Electronic Supplementary Material (ESI) for Dalton Transactions.

Highly photostable zinc selective molecular marker bearing flexible pivotal unit: opto-fluorescence enhancement effect and imaging applications in living system

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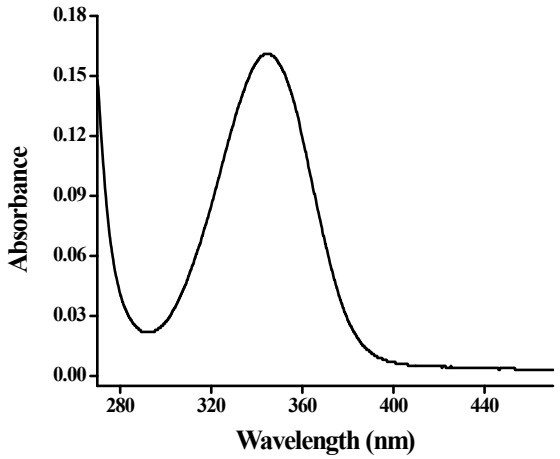

Fig. S1 UV-vis spectra of **L1** (20 μM) in dimethylformamide.

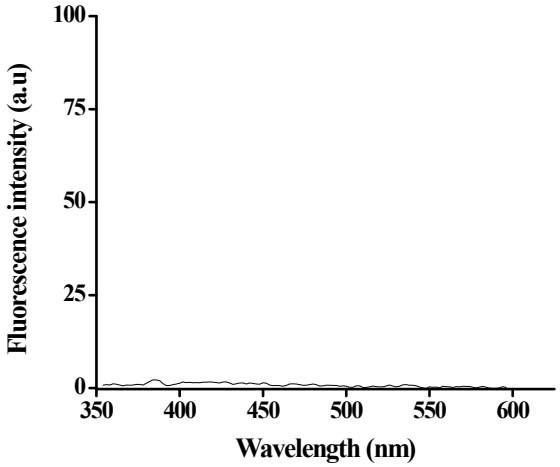

Fig. S2 Emission spectra of **L1** (20 μM) in dimethylformamide.

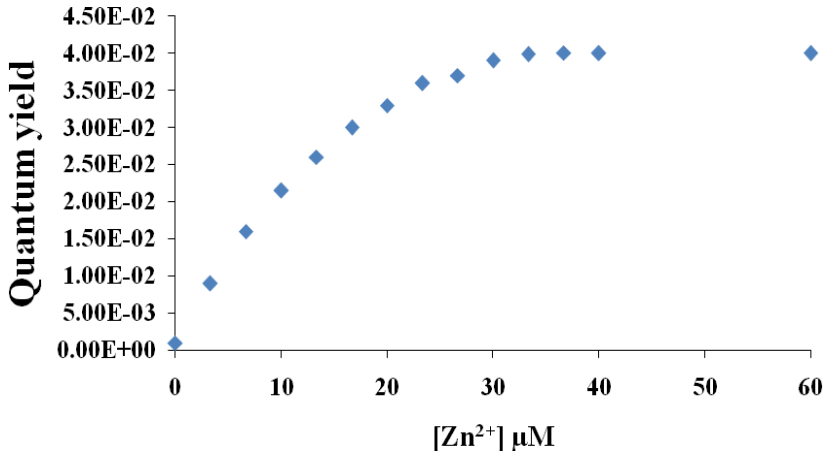

Fig. S3 Increase in quantum yield of **L1** (20 µM) in dimethylformamide in the presence of different concentration Zn^{2+}.

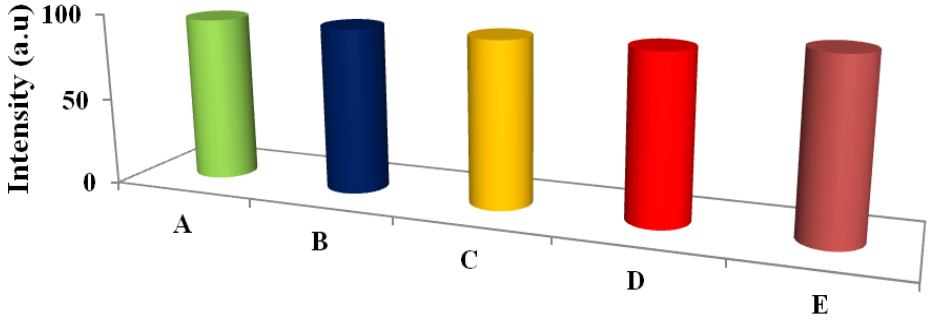

Fig. S4 Effect of competitive biologically important metal ions on Zn^{2+} induced fluorescence of **L1.** A, B, C, D, E implies $Na^{+} + Zn^{2+}$, $K^{+} + Zn^{2+}$, $Ca^{2+} + Zn^{2+}$, $Mg^{2+} + Zn^{2+}$, $Na^{+} + K^{+} + Ca^{2+} + Mg^{2+} + Zn^{2+}$ respectively.

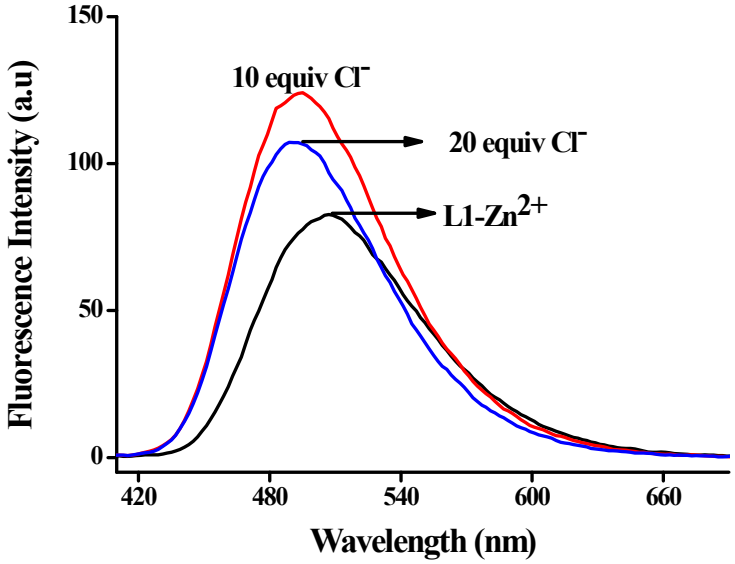

Fig. S5 Fate of Zn^{2+} induced fluorescence of **L1** in the presence of Cl^- (0, 10 and 20 equiv).

