **L2** in the presence of 20 μM Zn^{2+} in DMF.

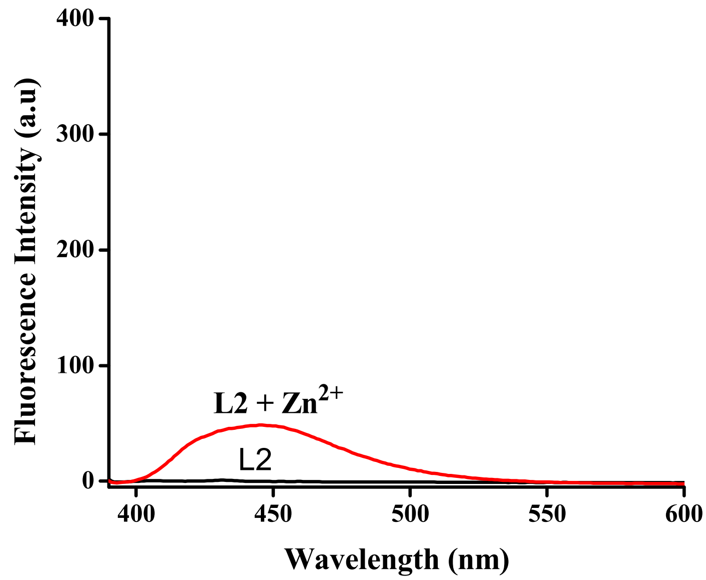

Figure S15. Emission spectra of **L2** (10 μM) in DMF in the presence of 20 μM of aqueous Zn^{2+} in filtered milliQ water.

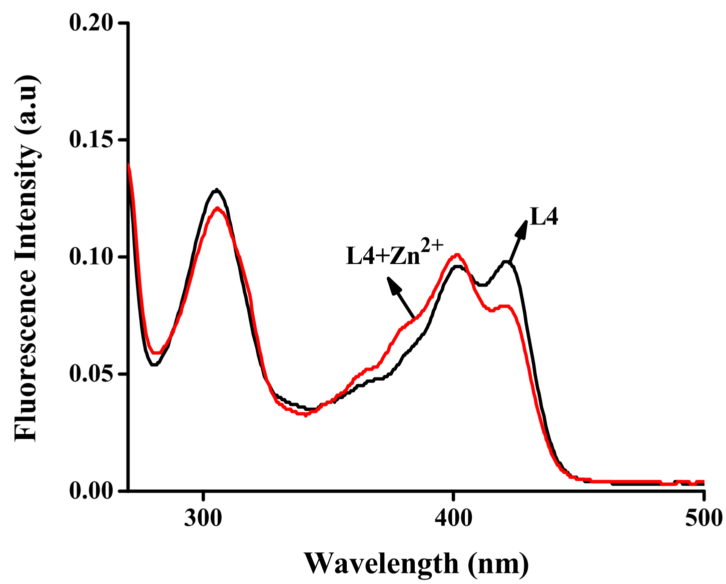

Figure S16. UV-vis absorption spectra of **L4** (10 μM) and **L4** in the presence of 20 μM Zn^{2+} in DMF.

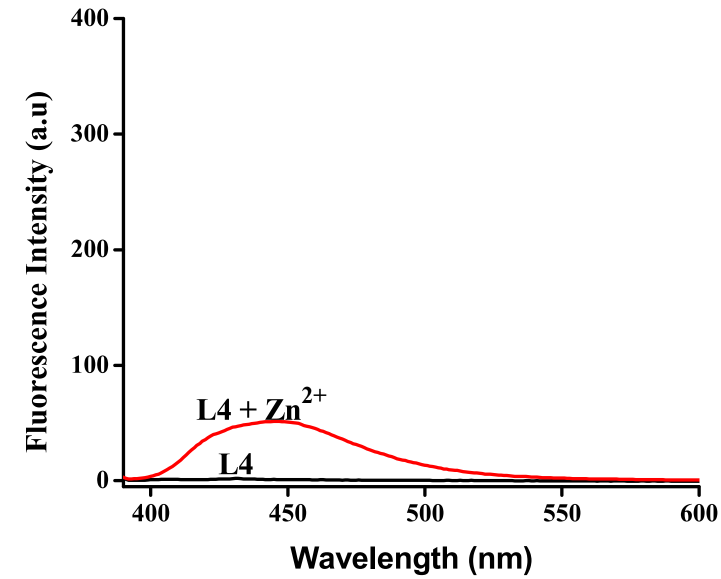

Figure S17. Emission spectra of **L4** (10 μM) in DMF in the presence of 20 μM of Zn^{2+} in filtered milliQ water.

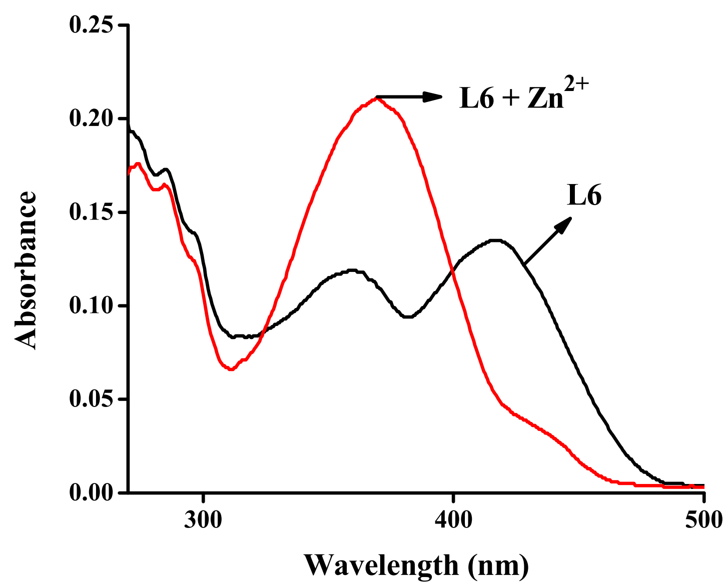

Figure S18. UV-vis absorption spectra of **L6** (10 μM) and **L6** in the presence of 20 μM Zn^{2+} in DMF.

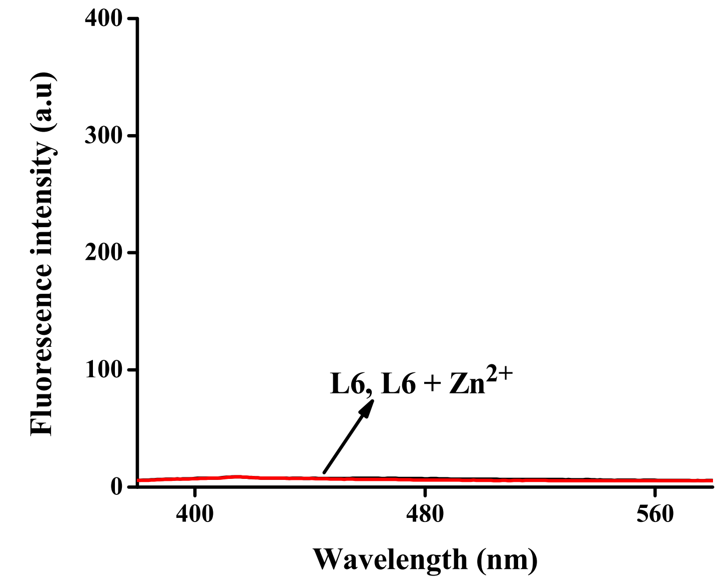

Figure S19. Emission spectra of **L6** (10 μM) in DMF in the presence of 20 μM of Zn^{2+} in filtered milliQ water.

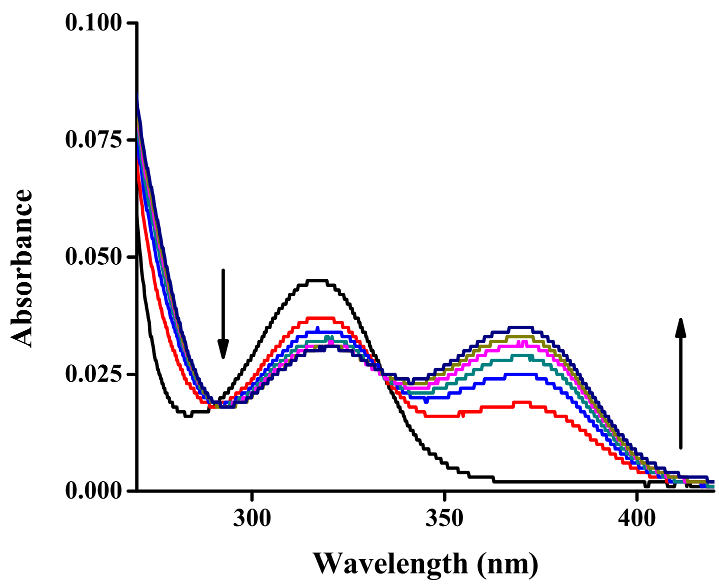

Figure S20. UV-vis absorption spectra of **L3** (10 μM) with increasing concentration of Zn^{2+} (0 – 20 μM) in DMF.

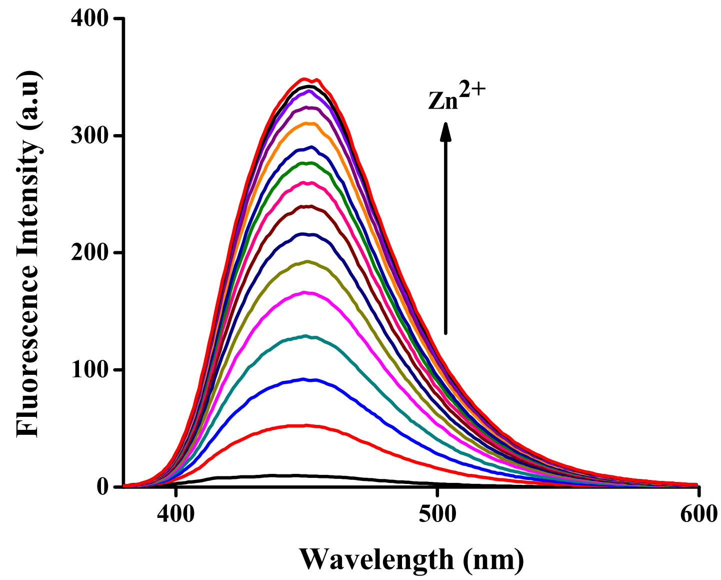

Figure S21. Emission spectrum of **L3** (10 μM) in DMF in the presence different concentration of Zn^{2+} (0 - 20 μM) in in filtered milliQ water.

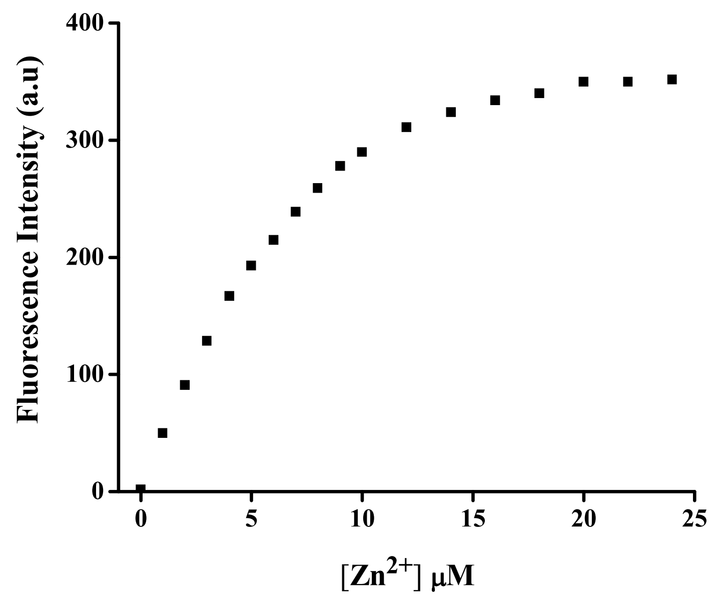

Figure S22. Changes in fluorescence intensity value of **L3** as a function of Zn^{2+} concentration.

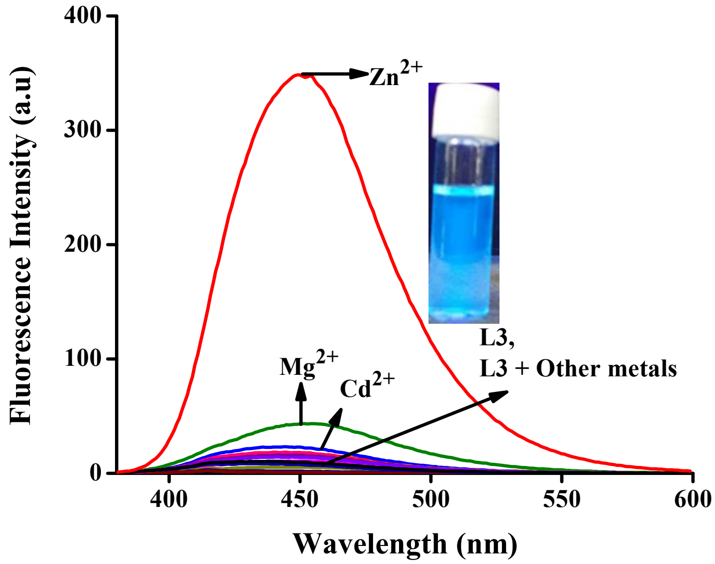

Figure S23. Emission spectra of **L3** (10 μM) in DMF in the presence of 20 μM of biologically and environmentally important metals in filtered milliQ water. Inset: Colour change of **L3** (10 μM) after addition of Zn^{2+} (20 μM) under 365nm UV-lamp.

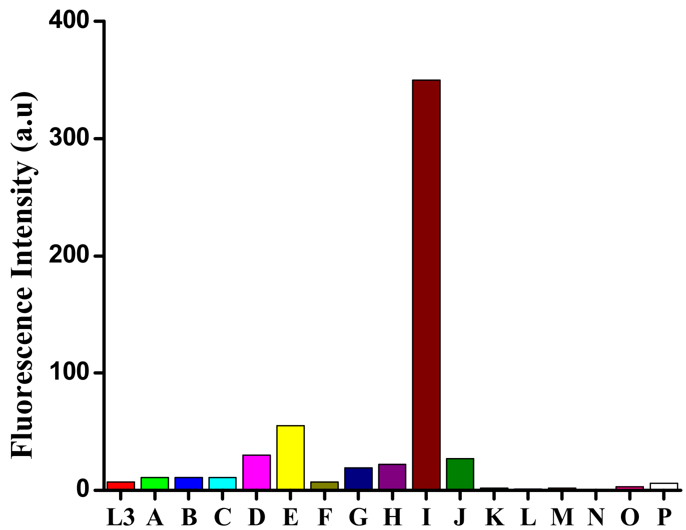

Figure S24. Emission spectra of **L3** (10 μM) in DMF in the presence of 100 μM of biologically and environmentally important metals in filtered milliQ water. A, B, C, D, E, F, G, H, I, J, K,L, M, N,O, P corresponds to Li^{+}, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Ba^{2+}, Sr^{2+}, Zn^{2+}(30 μM), Cd^{2+}, Pb^{2+}, Hg^{2+}, Cu^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+} respectively.

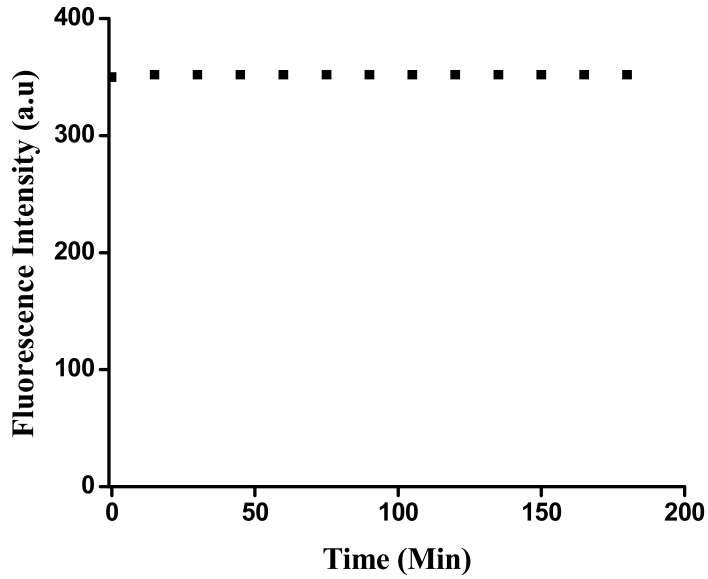

Figure S25. Effect of time on the emission spectrum of **L3**-Zn^{2+}.

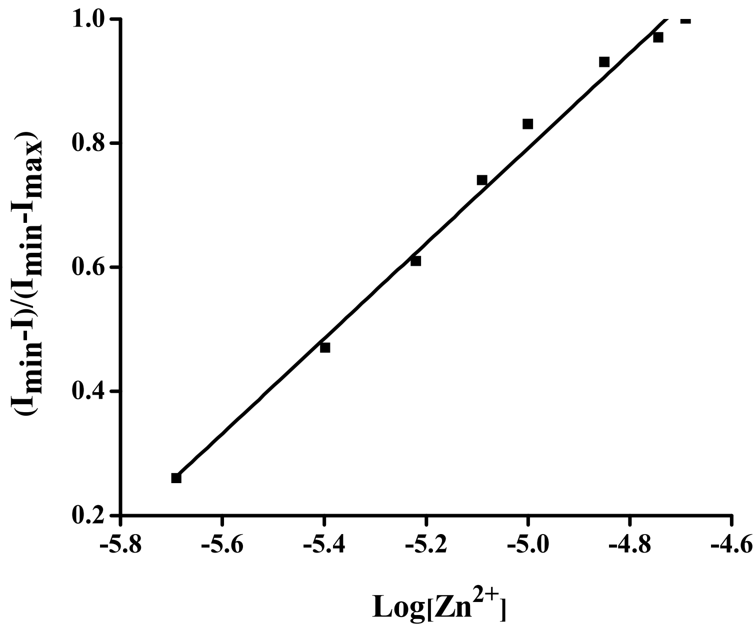

Figure S26. Calculation of limit of detection of Zn^{2+} by **L3**.