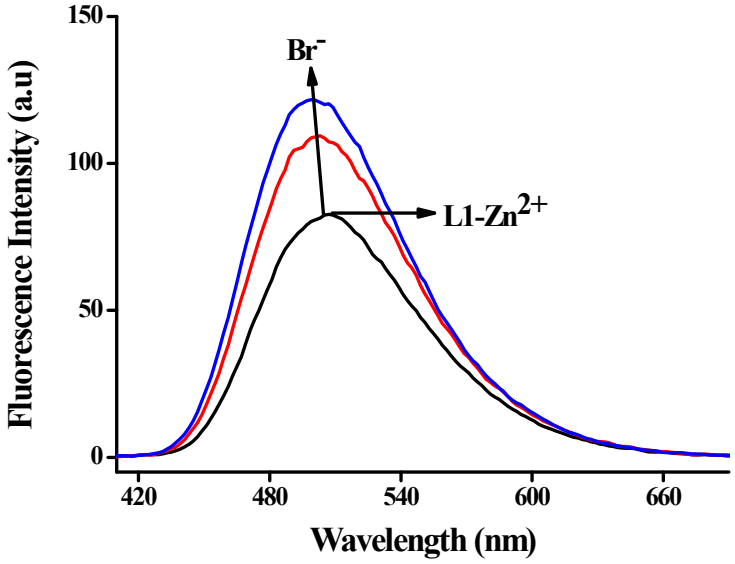

Fig. S6 Fate of Zn^{2+} induced fluorescence of **L1** in the presence of Br^- (0, 10 and 20 equiv).

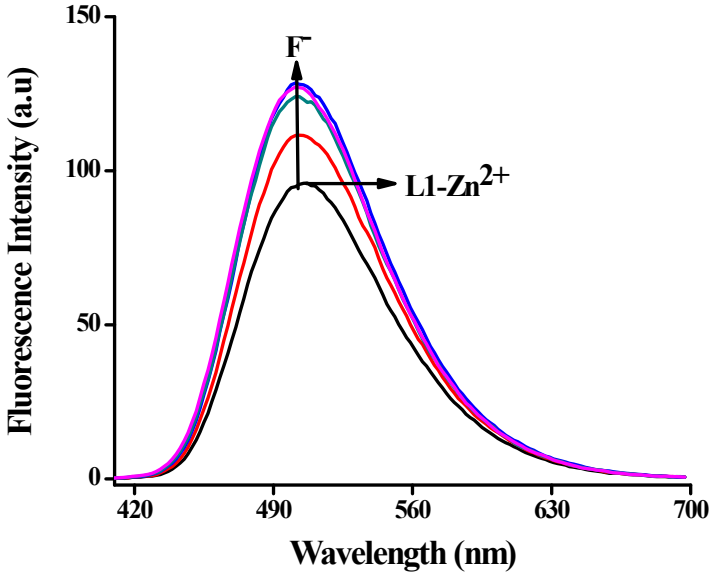

Fig. S7 Fate of Zn^{2+} induced fluorescence of **L1** in the presence of F^- (2, 4, 8 and 12 equiv).

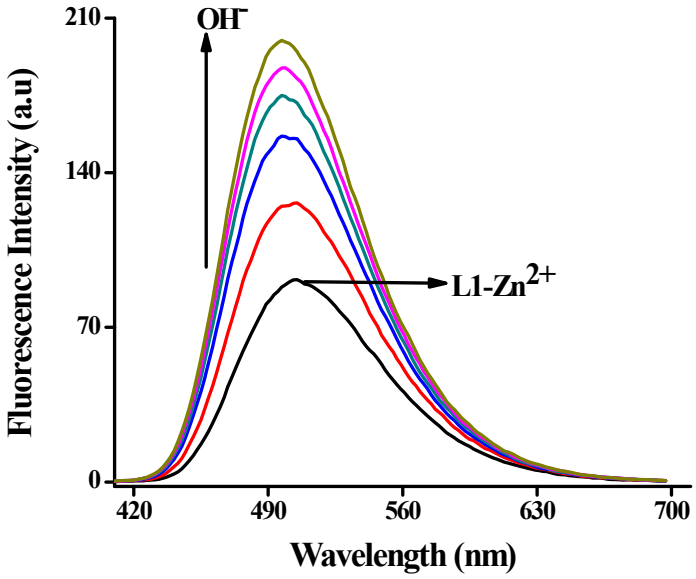

Fig. S8 Fate of Zn^{2+} induced fluorescence of **L1** in the presence of OH^- (5, 10, 15, 20 and 30 equiv).

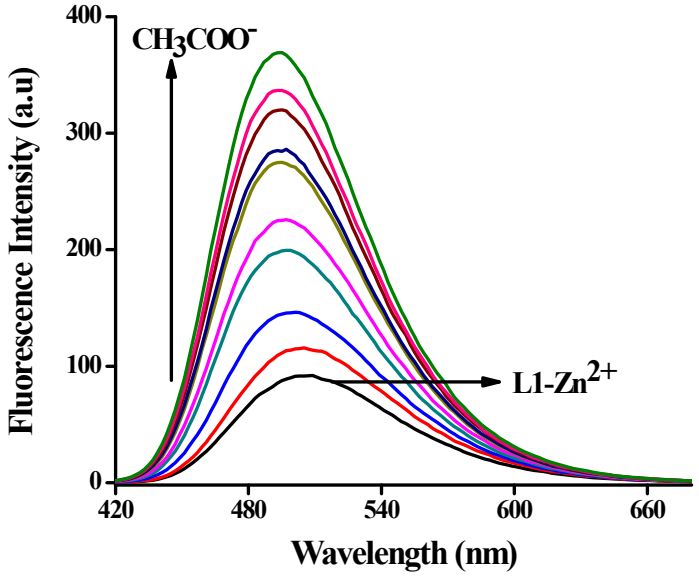

Fig. S9 Fate of Zn^{2+} induced fluorescence of **L1** in the presence of CH_3COO^- (2, 4, 6, 8, 10, 12, 16, 20 and 30 equiv).

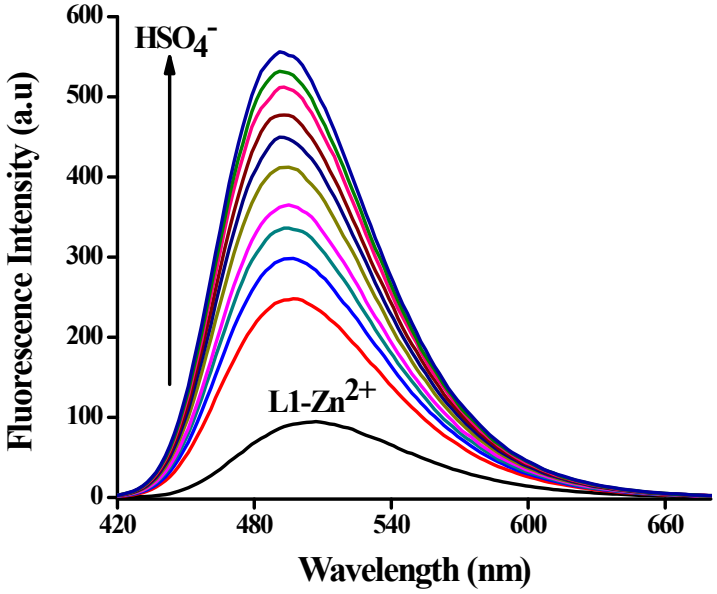

Fig. S10 Fate of Zn^{2+} induced fluorescence of **L1** in the presence of OH^- (1, 2, 3, 4, 6, 8, 10, 12, 16 and 20 equiv).