Detection limit: 0.93 µM

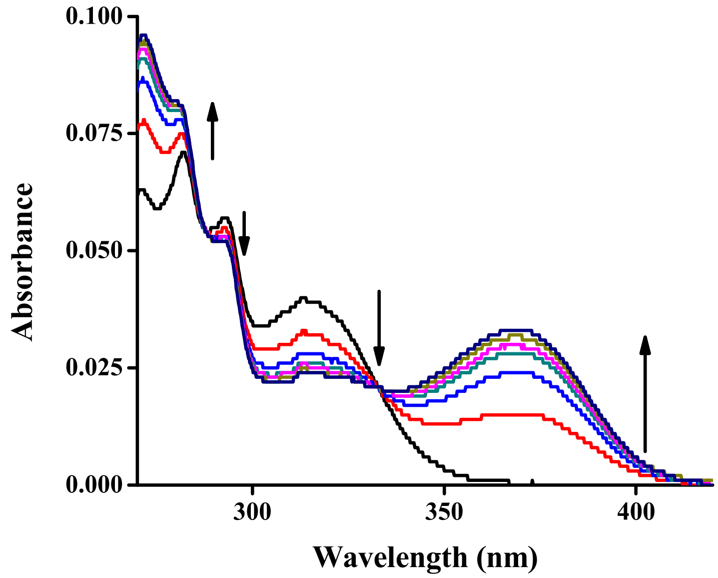

Figure S27. UV-vis absorption spectra of **L7** (10 μM) with increasing concentration of Zn^{2+} (0 – 20 μM) in DMF.

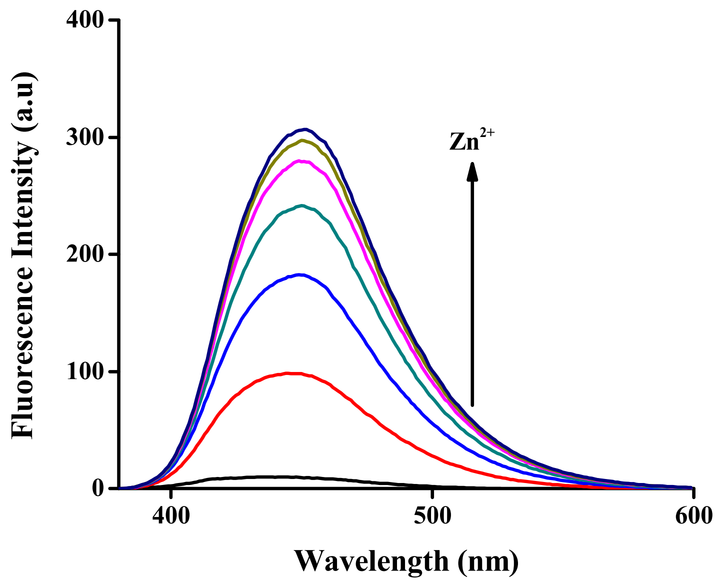

Figure S28. Emission spectrum of **L7** (10 μM) in DMF with increasing concentration of Zn^{2+} (0 – 20 μM) in filtered milliQ water.

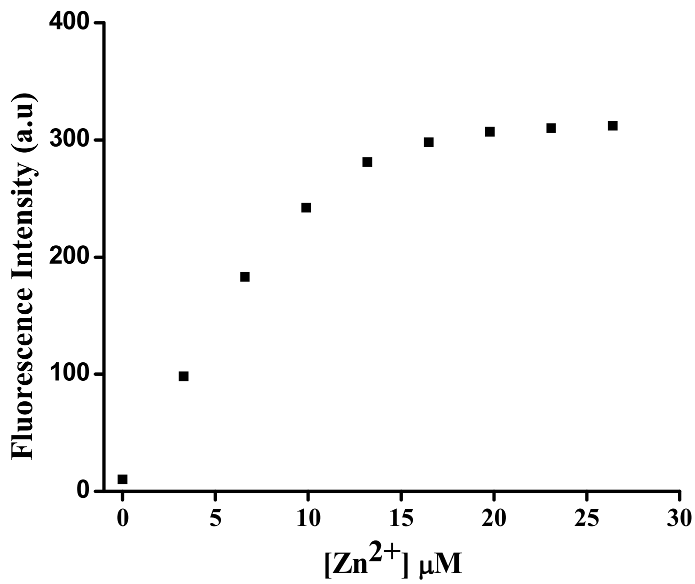

Figure S29. Changes in fluorescence intensity value of **L7** as a function of Zn^{2+} concentration.

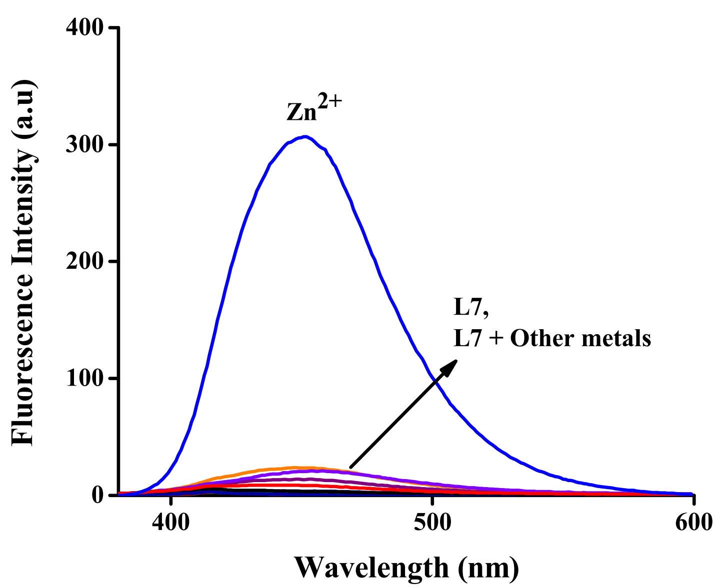

Figure S30. Emission spectra of **L7** (10 μM) in DMF in the presence of 20 μM of biologically and environmentally important metals in filtered milliQ water. Inset: Colour change of **L7** (10 μM) after addition of Zn^{2+} (20 μM) under 365nm UV-lamp.

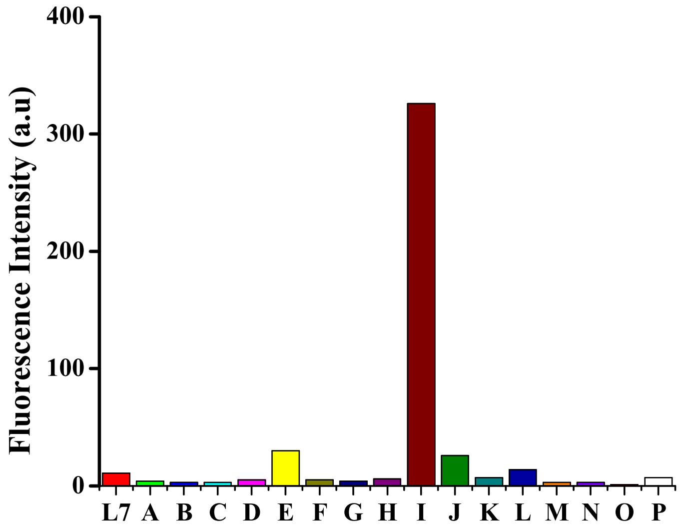

Figure S31. Emission spectra of **L7** (10 μM) in DMF in the presence of 100 μM of biologically and environmentally important metals in filtered milliQ water. A, B, C, D, E, F, G, H, I, J, K,L, M, N,O, P corresponds to Li^{+}, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Ba^{2+}, Sr^{2+}, Zn^{2+}(30 μM), Cd^{2+}, Pb^{2+}, Hg^{2+}, Cu^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+} respectively.

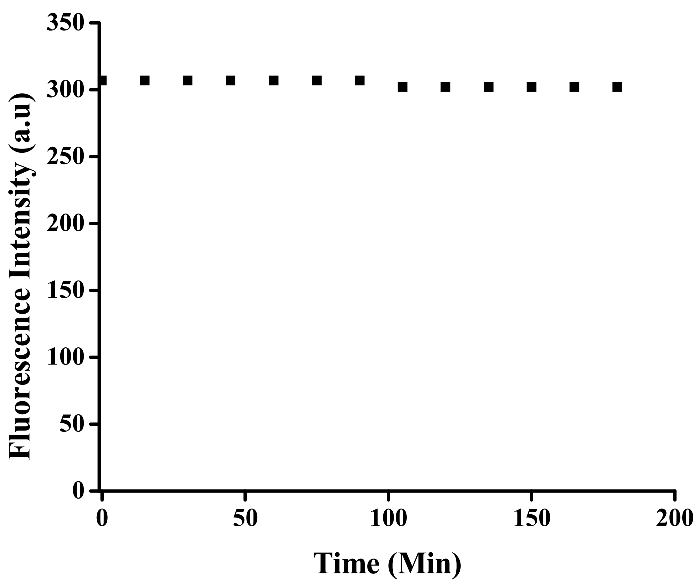

Figure S32. Effect of time on the emission spectrum of **L7**-Zn^{2+}.

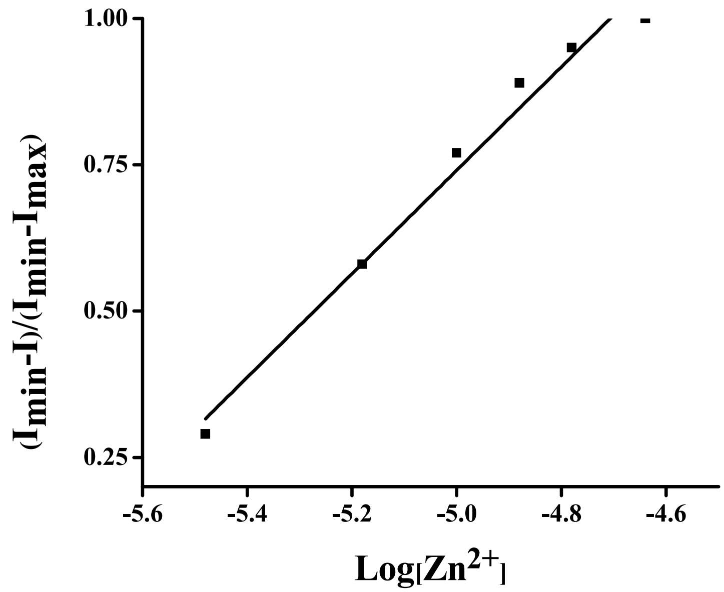

Figure S33. Calculation of limit of detection of Zn^{2+} by **L7.**

Detection limit: 1.38 μM

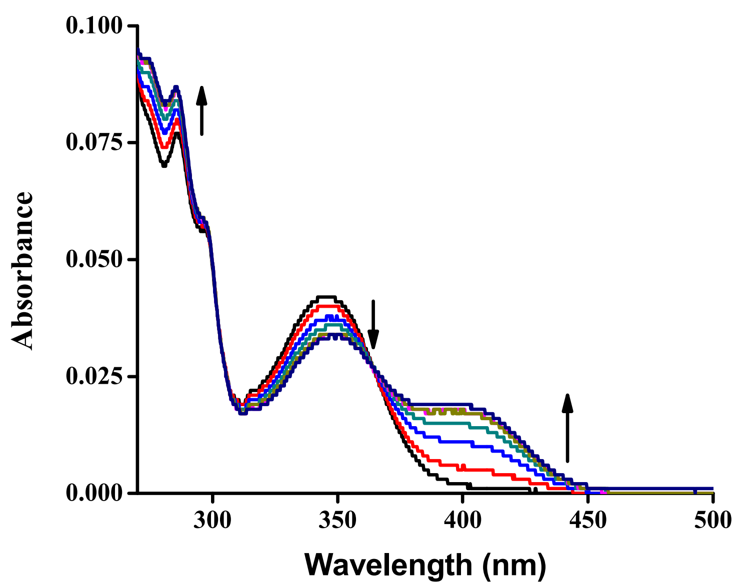

Figure S34. UV-vis absorption spectra of **L5** (10 μM) with increasing concentration of Zn^{2+} (0 – 20 μM) in DMF.

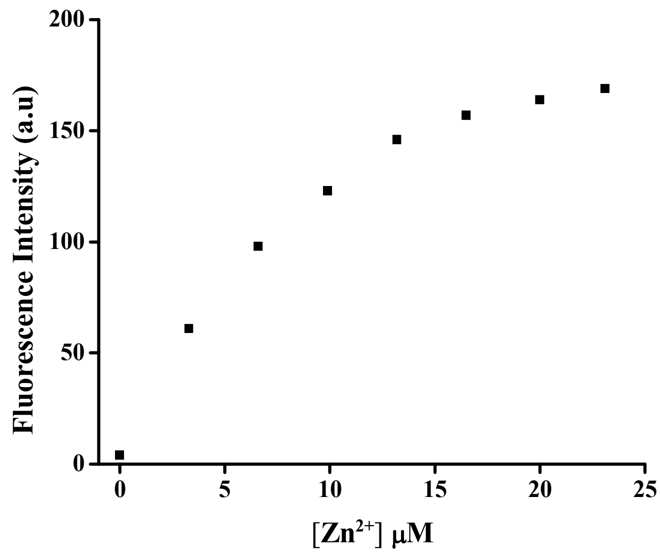

Figure S35. Changes in fluorescence intensity value of **L5** as a function of Zn^{2+} concentration.

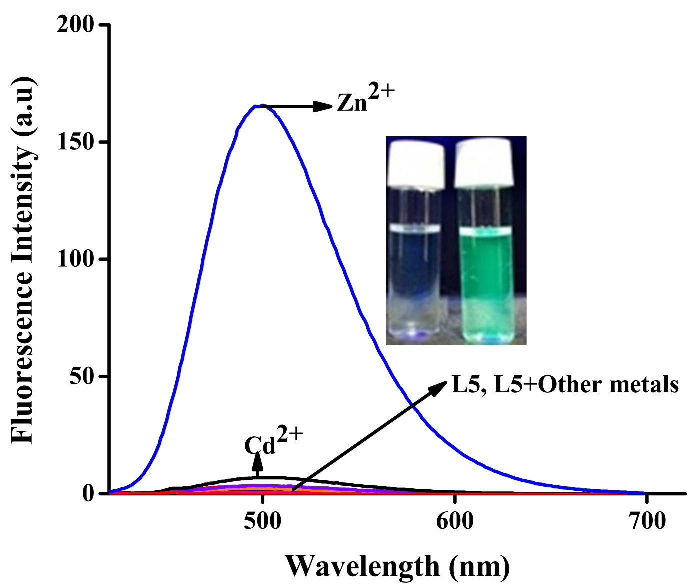

Figure S36. Emission spectra of **L5** (10 μM) in DMF in the presence of 20 μM of biologically and environmentally important metals in filtered milliQ water. Inset: Colour change of **L5** (10 μM) before and after addition of Zn^{2+} (20 μM) under 365nm UV-lamp.

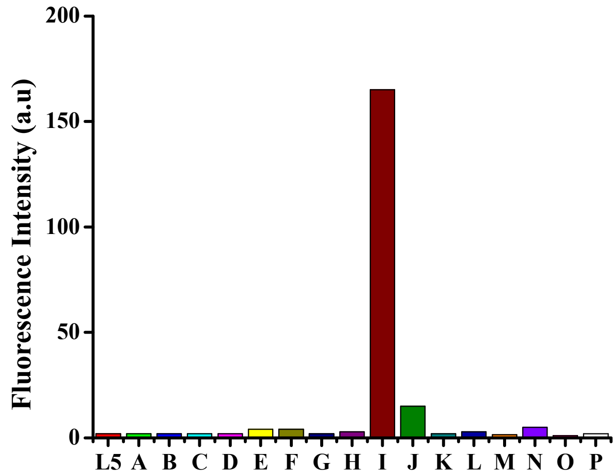

Figure S37. Emission spectra of **L5** (10 μM) in DMF in the presence of 100 μM of biologically and environmentally important metals in filtered milliQ water. A, B, C, D, E, F, G, H, I, J, K,L, M, N,O, P corresponds to Li^{+}, Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Ba^{2+}, Sr^{2+}, Zn^{2+} (30 μM), Cd^{2+}, Pb^{2+}, Hg^{2+}, Cu^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+} respectively.

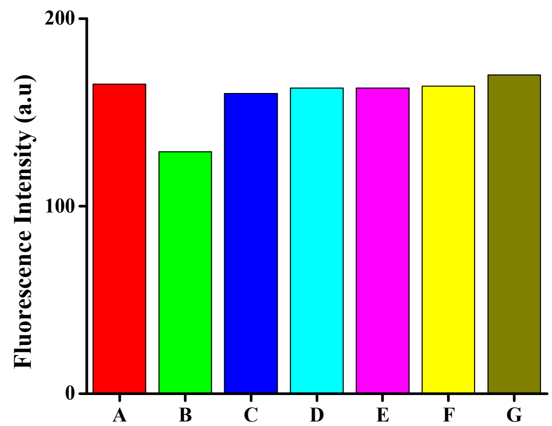

Figure S38. Fluorescence intensity as bar graph. A, B, C, D, E, F, G represents **L5** + Zn^{2+}, **L5** + Cd^{2+}+ Zn^{2+}, **L5** + Hg^{2+}+ Zn^{2+}, **L5** + Na^{+}+ Zn^{2+}, **L5** + K^{+}+ Zn^{2+}, **L5** + Ca^{2+}+ Zn^{2+}, **L5** + Mg^{2+}+ Zn^{2+}.

Table 1.

S No	Ensemble	$\lambda_{absorption}$	λ_{ex}	λ_{em}	$\lambda_{em(max)}$	Q.Y	Interaction with Na^+, K^+, Ca^{2+}, Mg^{2+}, Pb^{2+}, Cd^{2+}
1	**L1**-Zn^{2+}	269-281,282-293, 294-333, 334-418	370	381-581	448	0.33	Negligible fluorescence enhancement was observed in the presence of Cd^{2+}.
2	**L3**-Zn^{2+}	290-339, 340-418	370	381-600	448	0.40	Little fluorescence enhancement was observed in the presence of Ca^{2+}, Mg^{2+}, Cd^{2+}.
3	**L5**-Zn^{2+}	279-292,293-314, 315-375, 376-450	400	421-700	498	0.31	Negligble interference of Cd^{2+}.
4	**L7**-Zn^{2+}	277-288,289-304, 305-340, 347-410	370	380-598	448	0.35	Negligble interference of Cd^{2+}.

Figure S39. Colour changes of all the ligands **L1, L6, L5, L2, L3, L4, L7** (10 μM) after addition of Zn^{2+} (20 μM) under 365nm UV-lamp.

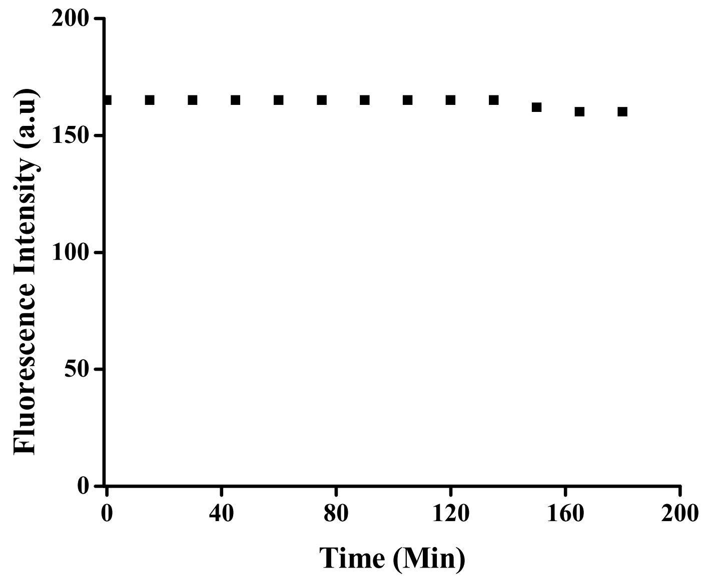

Figure S40. Effect of time on the emission spectrum of **L5** -Zn^{2+}.

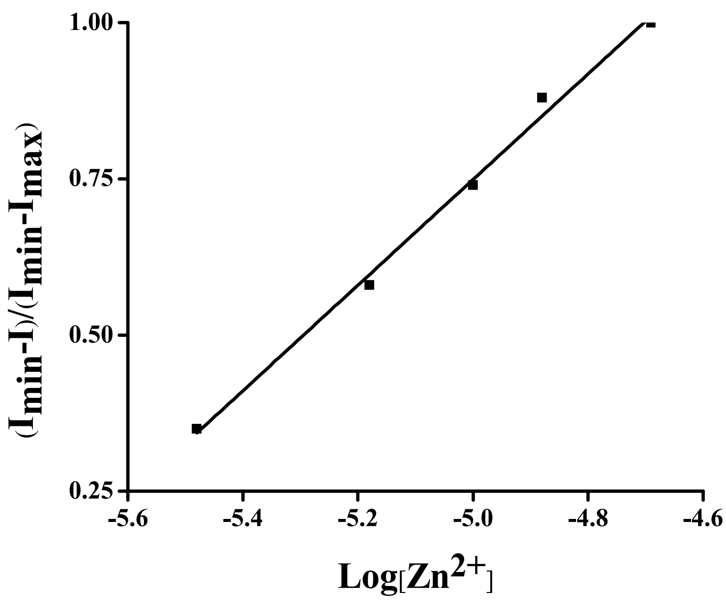

Figure S41. Calculation of limit of detection of Zn^{2+} by **L5**.

Detection limit: 1.31 µM

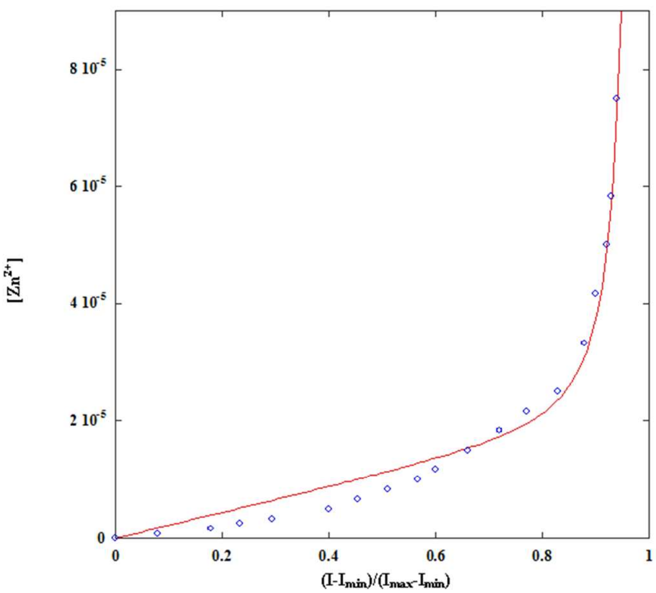

Figure S42. Binding constant calculation for the **L5**-Zn^{2+} complex.

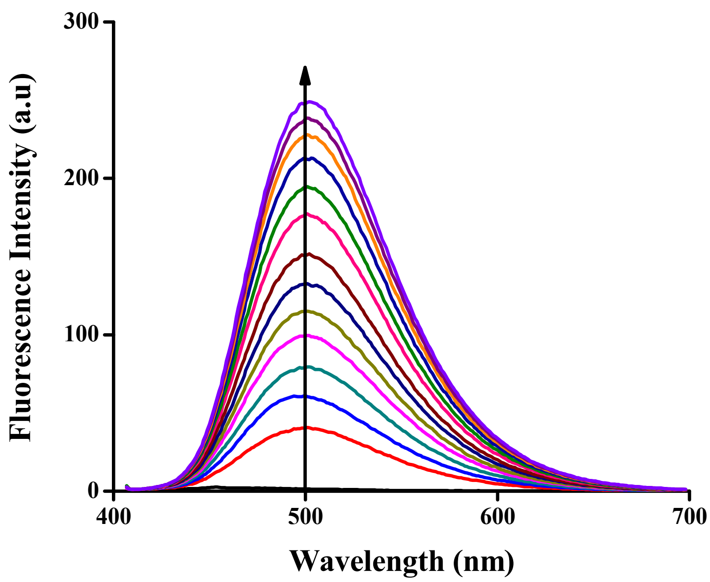

Figure S43. Emission spectrum of **L5** (50µM) in 10% HEPES buffered DMF (pH~7.4), in the presence of increasing amount of Zn^{2+} (50µM to 1000 µM).

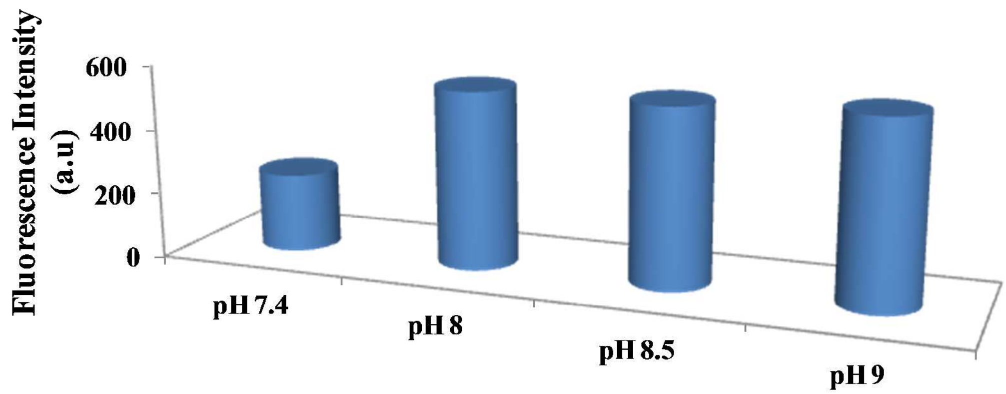

Figure S44. Fluorescence intensity of probe **L5 (**50 μM) in different pH in the presence of Zn^{2+} (1000 μM).

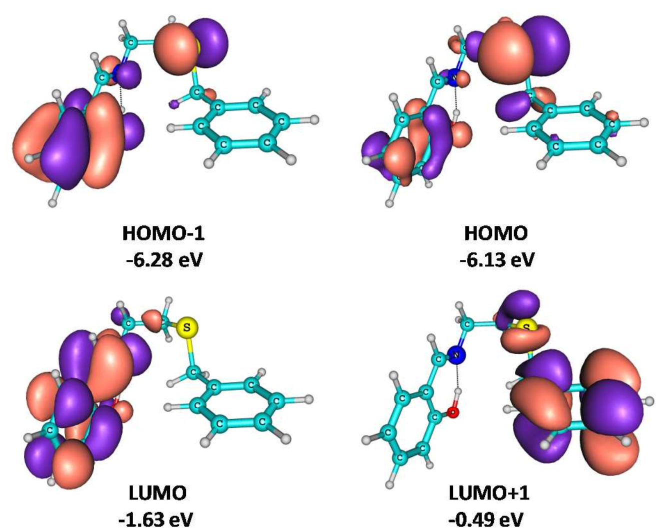

Figure S45. Frontier molecular orbitals of **L3** (isocontour at 0.03 a. u.)

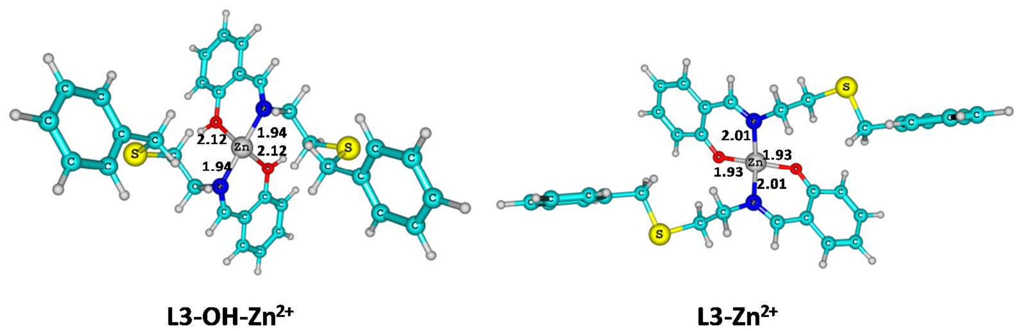

Figure S46. Optimized structure of **L3-OH-Zn^{2+}** and **L3-Zn^{2+}**

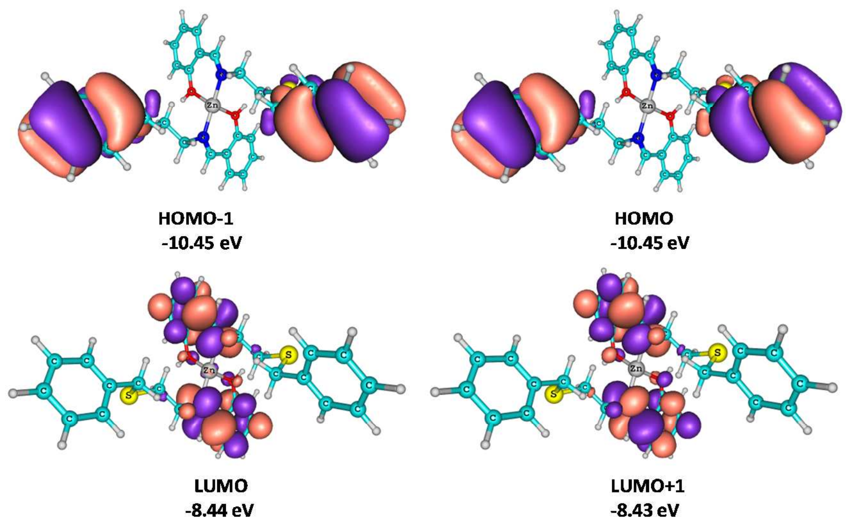

Figure S47. Frontier molecular orbitals of **L3-OH-Zn^{2+}** (isocontour at 0.03 a. u.)

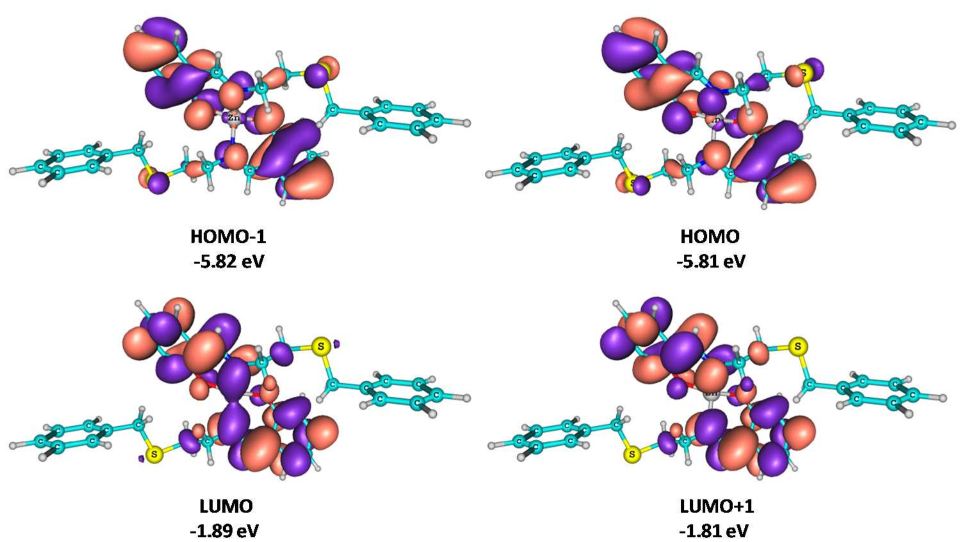

Figure S48. Frontier molecular orbitals of **L3-Zn^{2+}** (isocontour at 0.03 a. u.)

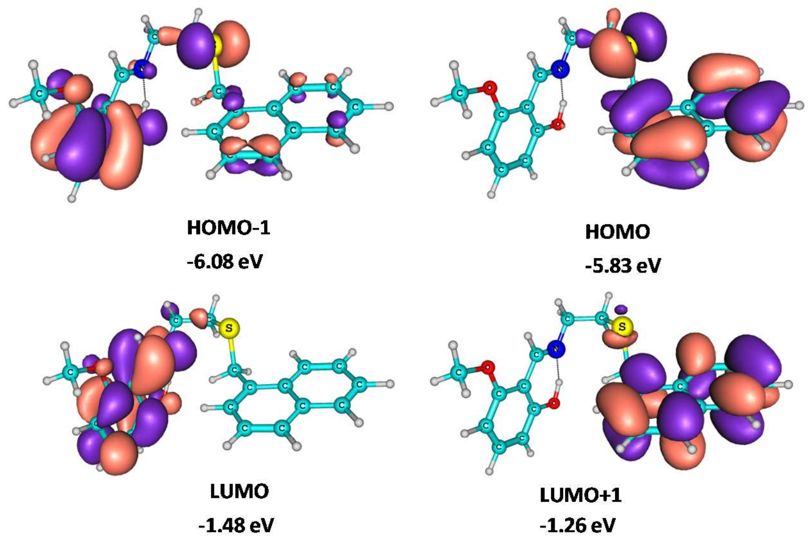

Figure S49. Frontier molecular orbitals of **L5** (isocontour at 0.03 a. u.)

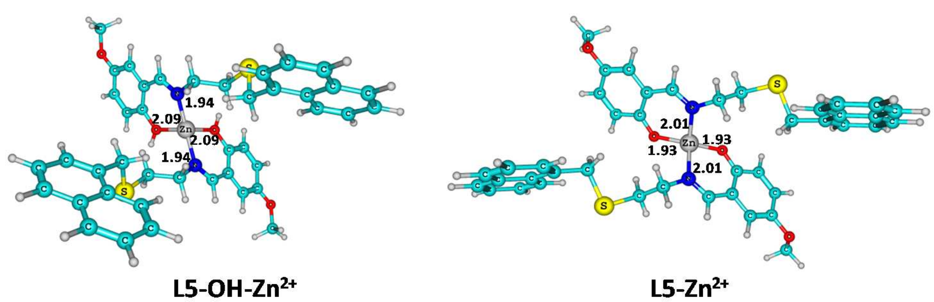

Figure S50. Optimized structure of **L5-OH-Zn^{2+}** and **L5-Zn^{2+}**

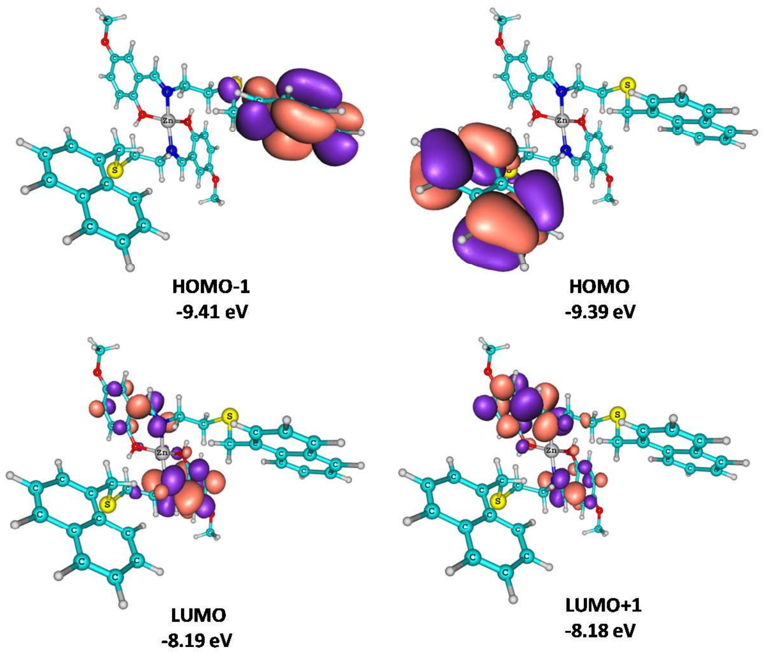

Figure S51. Frontier molecular orbitals of **L5-OH-Zn^{2+}** (isocontour at 0.03 a. u.)