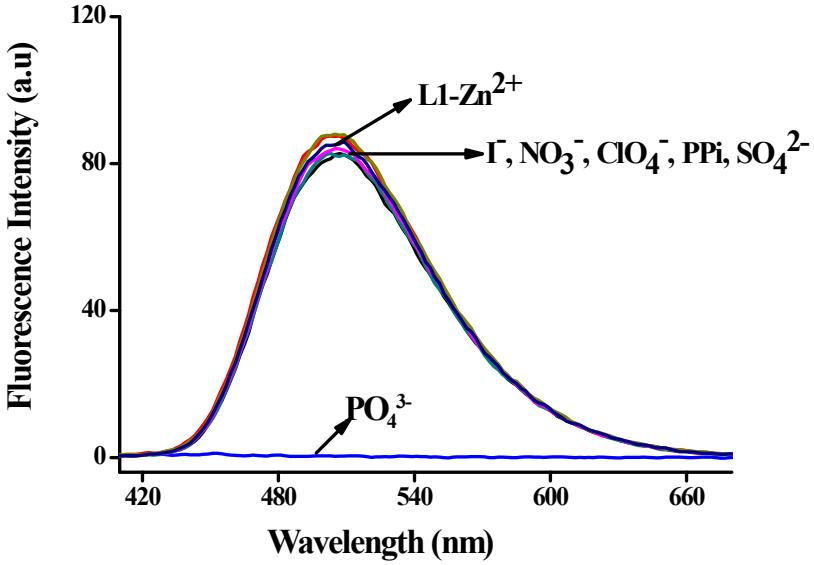

Fig. S11 Fate of Zn^{2+} induced fluorescence of **L1** in the presence of 30 equiv of I^-, NO_3^-, ClO_4^- SO_4^{2-}, $P_2O_7^{4-}$ and 3 equiv of PO_4^{3-}.

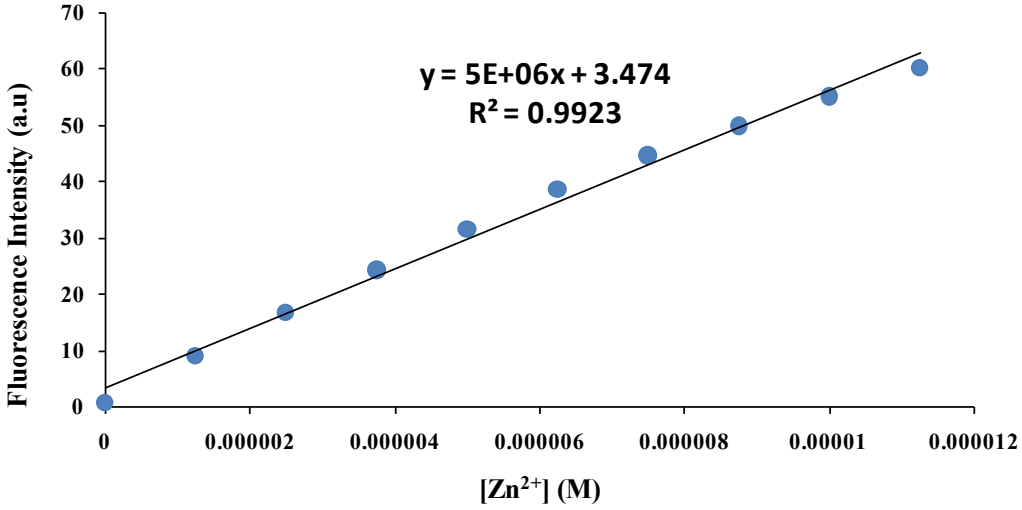

Fig. S12 Limit of detection calculation

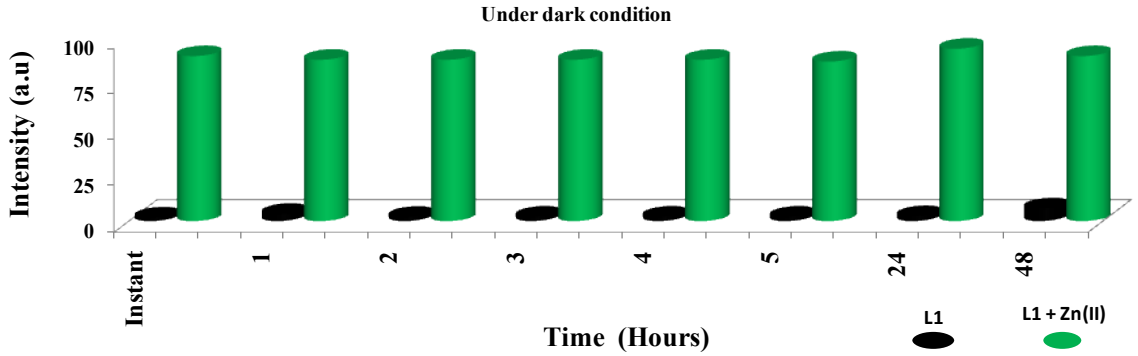

Fig. S13 Effect of time on the emission of **L1** and corresponding Zn^{2+} induced fluorescence enhancement in dark condition (First emission profile of 20 μM **L1** was recorded at one particular time, followed by recording the emission of **L1** after addition of 40 μM of Zn^{2+}, the solutions were kept in dark condition).

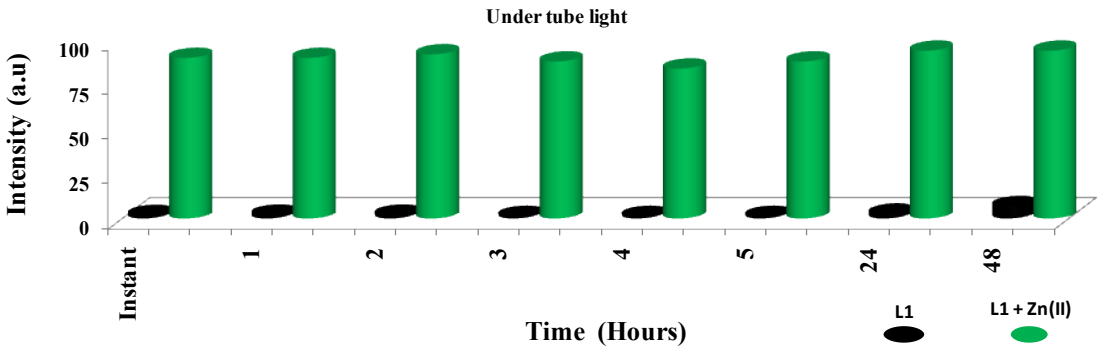

Fig. S14 Effect of time on the emission of **L1** and corresponding Zn^{2+} induced fluorescence enhancement under normal tube light (First emission profile of 20 μM **L1** was recorded at one particular time, followed by recording the emission of **L1** after addition of 40 μM of Zn^{2+}, the solutions were kept under normal tube light.