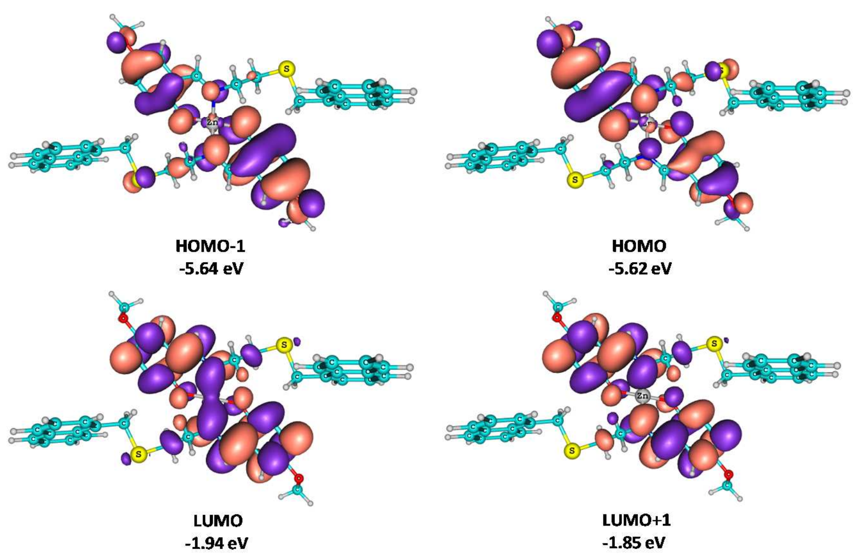

Figure S52. Frontier molecular orbitals of **L5-Zn^{2+}** (isocontour at 0.03 a. u.)

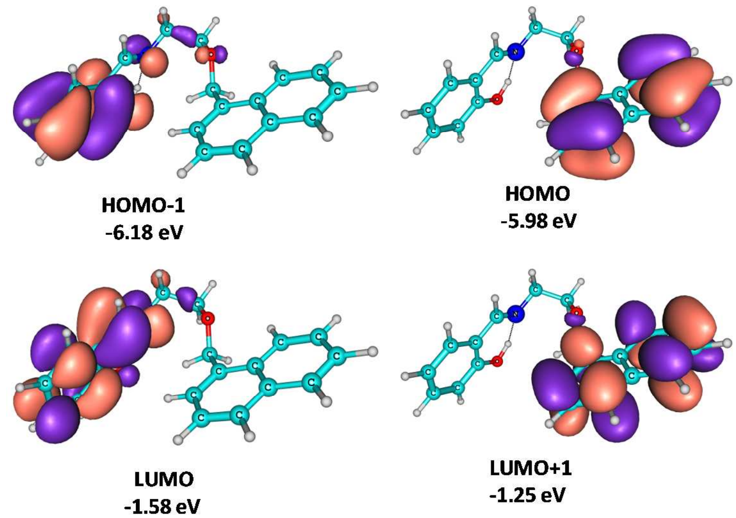

Figure S53. Frontier molecular orbitals of **L7** (isocontour at 0.03 a. u.)

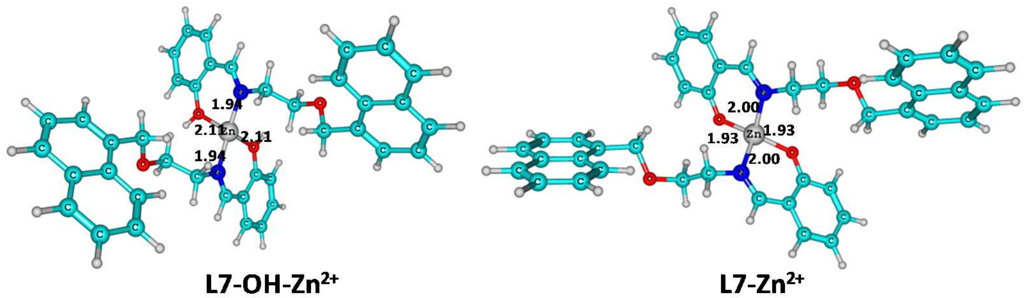

Figure S54. Optimized structure of **L7-OH-Zn^{2+}** and **L7-Zn^{2+}**

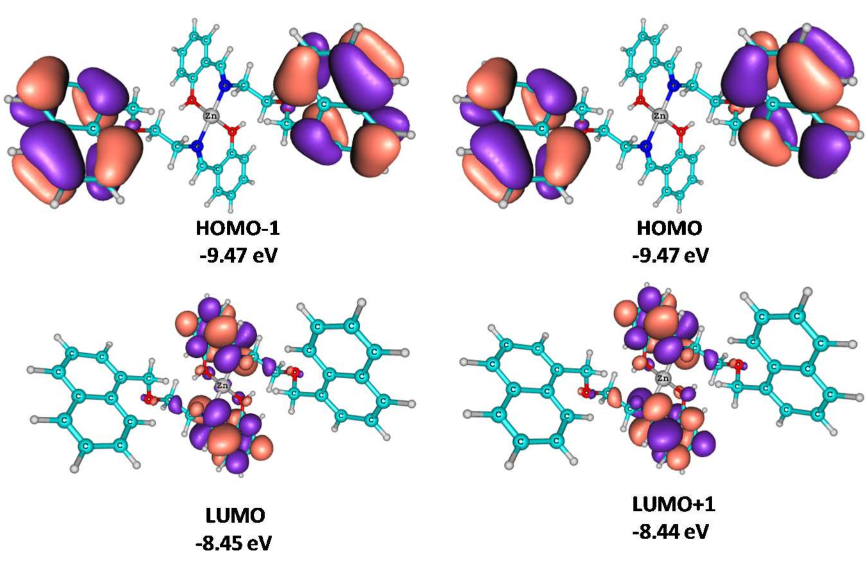

Figure S55. Frontier molecular orbitals of **L7-OH-Zn^{2+}** (isocontour at 0.03 a. u.)

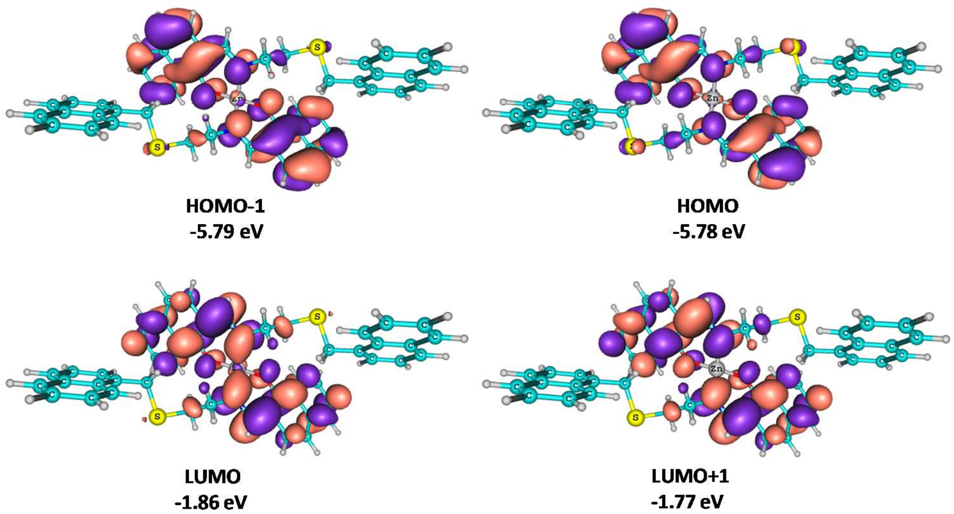

Figure S56. Frontier molecular orbitals of **L7-Zn^{2+}** (isocontour at 0.03 a. u.)

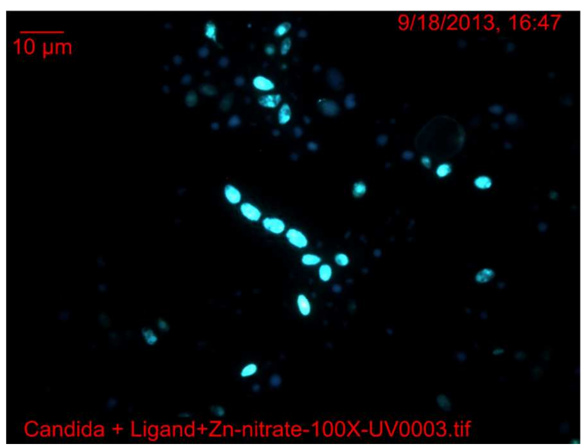

Figure S57. *Candida albicans* cells treated with **L5** followed by Zn^{2+}

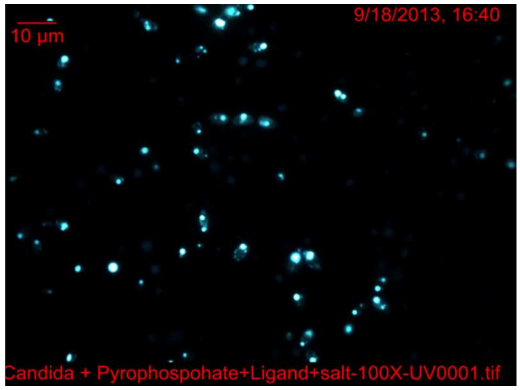

Figure S58. *Candida. albicans* cells treated with pyrophosphate followed by **L5** and then finally with Zn^{2+}

MTT assay for cell viability testing[5-8]

For assaying toxicity of a compound in in-vitro cell culture experiment, MTT assay is the assay of choice. It is commonly known as cell viability and proliferation assay. MTT stands for (3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide).

The assay depends on the reductive cleavage of the yellow tetrazolium salt MTT into dark purple coloured formazan crystals by metabolically active cells which can be measured spectrophotometrically at 570 nm.

Materials

1. Freshly prepared acidified Isopropanol solution (0.1 N HCl in absolute isopropanol)
2. MTT (5 mg/ml in PBS) – freshly prepared, filtered and kept in dark.
3. Stock solutions of Salt
4. Stock solutions of ligand
5. PBS-Phosphate buffer saline, pH 7

Procedure:

1.5 ml exponentially growing broth culture of *Candida albicans* (IMTECH No. 3018) grown in Potato Dextrose Agar medium (pH 6.0, incubation temperature, 37 ^{0}C) was centrifuged at 6,000 rpm for 5 minutes and the pellet was washed twice with normal saline and finally suspended in various solutions (as mentioned below) at cell density of ~10^{8} cell/ml. Following four experimental sets (each set composed of three tubes) are prepared.

i) Set I- Each tube contains the cells suspension without salt or ligand and incubated for 2 hours.

ii) Set II- Each tube contains the cells suspended in 200µl salt (200nM), incubated for 2 hours, then 200µl ligand (400nM) is added and again incubated for 2 hours.

iii) Set III- Each tube contains the cells suspended in 200µl salt (2µM) and incubated for 2 hours, then 200µl ligand (4µM) is added and again incubated for 2 hours.

iv) Set IV- Each tube contains the cells suspended in 200µl salt (3mM) and incubated for 2 hours, and then 200µl ligand (3mM) is added and again incubated for 2 hours.

After the incubation periods were over the cells were centrifuged at 6,000 rpm for 5 minutes and the pellet was washed twice with normal saline. Next each pellet was suspended in 3 ml PBS. 300 µl MTT was added to each tube. Two additional sets were prepared. The blank set was prepared by adding 3.3 ml PBS with no MTT. Another set is prepared which consisted of 3 ml PBS and 300 µl MTT which remained uninoculated.

All sets were incubated for 4 hrs in dark at 37^{0} C. After incubation 3 ml of freshly prepared acidic isopropanol solution was added to each tube of all seven experimental sets. Contents were mixed well and incubated for another 1 hr in dark at 37^{0} C for solubilizing the complex. After incubation O.D. of all tubes of each set were measured at 570 nm.

Assay result for MTT assay for Candida cells.

Incubation conditions	OD at 570 nm
A. Only Candida	2.89
B. Candida + Salt (200 nM) +Ligand (400 nM)	2.69
C. Candida + Salt (2μM) +Ligand (4 μM)	2.61
D. Candida + Salt (3 mM) +Ligand (3 mM)	2.43
E. Control without inoculum (with MTT Salt only)	0.21

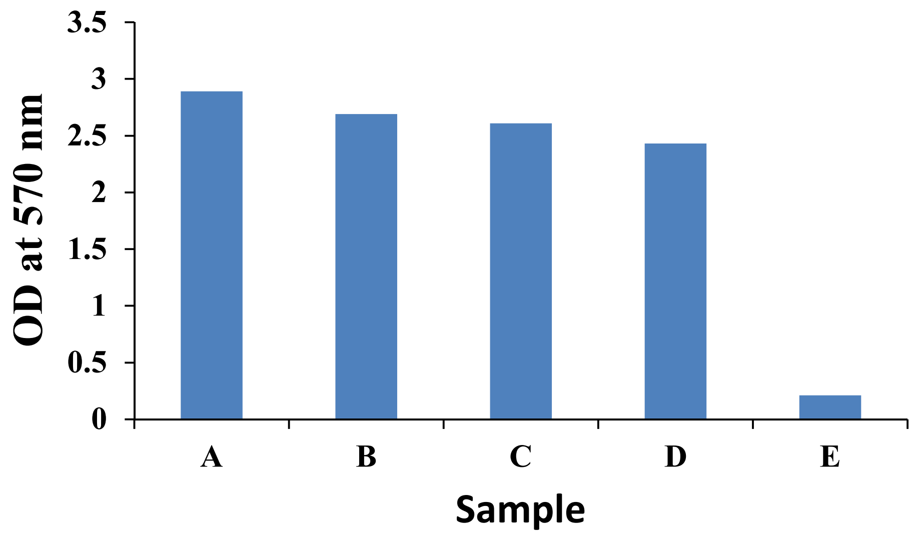

Figure S59. Graphical presentation of results of MTT assay: (A) Only *Candida*; (B) *Candida* + Zn^{2+} (200 nM) + **L5** (400 nM); (C) *Candida* + Zn^{2+} (2 μM) + **L5** (4 μM); (D) *Candida* + Zn^{2+} (3 mM) + **L5** (3 mM); (E) Control without inoculum (with MTT Salt only).