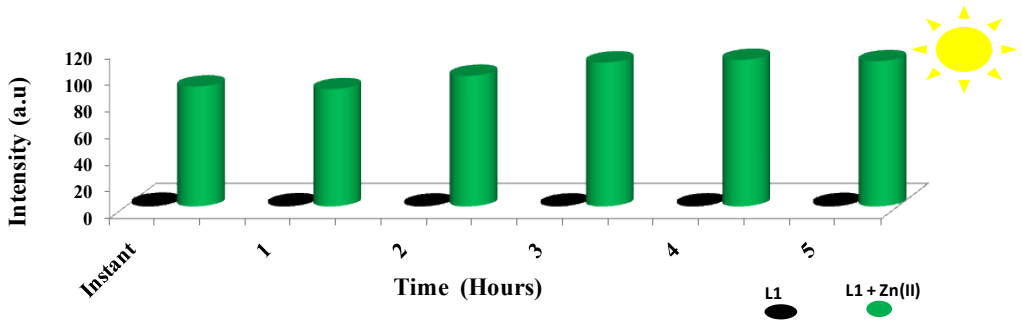

Fig. S15 Effect of time on the emission of **L1** and corresponding Zn^{2+} induced fluorescence enhancement when kept under sunlight for a particular time (First emission profile of 20 μM **L1** was recorded at one particular time, followed by recording the emission of **L1** after addition of 40 μM of Zn^{2+}, the solutions were kept under sunlight for a particular time).

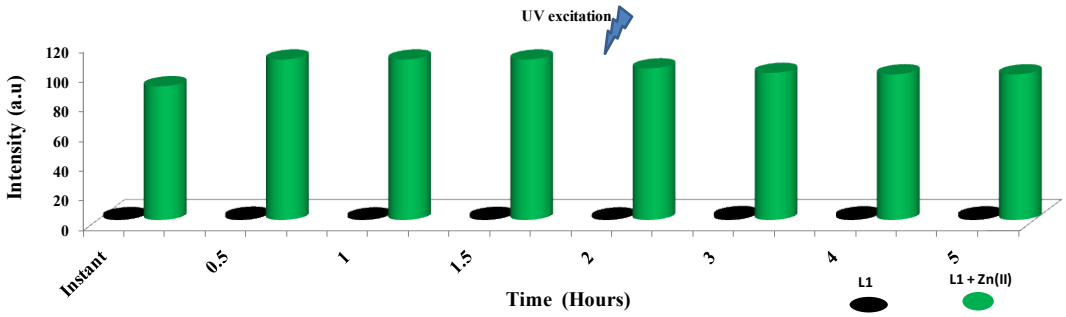

Fig. S16 Effect of time on the emission of **L1** and corresponding Zn^{2+} induced fluorescence enhancement when kept under UV light for a particular time (First emission profile of 20 μM **L1** was recorded at one particular time, followed by recording the emission of **L1** after addition of 40 μM of Zn^{2+}, the solutions were kept under UV light for a particular time).

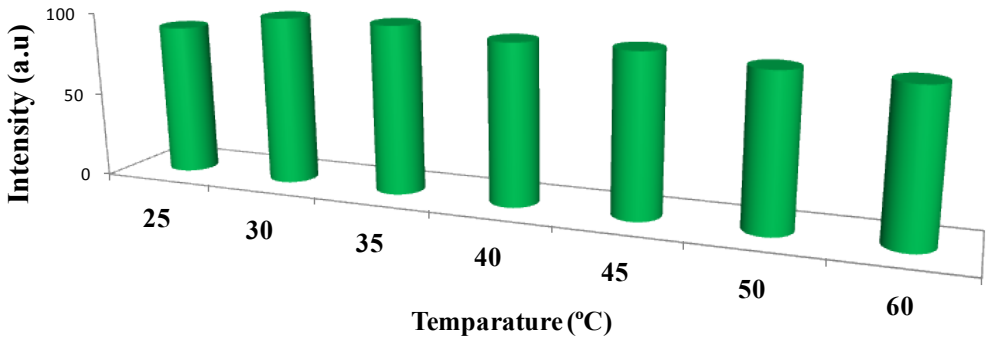

Fig. S17 Effect of temperature on the emission of **L1**-Zn^{2+} complex.

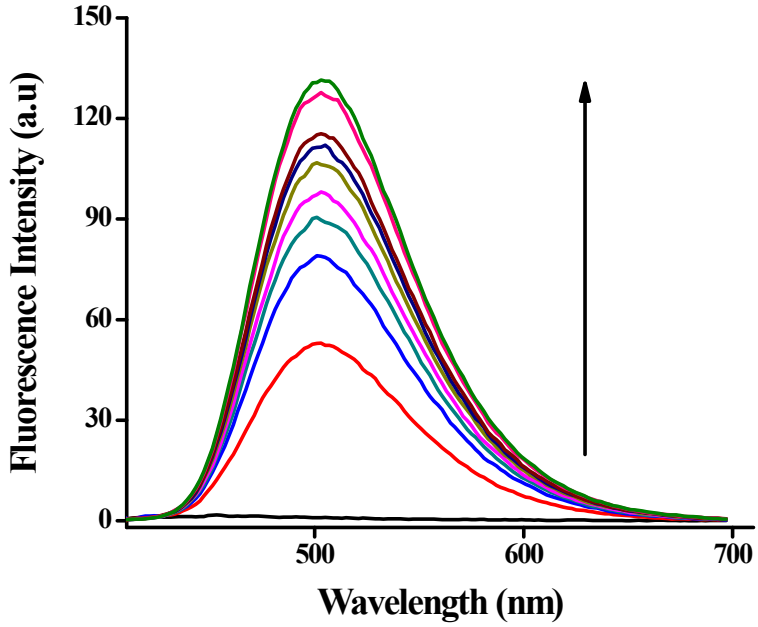

Fig. S18 Emission spectrum of **L1** (80μM) in 10% HEPES buffered dimethylformamide (pH~7.4), in the presence of increasing amount of Zn^{2+} (0-20 equiv).

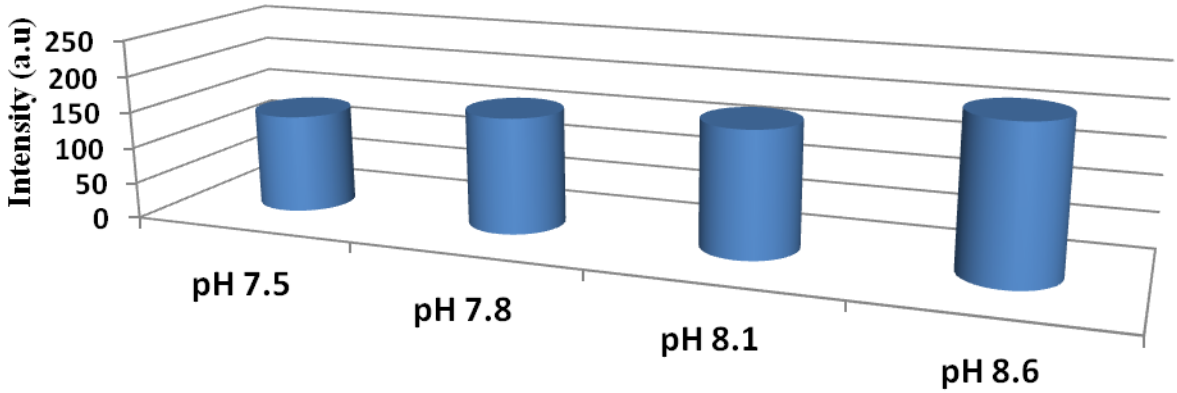

Fig. S19 Fluorescence intensity of probe **L1 (**80 μM) in different pH in the presence of 20 equiv of Zn^{2+}.

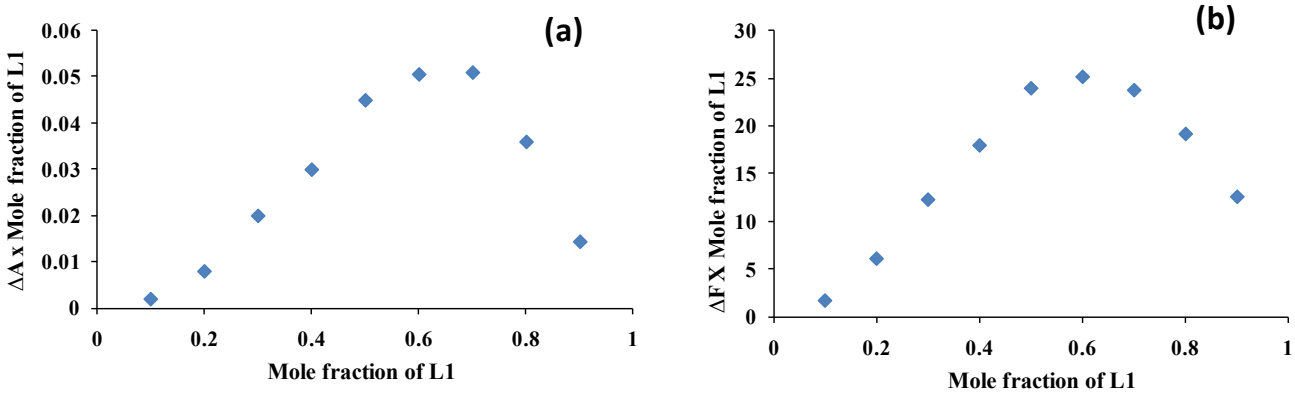

Fig. S20 Job plot for the identification of **L1**-Zn^{2+} complex stoichiometry using (a) absorbance values recorded at 400 nm, and (b) fluorescence intensities at 500 nm.