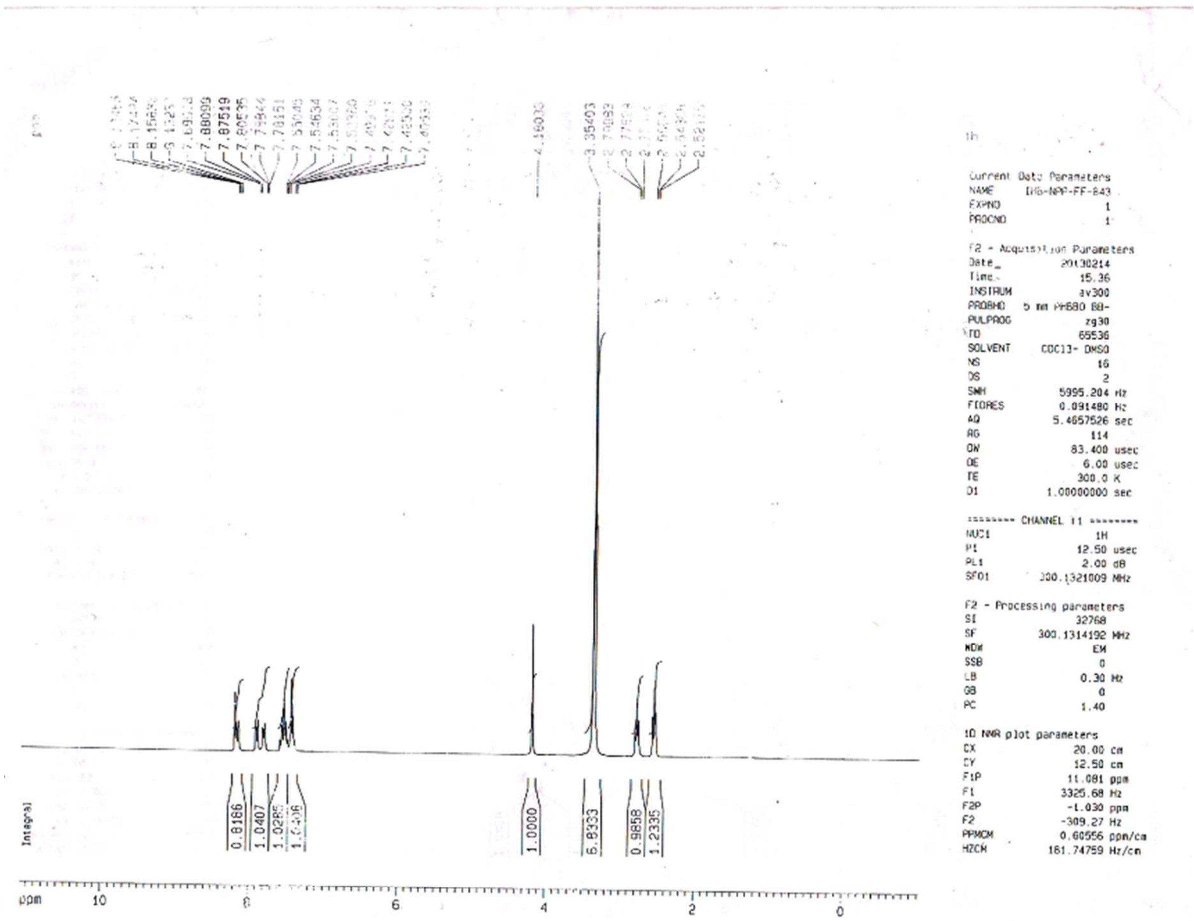

Figure S60. ^{1}H NMR of **β-naphthaylmercaptoethylamine**

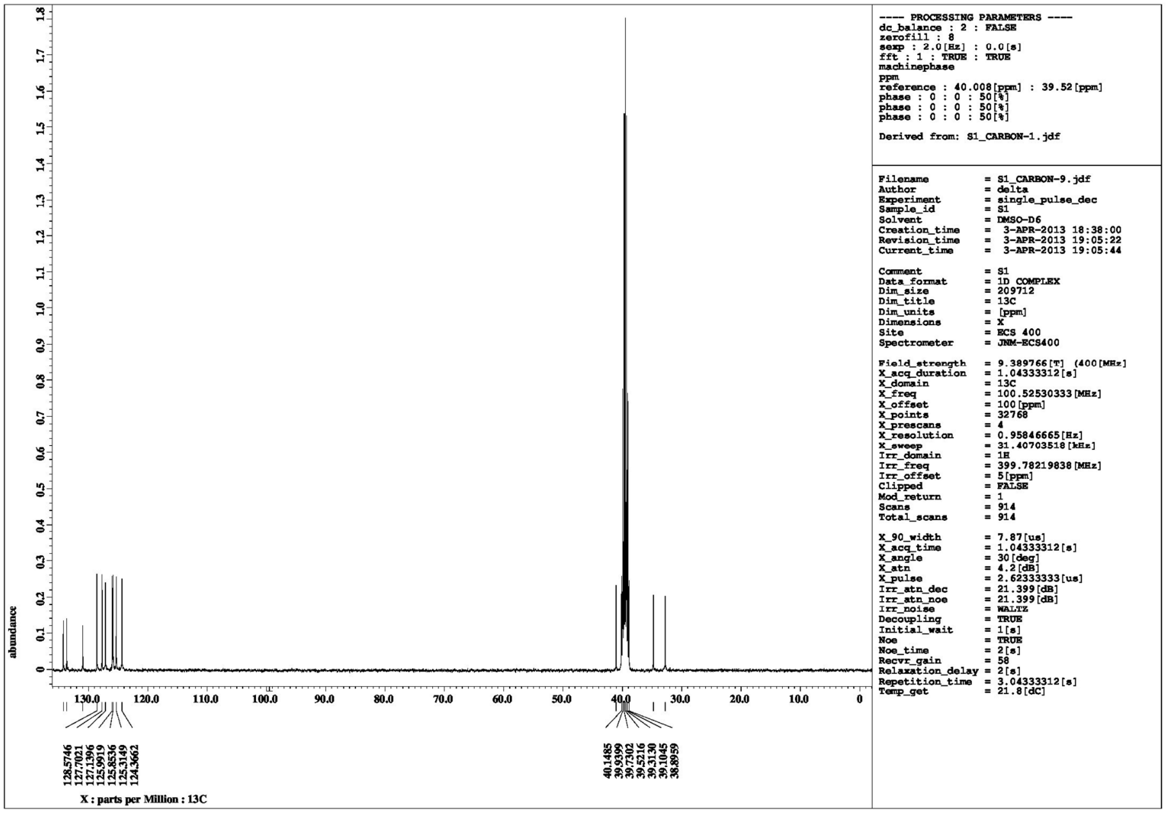

Figure S61. ^{13}C NMR of β-naphthaylmercaptoethylamine

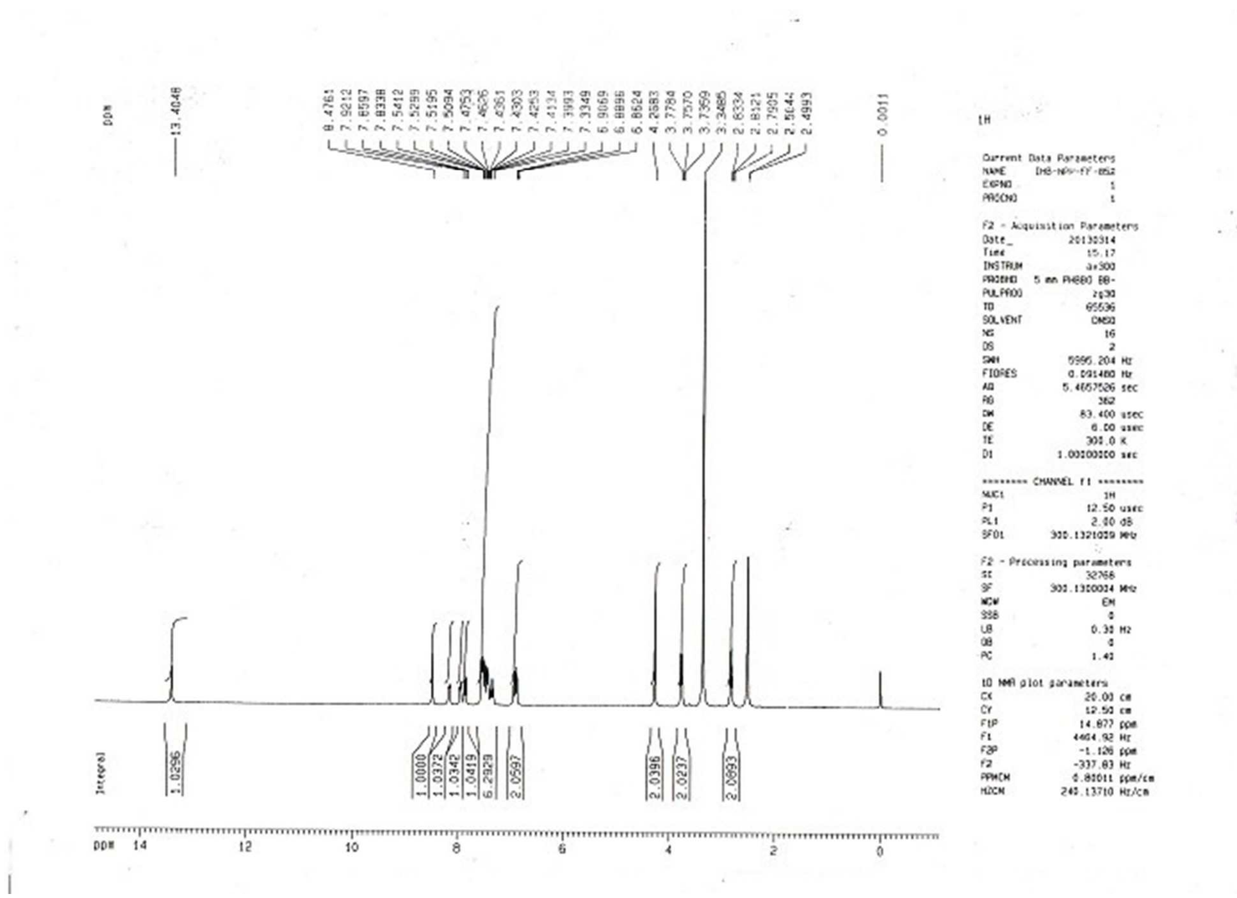

Figure S62. ^{1}H NMR OF **L1**

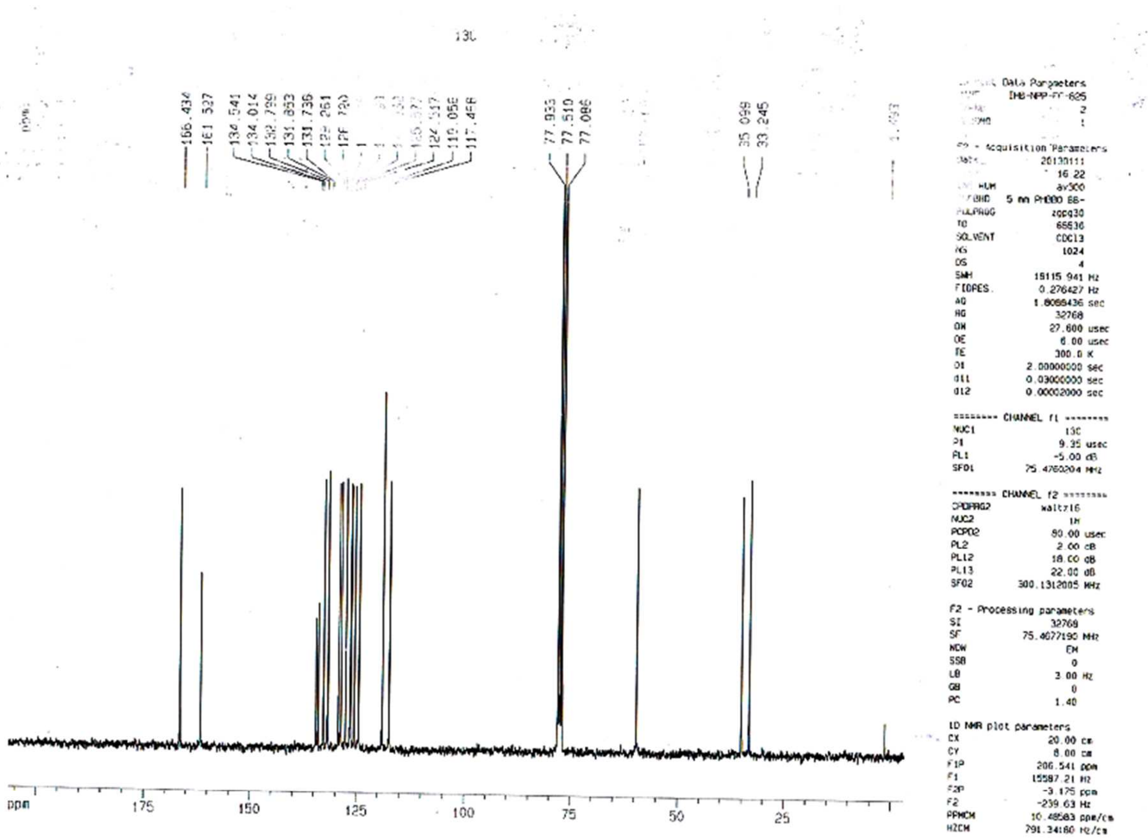

Figure S63. ^{13}C NMR OF **L1**

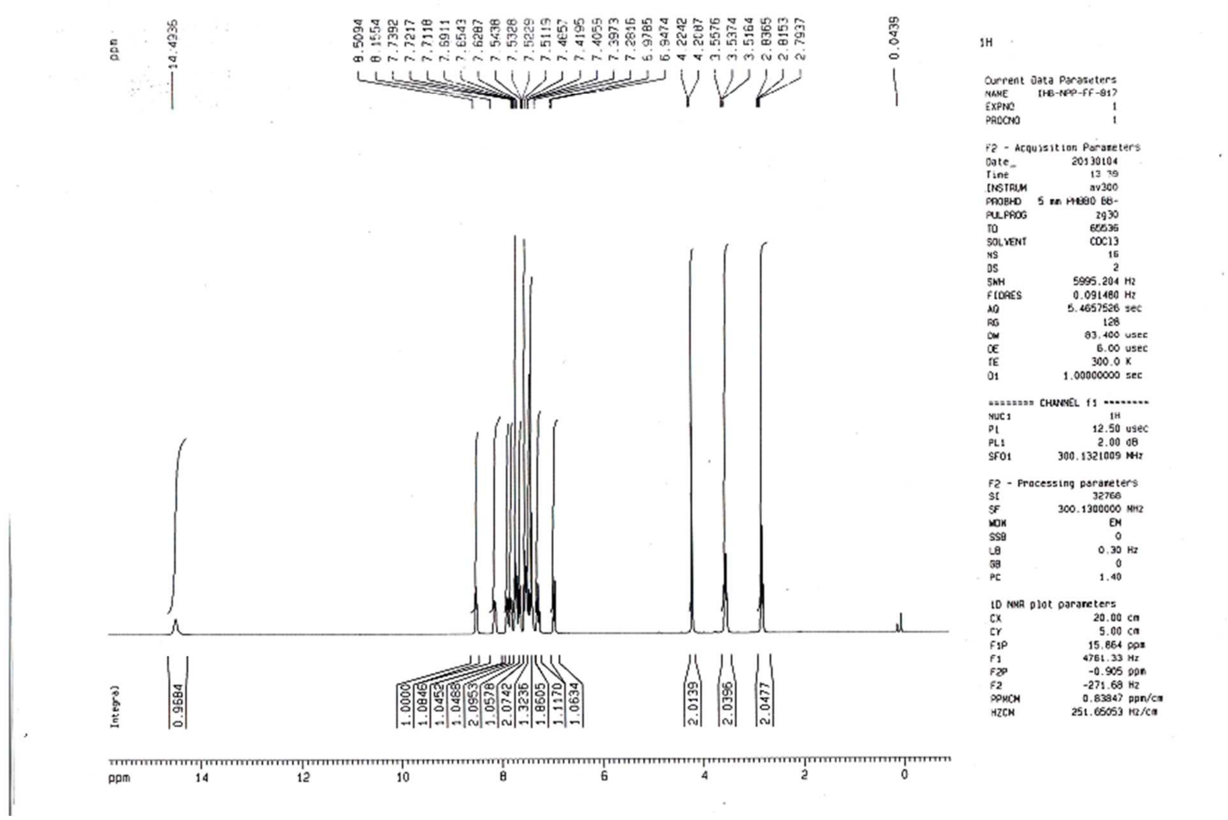

Figure S64. ^{1}H NMR OF **L2**

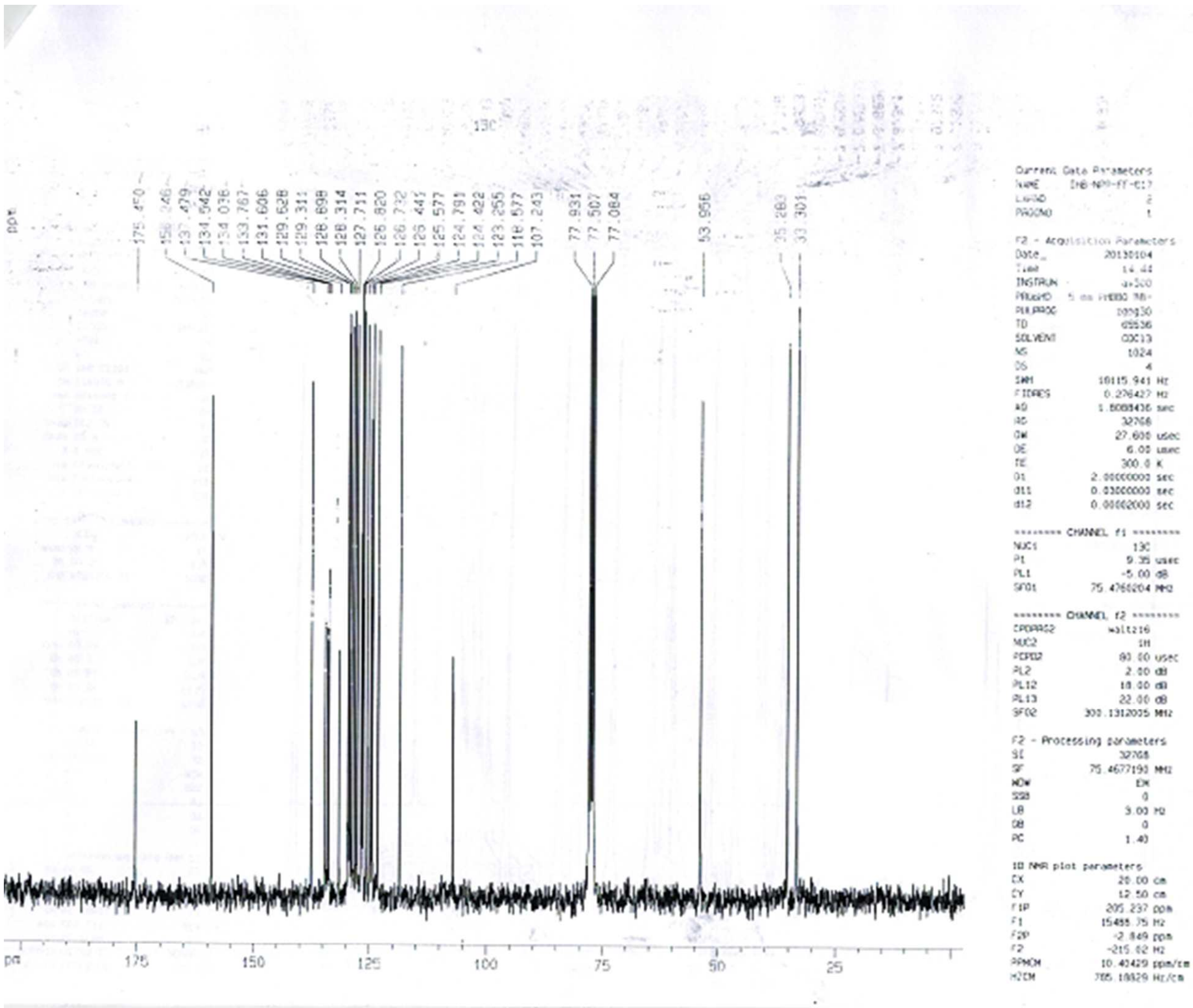