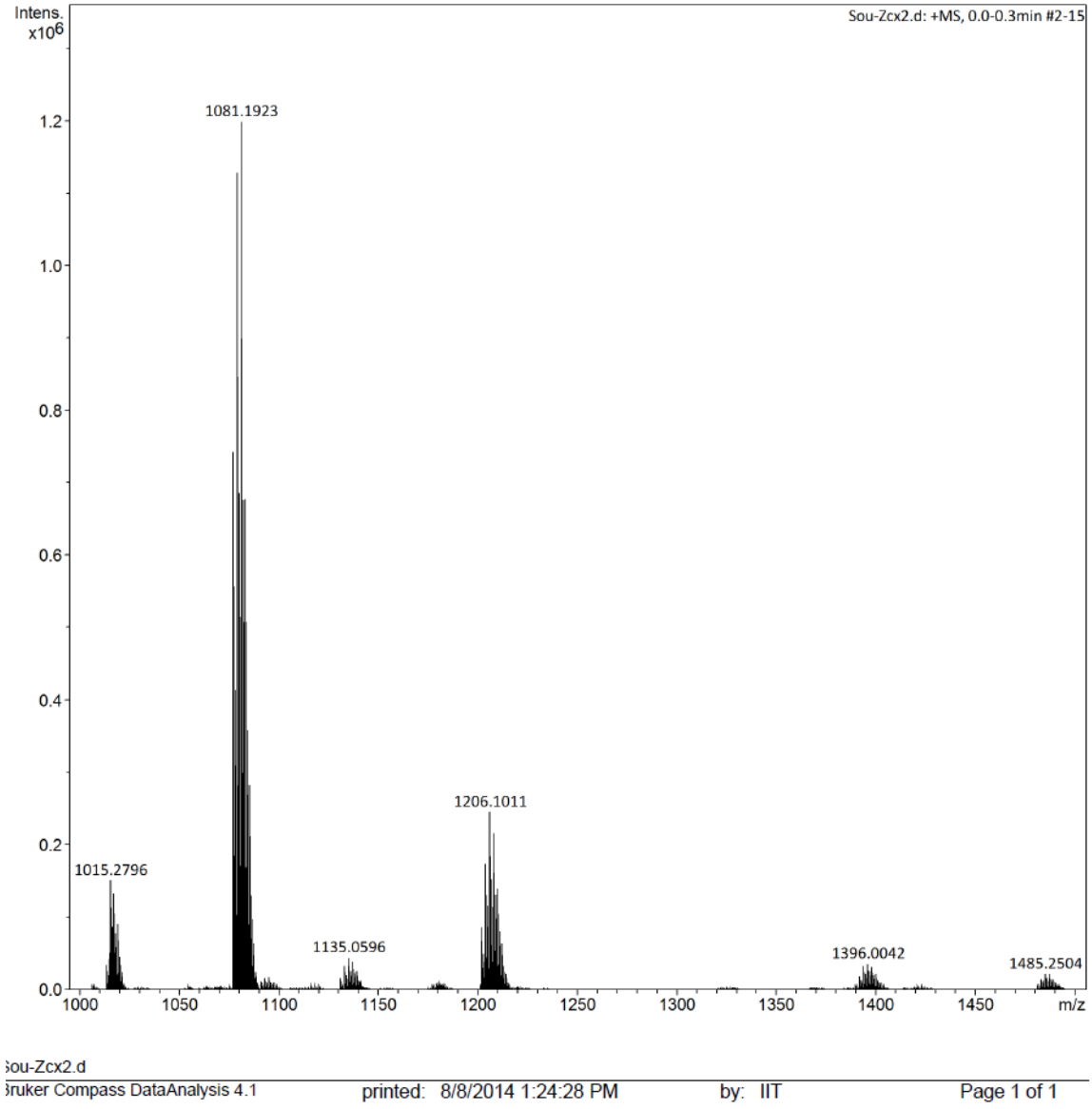

Fig. S21 High-resolution mass spectra of **L1**-Zn^{2+}

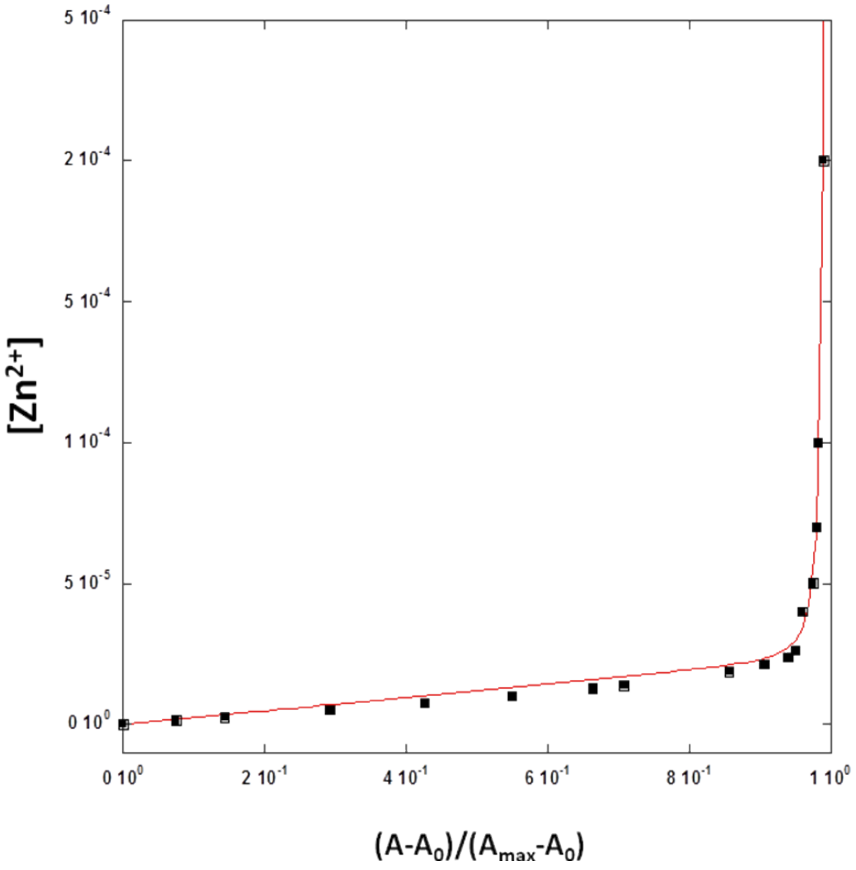

Fig. S22 Binding constant calculation for the **L1**-Zn^{2+} complex.

MTT assay for cell viability testing[1-4]

Introduction

MTT assay, known as cell viability and proliferation assay is the assay of choice to assay the toxicity of a newly developed compound in *in-vitro* cell culture experiment. The full form of MTT is 3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide.

Principle

This assay mainly relies on the reductive cleavage of the yellow tetrazolium salt MTT, 3-[4,5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide, into dark purple colored formazan by metabolically dynamic cells which can be evaluated spectrophotometrically at 570 nm.

Materials:

1. Phosphate buffer saline of pH 7
2. Freshly prepared and filtered MTT (5 mg/ml in PBS); kept in dark condition.
3. Freshly prepared acidified isopropanol solution (0.1 N HCl in absolute isopropanol)
4. Stock solutions of zinc nitrate (200 nM, 2 μM and 3 mM)
5. Stock solutions of probe **L1** (400 nM, 4 μM and 3 mM)

Procedure:

1.5 ml exponentially growing broth culture of *Candida albicans* (IMTECH No. 3018), grown in Potato Dextrose Agar medium of pH 6.0, incubated at 37°C temperature was centrifuged at 6,000 rpm for 5 minutes. Then the pellet were washed carefully twice with normal saline solution and suspended in various solutions as mentioned below around 108 cell/ml cell density.

Following four experimental sets (each set composed of three tubes) were prepared for each type of cell culture.

i) Set A: Each tube contains only the *Candida albicans* cells. This is used as positive control.

ii) Set B: Each tube contains the cells suspended in 200 μl probe **L1** (3 mM) and incubated for 2 hours.

iii) Set C: Each tube contains the cells suspended in 200 μl zinc nitrate (2 μM), incubated for 2 hours followed by incubation of 200 μl probe **L1** (4 μM) for 2 hours.

iv) Set D: Each tube contains the cells suspended in 200 μl zinc nitrate (3 mM), incubated for 2 hours, then 200 μl probe **L1** (3 mM) is added and again incubated for 2 hours.

After the incubation, the cells were again centrifuged at 6,000 rpm for 5 minutes and the pellet was washed twice with normal saline solution. Each pellet was suspended in 3 ml PBS buffer. 300 μl MTT was added to each tube. All sets were incubated for 4 hrs in dark condition at 37°C temperature. After incubation, 3 ml of freshly prepared acidic isopropanol solution was added to

each tube of all six 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.

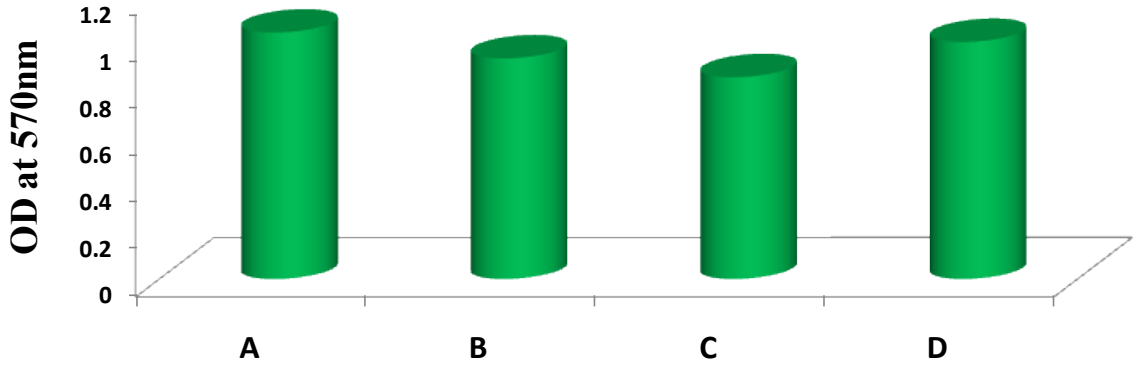

Fig. S23 Graphical presentation of results of MTT assay: (A) Only Candida, (B) Candida + **L1** (3 mM), (C) Candida + Zn^{2+} (2 µM) + **L1** (4 µM), (D) Candida + Zn^{2+} (200 nM) + **L1** (400 nM)

Table S1 Result for MTT assay of *Candida* cells for probe **L1**

Incubation conditions	OD at 570nm *Candida*
(A) Only Candida cells	1.05
(B) Candida cells + probe **L1** (3 mM)	0.94
(C) Candida cells + 2 µM zinc nitrate+ 4 µM probe **L1**	0.86
(D) Candida cells + 200 nM zinc nitrate+ 400 nM probe **L1**	1.01