Figure S65. ^{13}C NMR OF **L2**

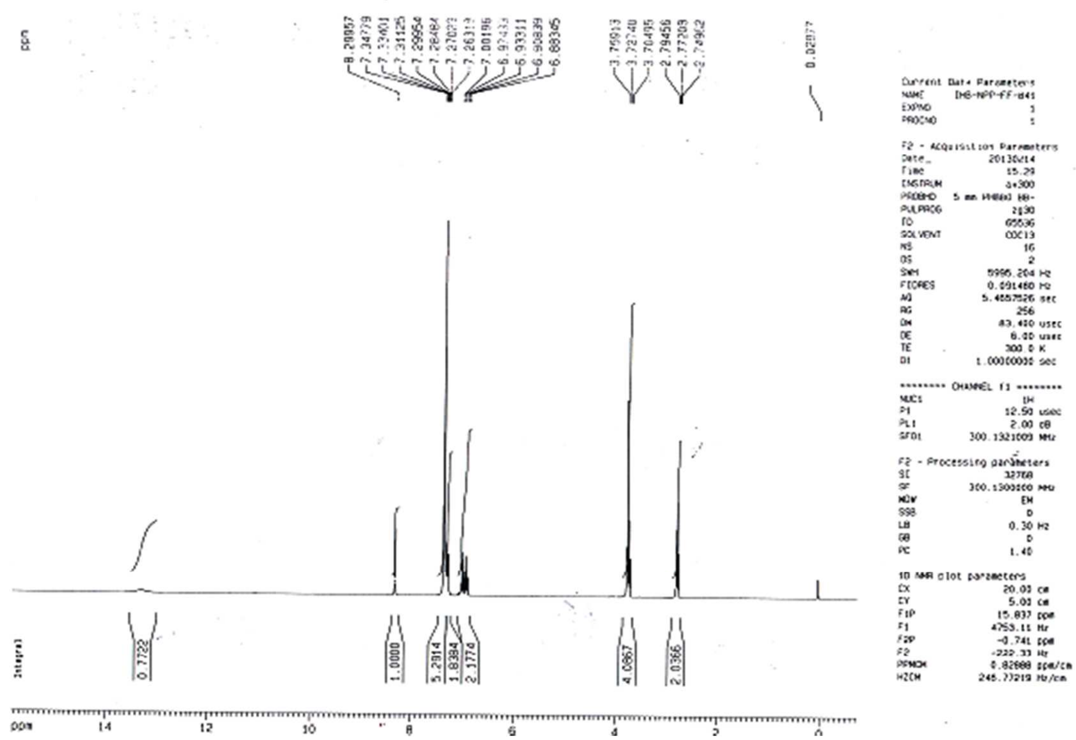

Figure S66. ^{1}H NMR OF **L3**

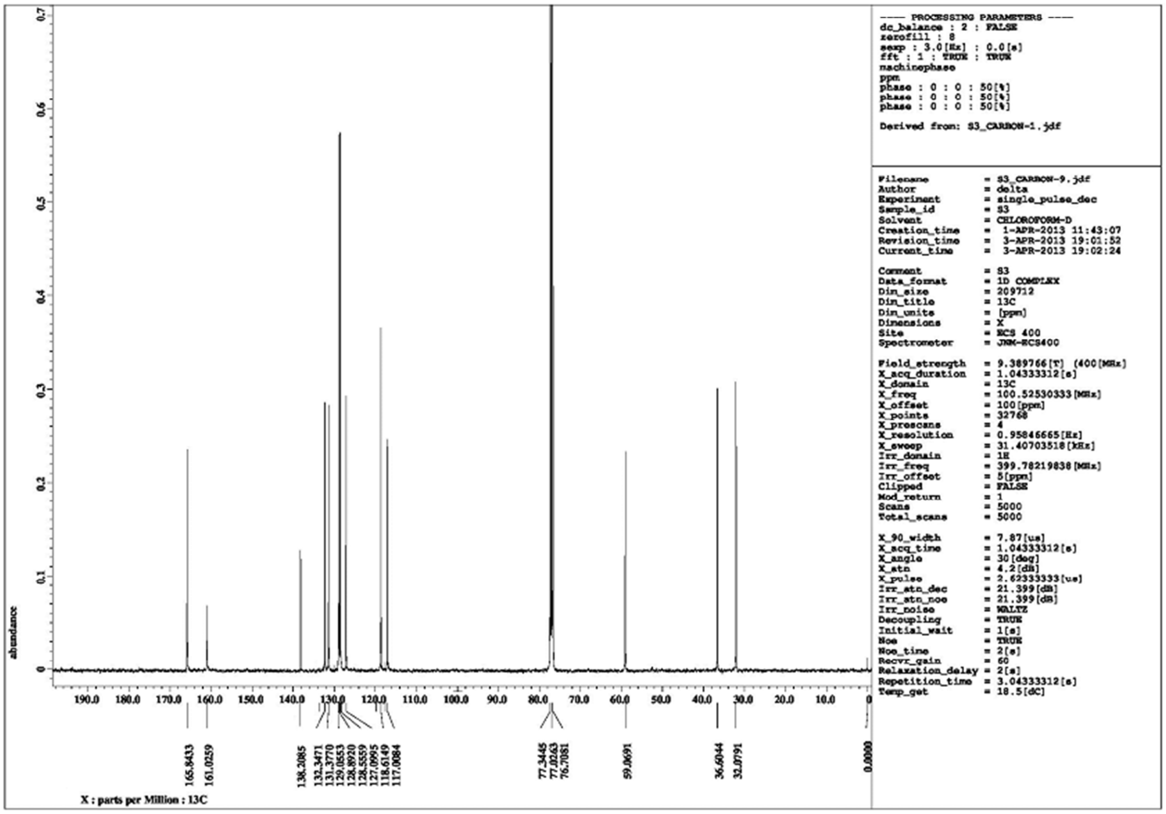

Figure S67. ^{13}C NMR OF **L3**

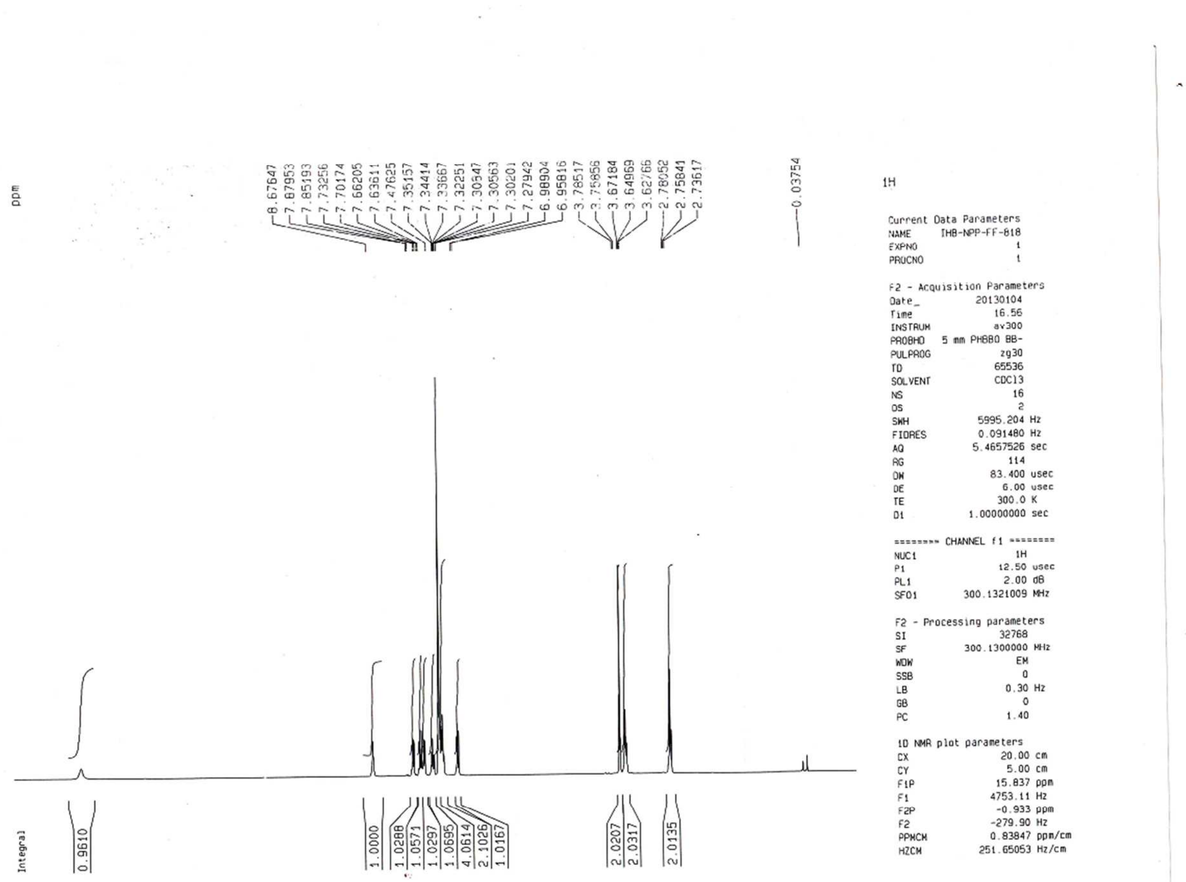

Figure S68. ^{1}H NMR OF **L4**

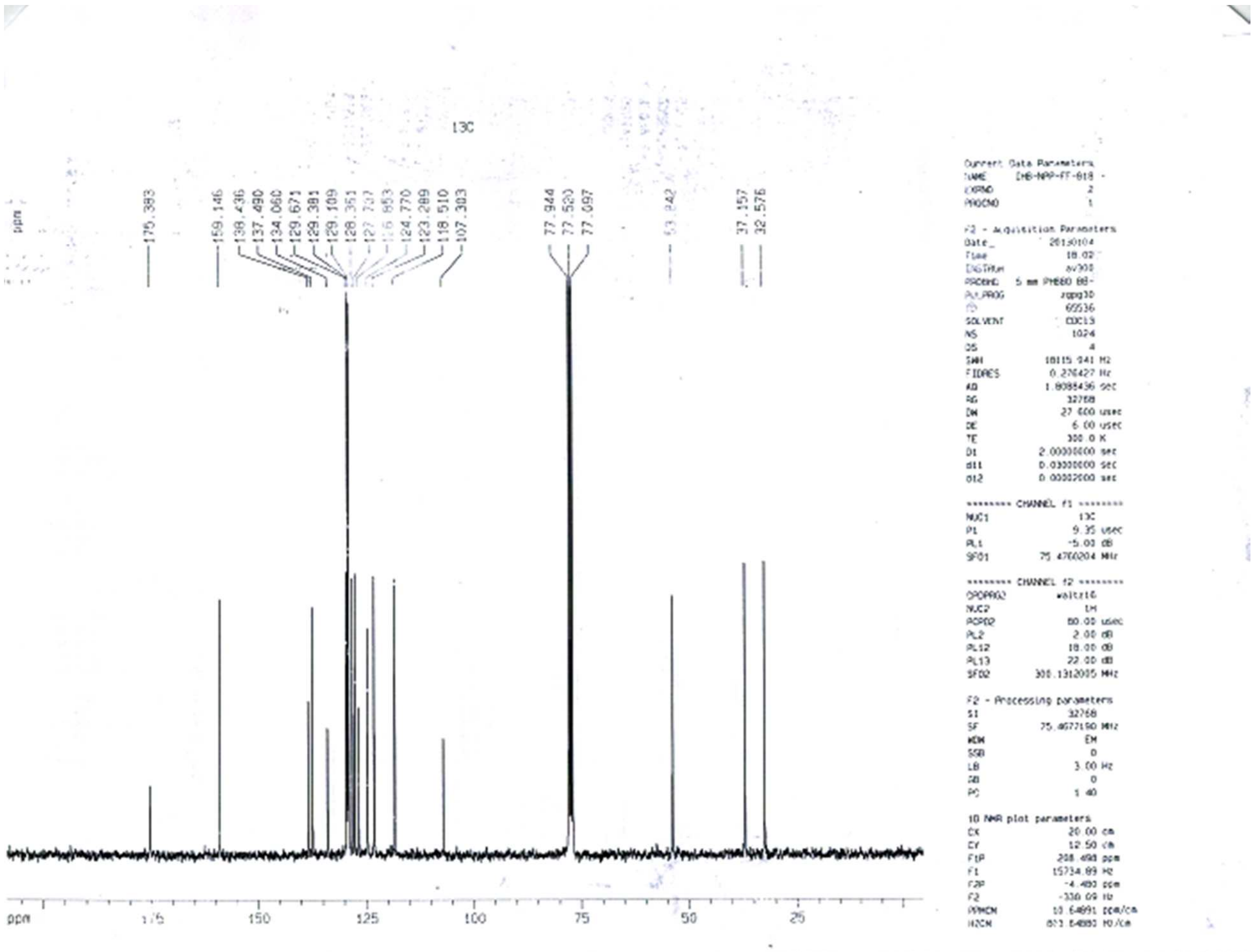

Figure S69. ^{13}C NMR OF **L4**

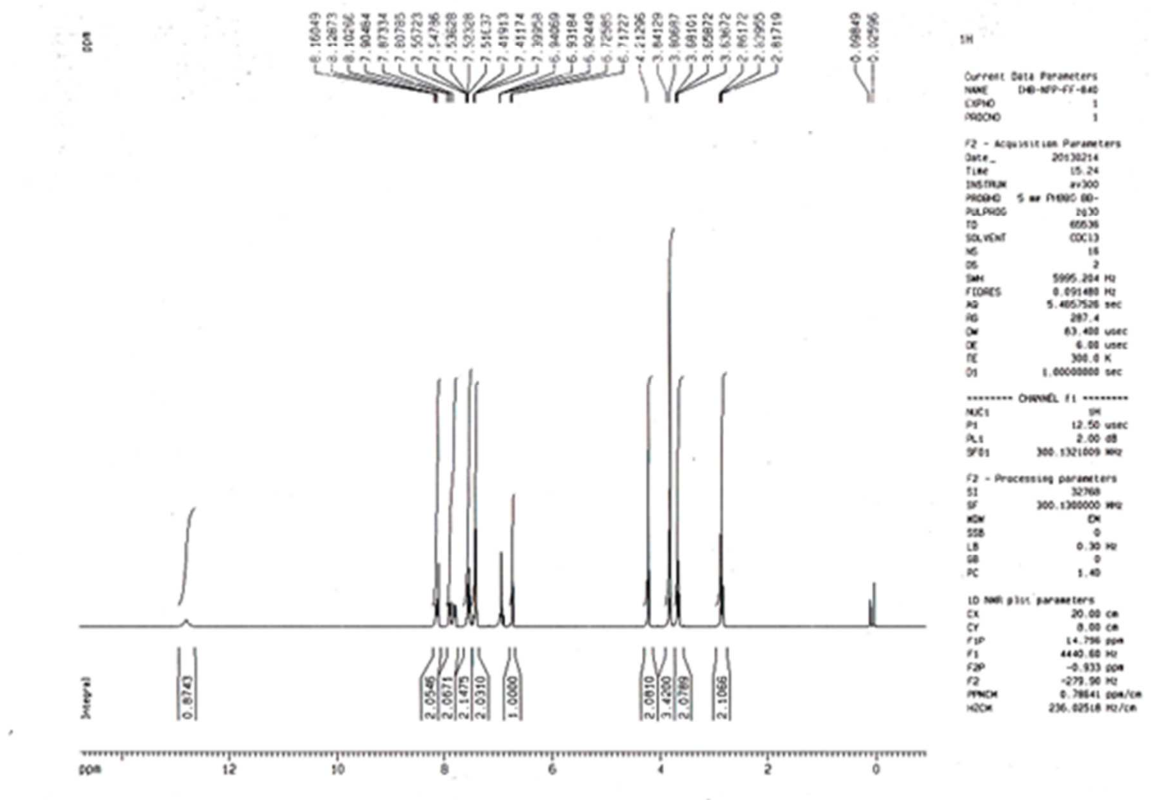

Figure S70. ^{1}H NMR OF **L5**

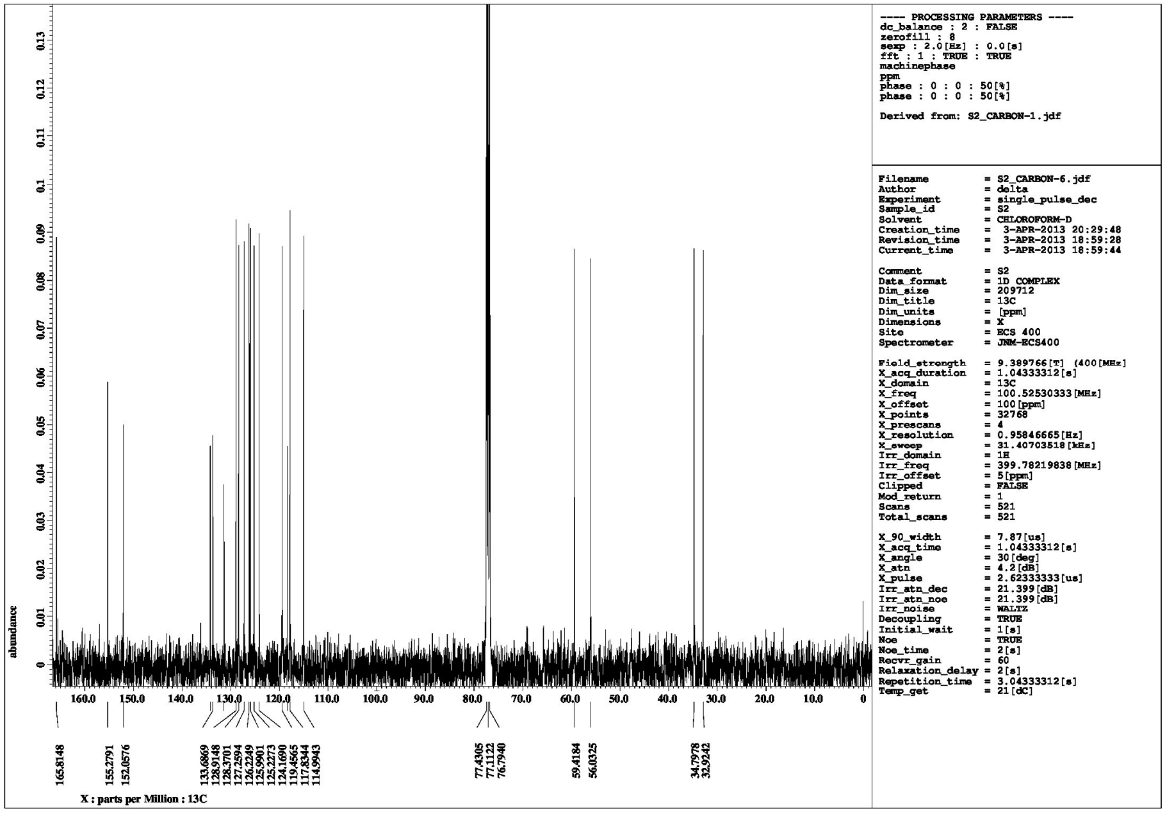

Figure S71. ^{13}C NMR OF **L5**

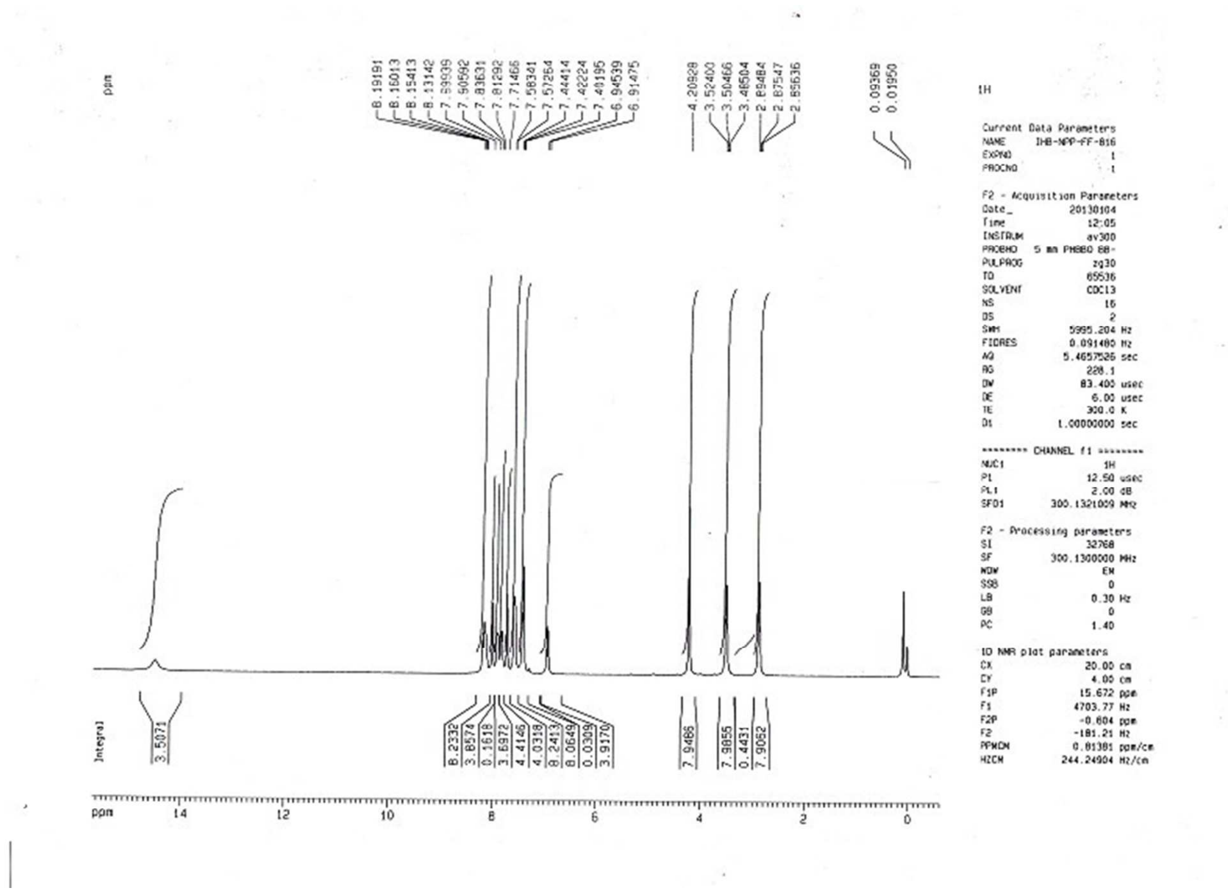

Figure S72. ^{1}H NMR OF **L6**

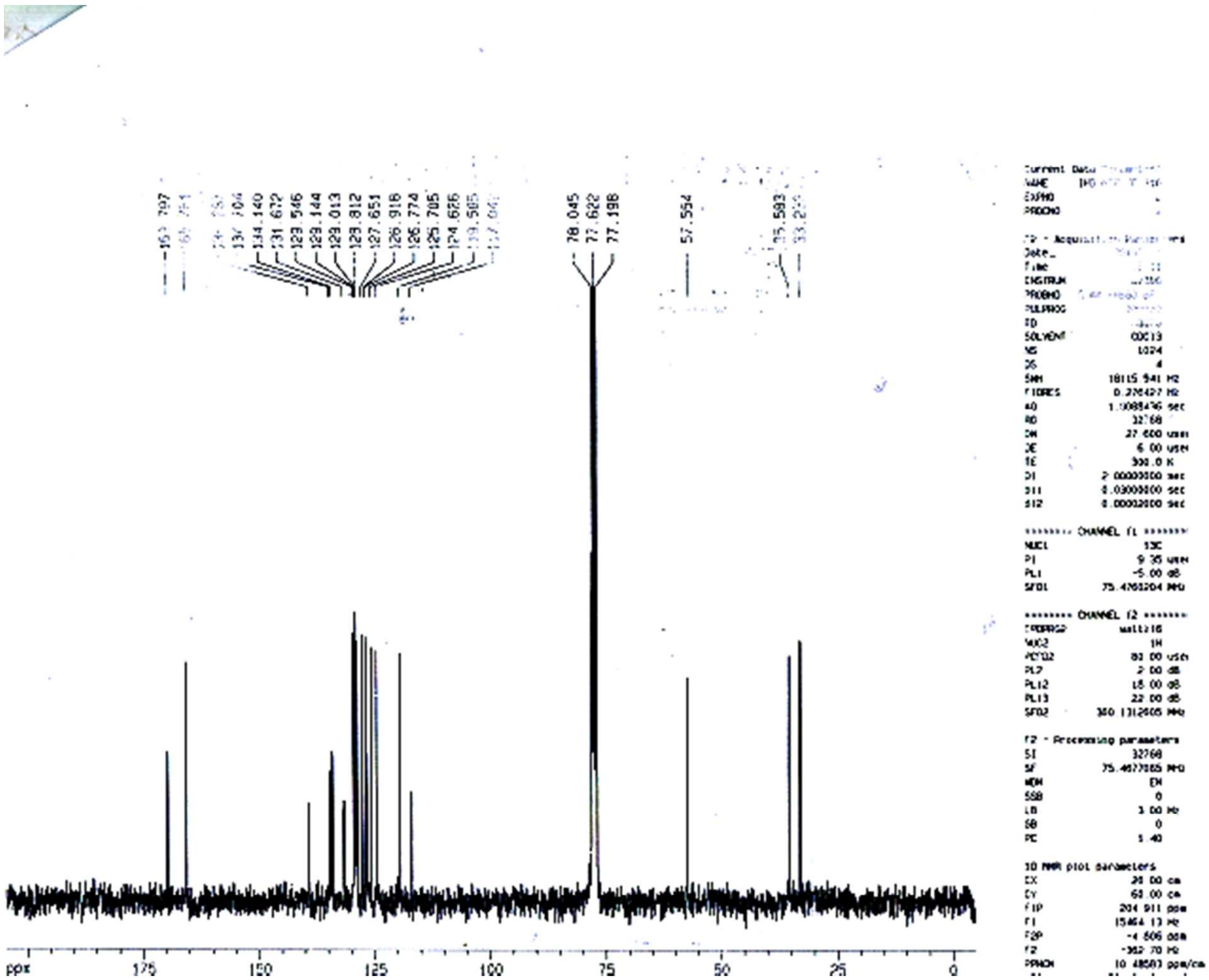

Figure S73. ^{13}C NMR OF **L6**

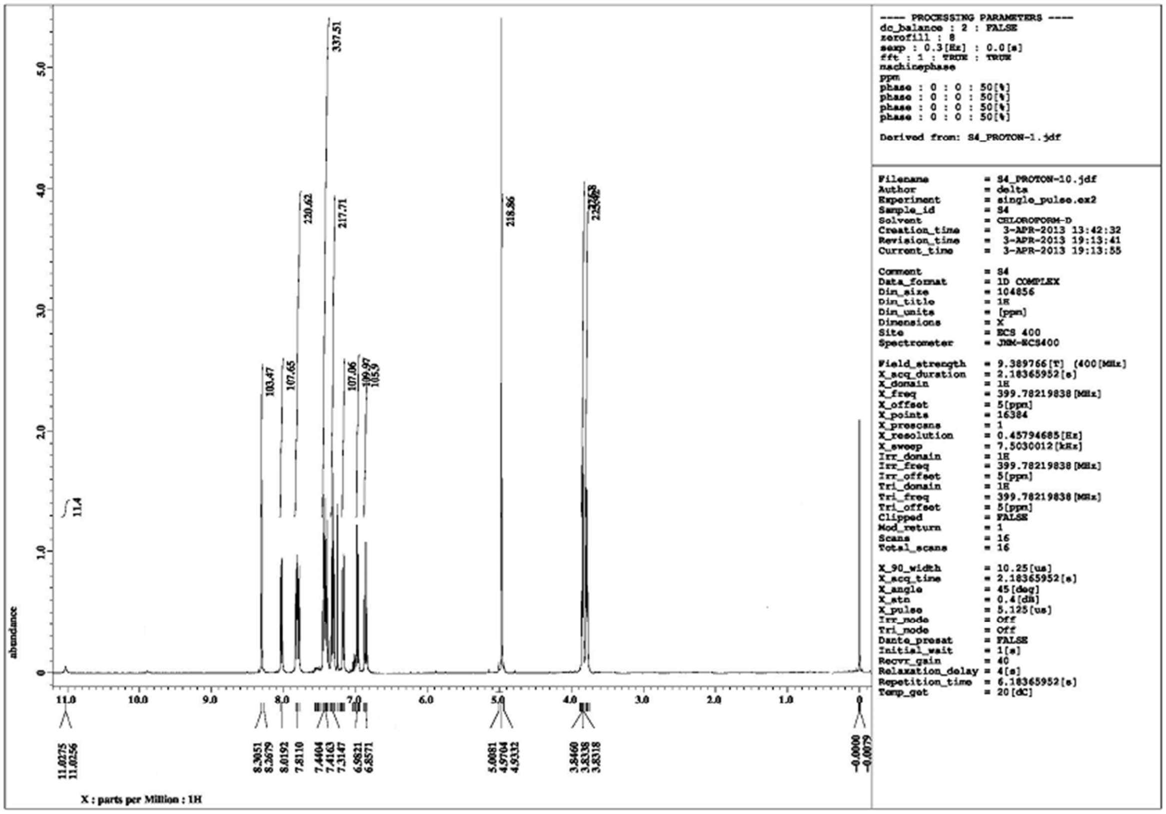

Figure S74. ^{1}H NMR OF **L7**

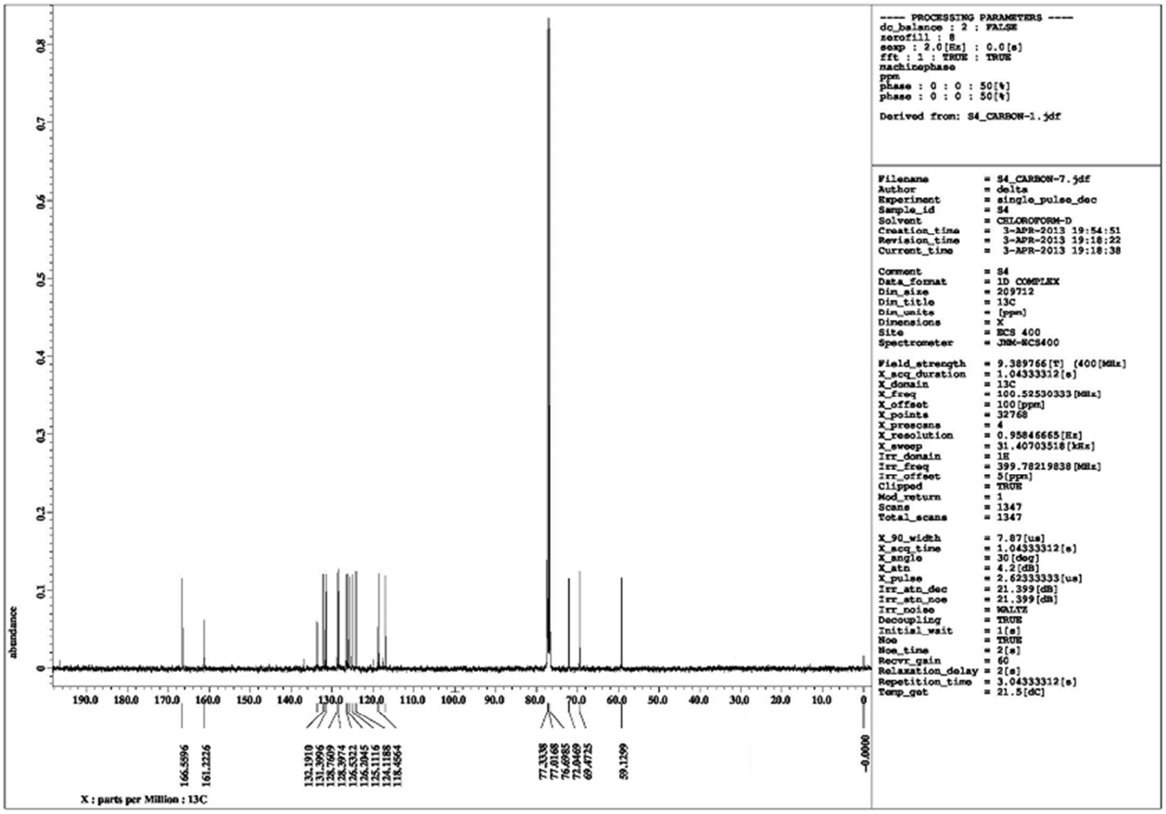

Figure S75. ^{13}C NMR OF **L7**

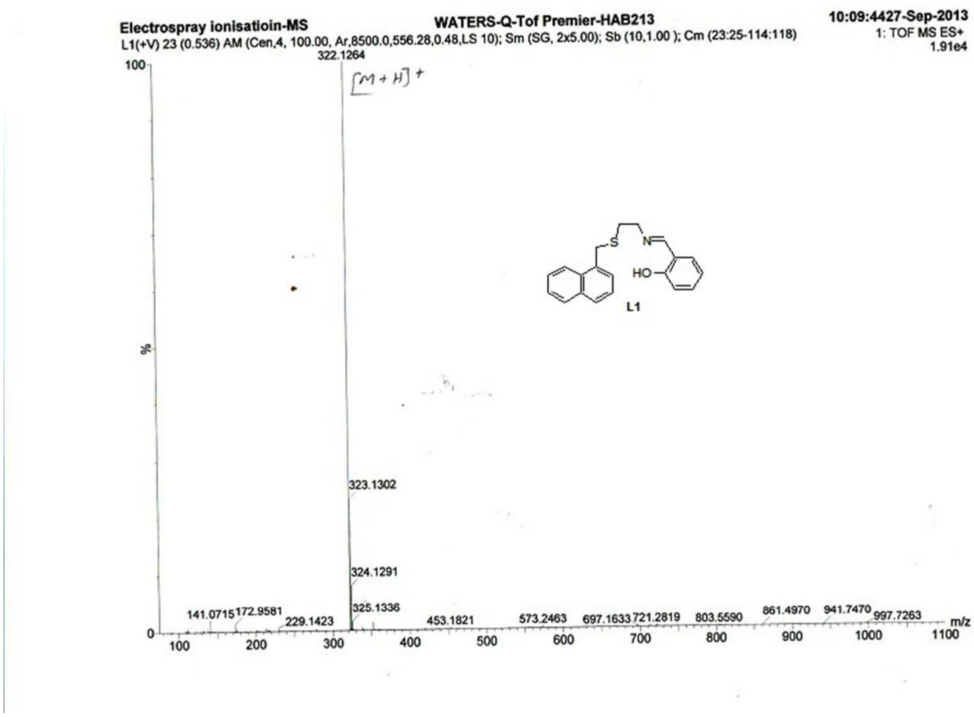

Figure S76. HRMS spectra of **L1**

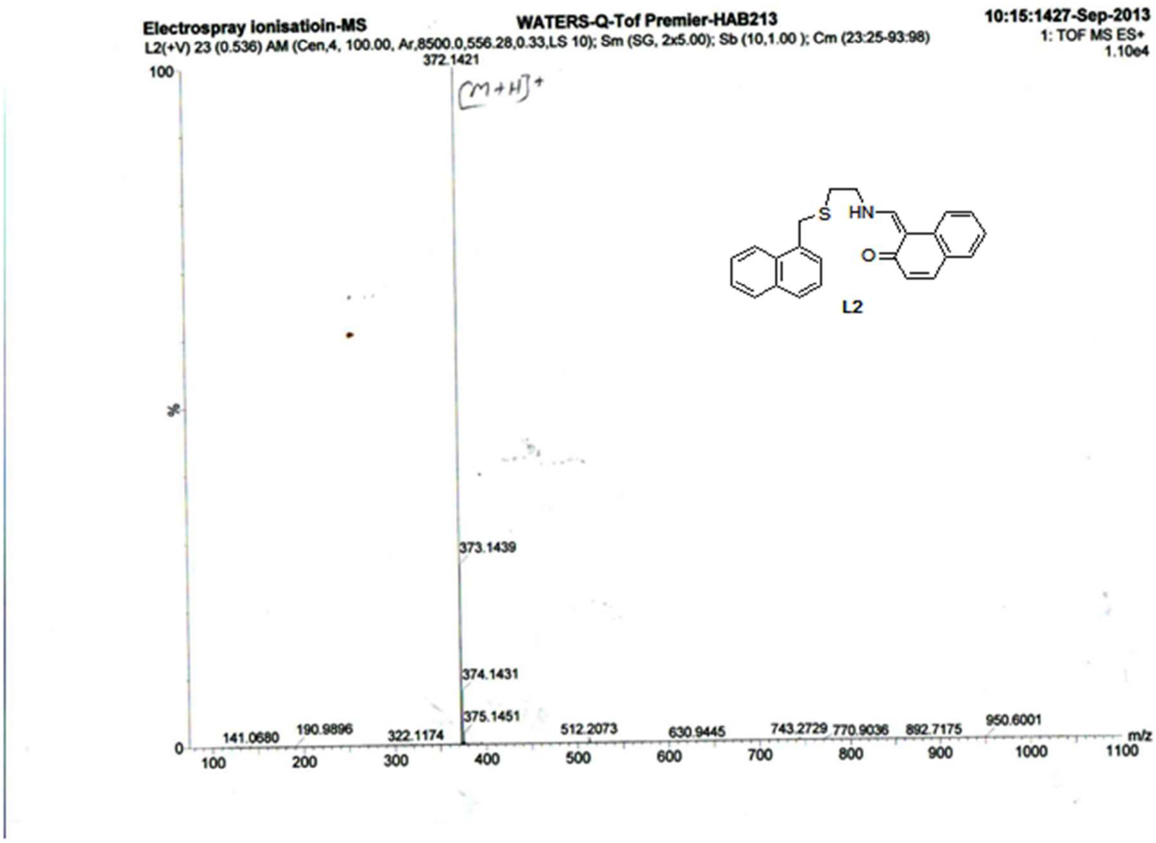