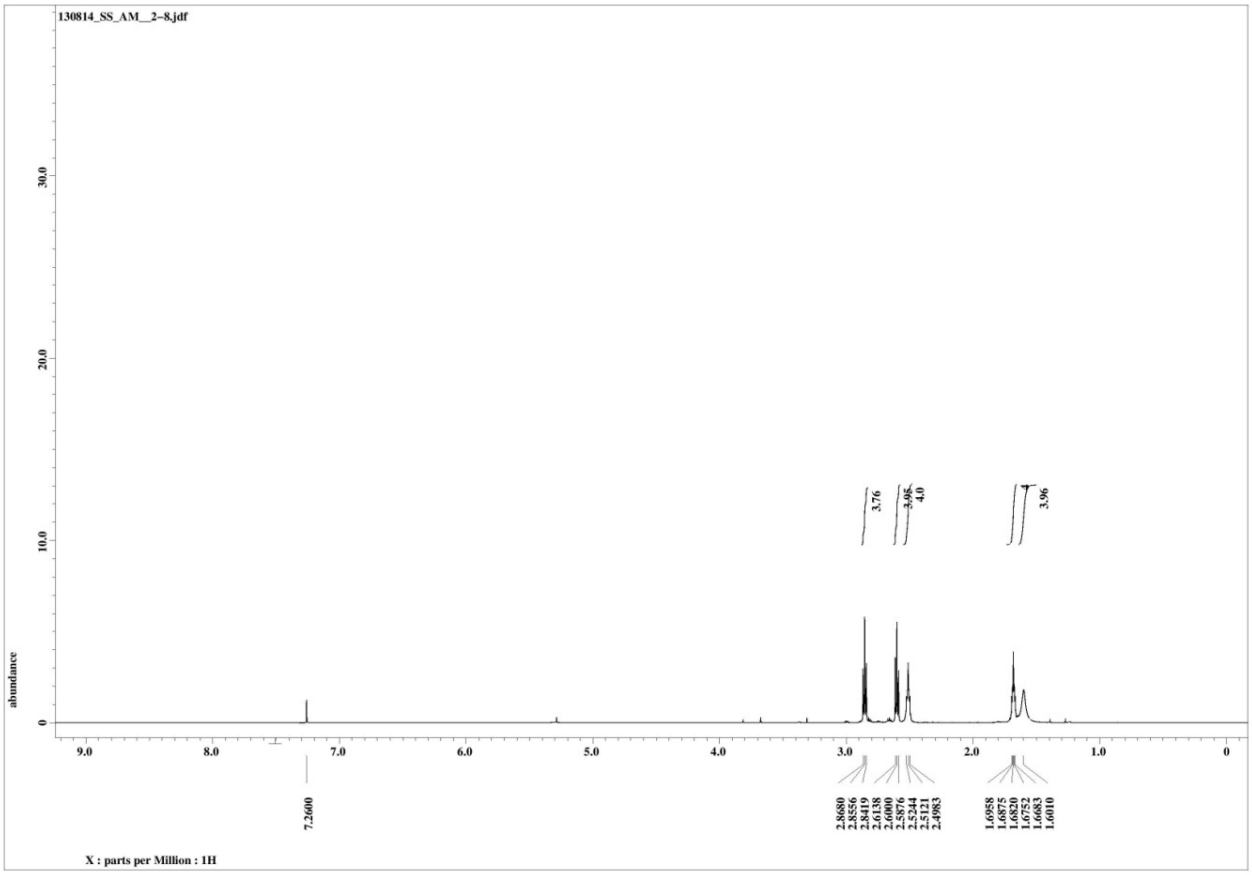

Fig. S24 ^{1}H-NMR of compound **3** in $CDCl_3$

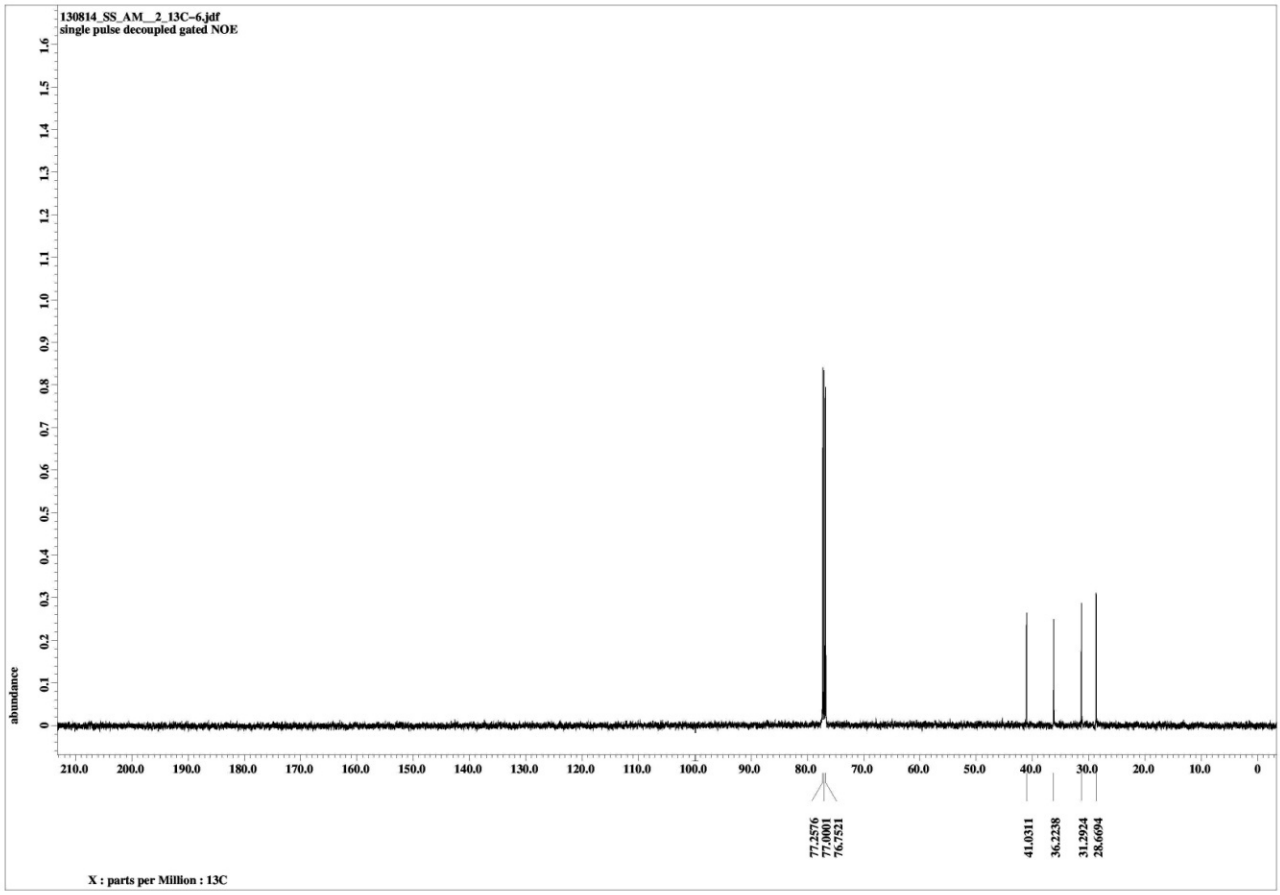

Fig. S25 ^{13}C NMR of compound **3** in $CDCl_3$

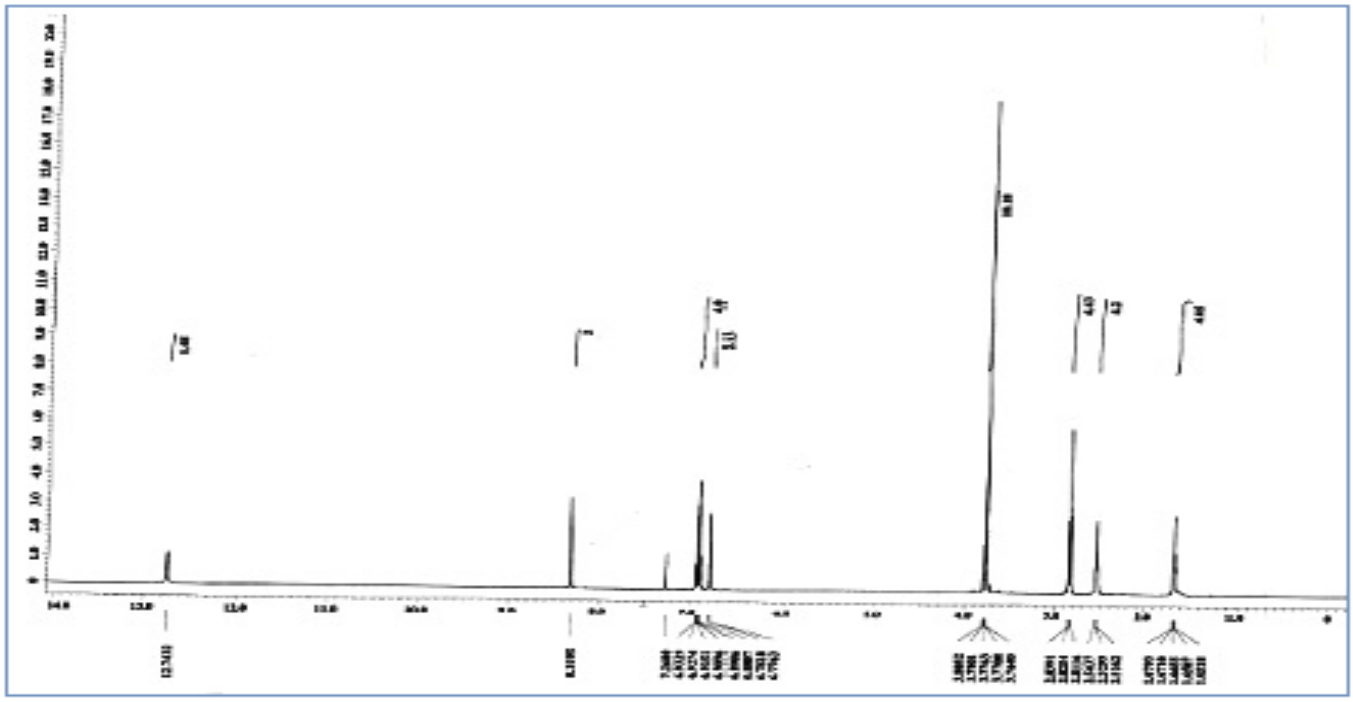

Fig. S26 ^{1}H-NMR of compound **L1** in $CDCl_3$

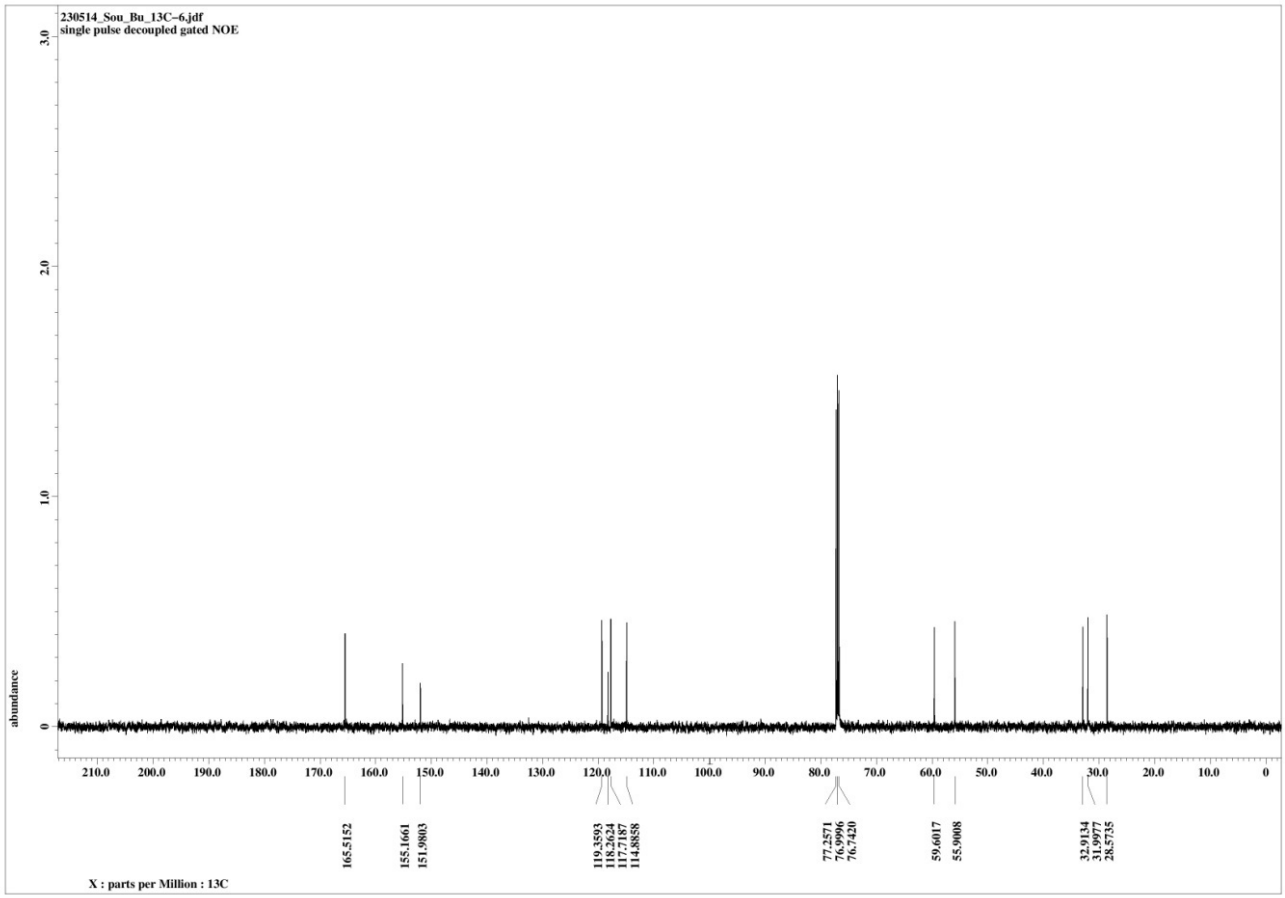

Fig. S27 ^{13}C NMR of compound **L1** in $CDCl_3$

References:

1. J. Meerloo, et al. Cell Sensitivity Assays: The MTT Assay. Cancer Cell Culture: Methods and Protocols, 2nd ed.; Methods in Molecular Biology; Springer: New York, 2011; DOI 10.1007/978-1-61779-080-5_20; vol. 731.

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