Figure S77. HRMS spectra of **L2**

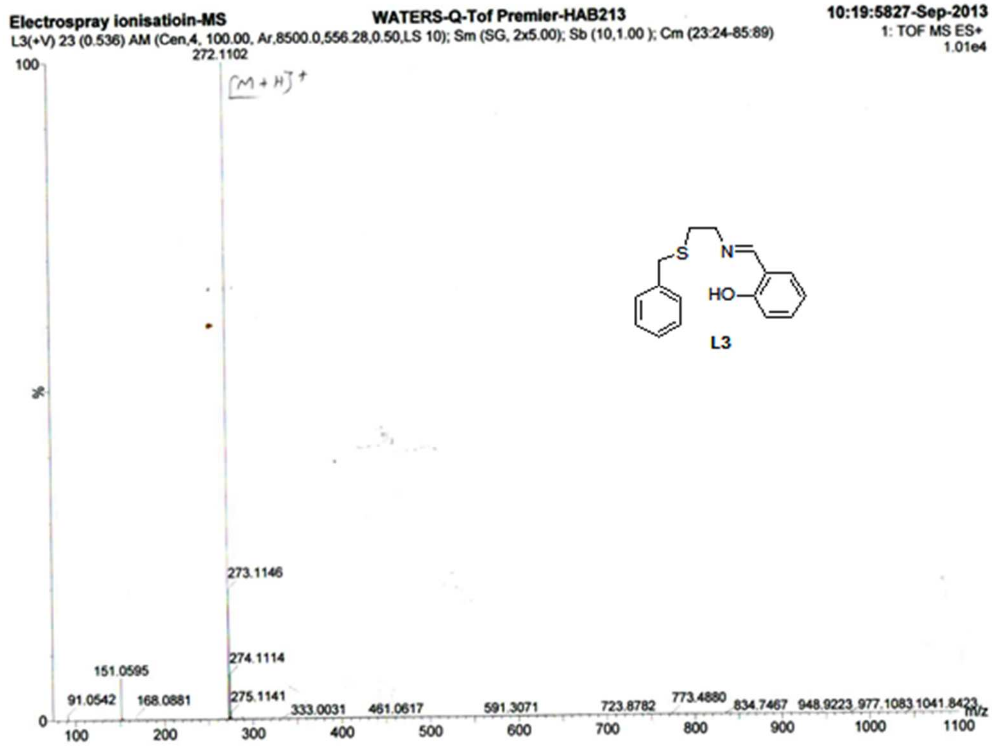

Figure S78. HRMS spectra of **L3**

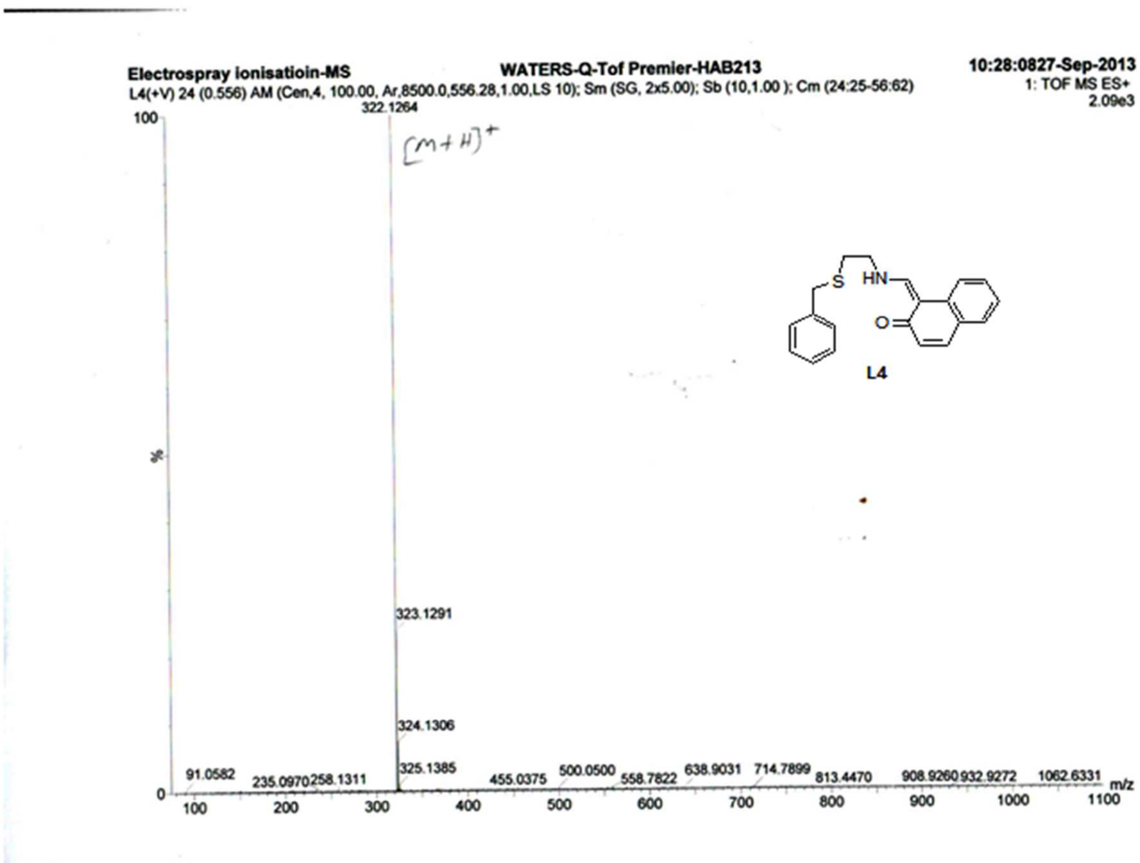

Figure S79. HRMS spectra of **L4**

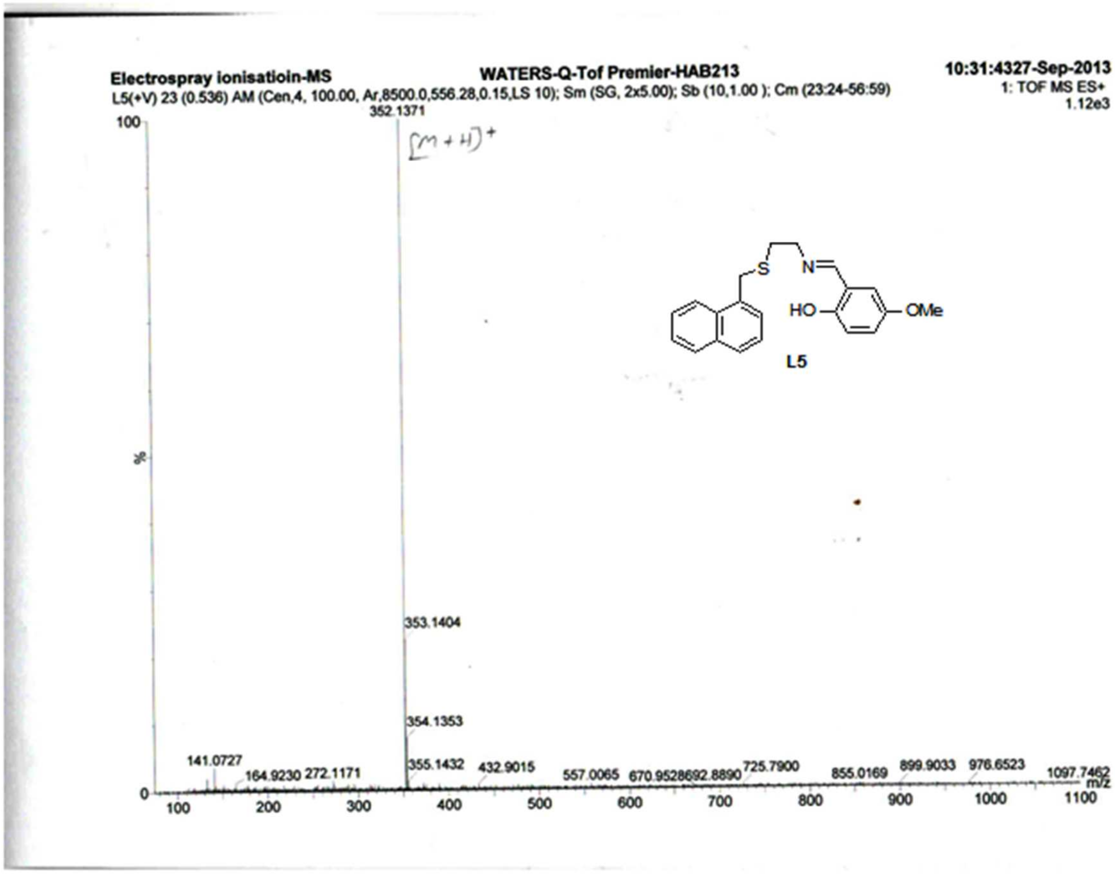

Figure S80. HRMS spectra of **L5**

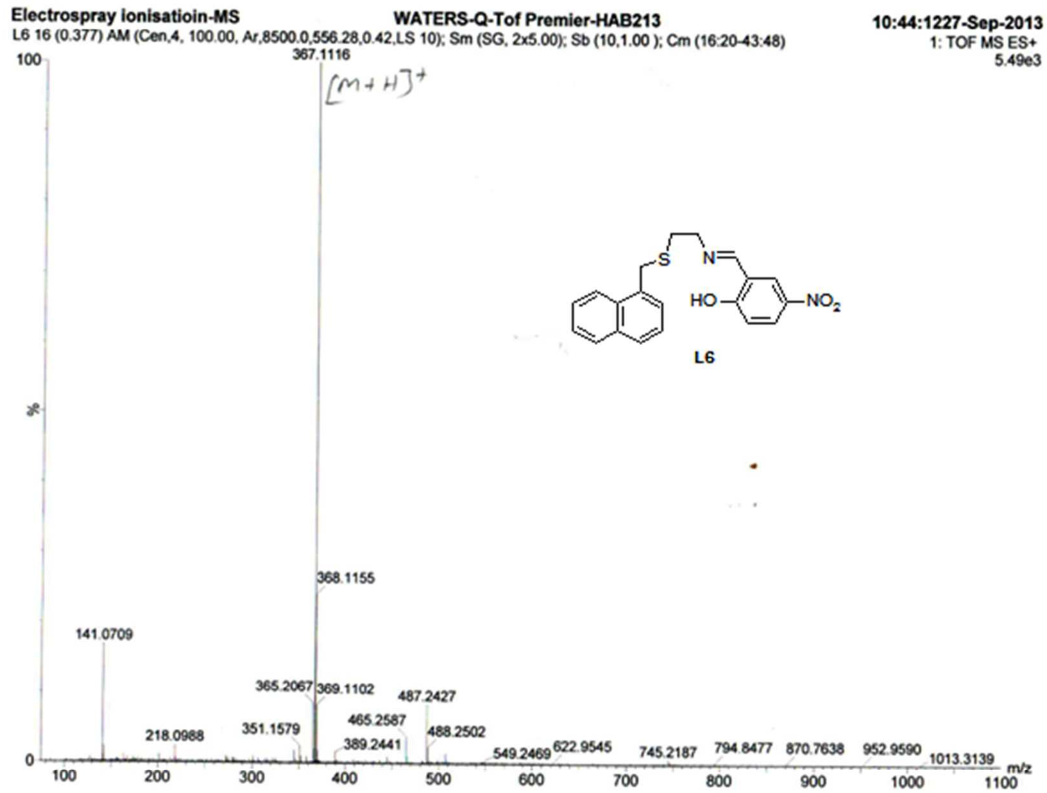

Figure S81. HRMS spectra of **L6**

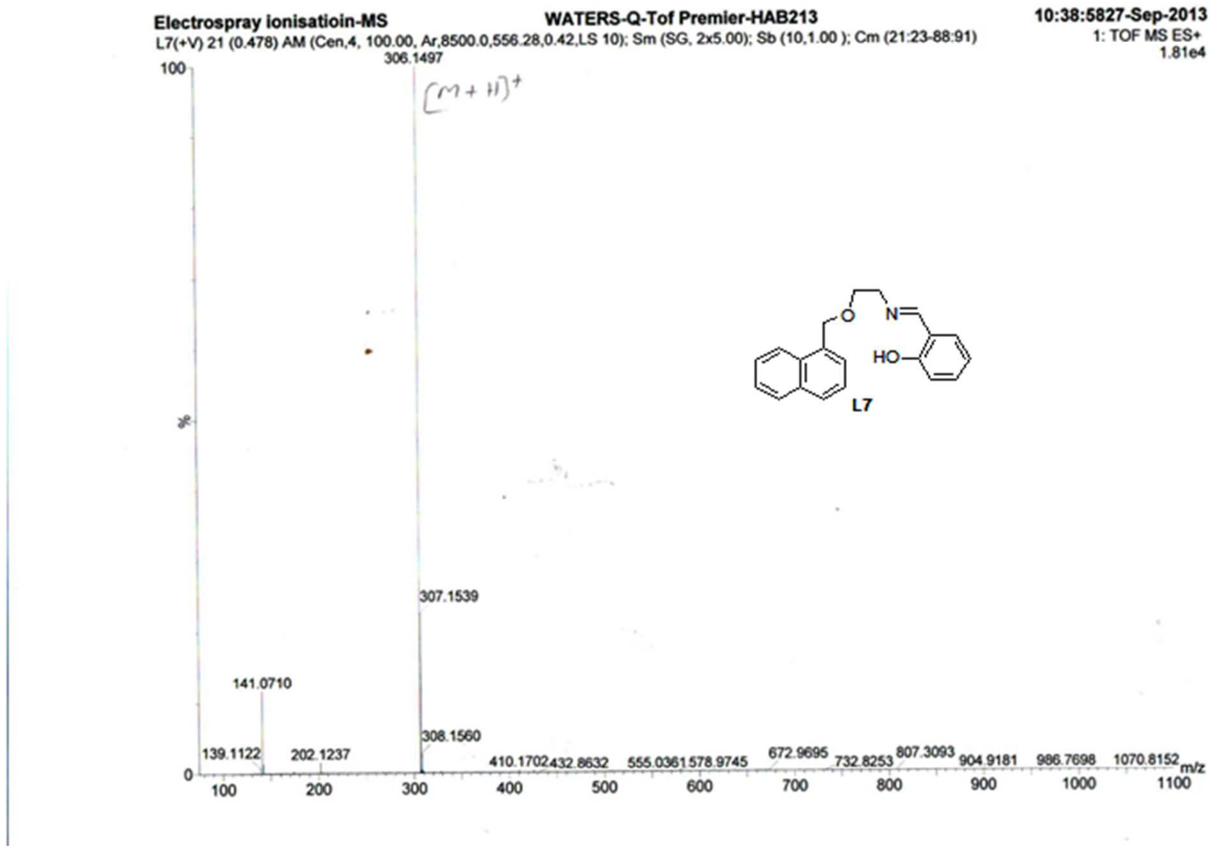

Figure S82. HRMS spectra of **L7**

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