Perimidine based selective colorimetric and fluorescent turn-off chemosensor of aqueous Cu^{2+}: Studies on its antioxidant property alongwith its interaction with calf thymus-DNA

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1. **^{1}H NMR (DMSO-d6, 300 MHz) spectra of 1:**

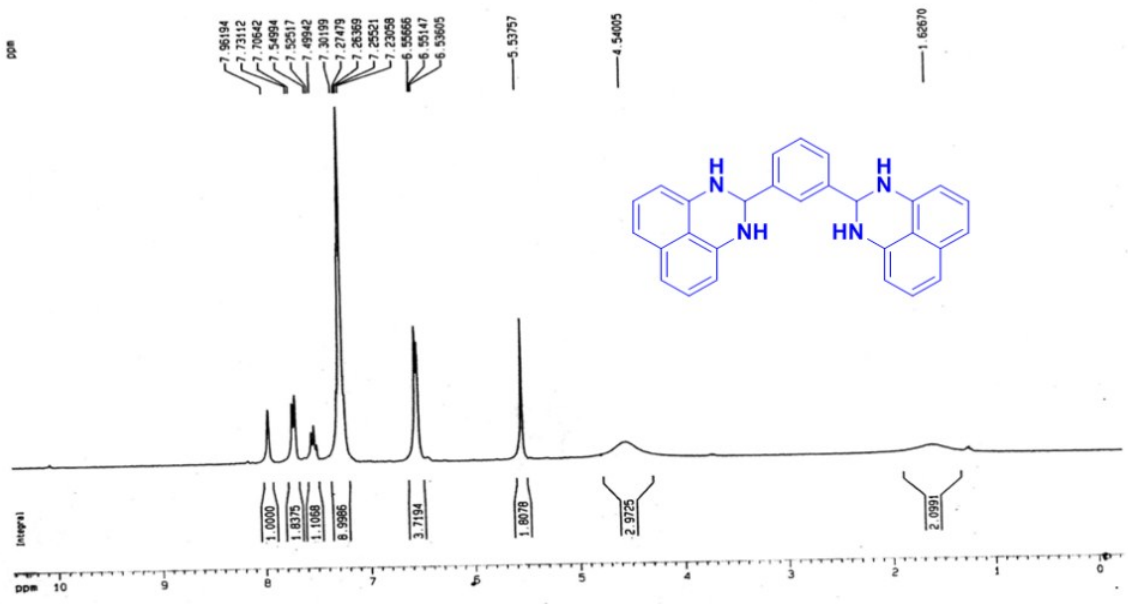

Fig. S1. ^{1}H NMR spectra of **1**

2. ^{13}C NMR (DMSO-d6, 75 MHz) spectra of 1:

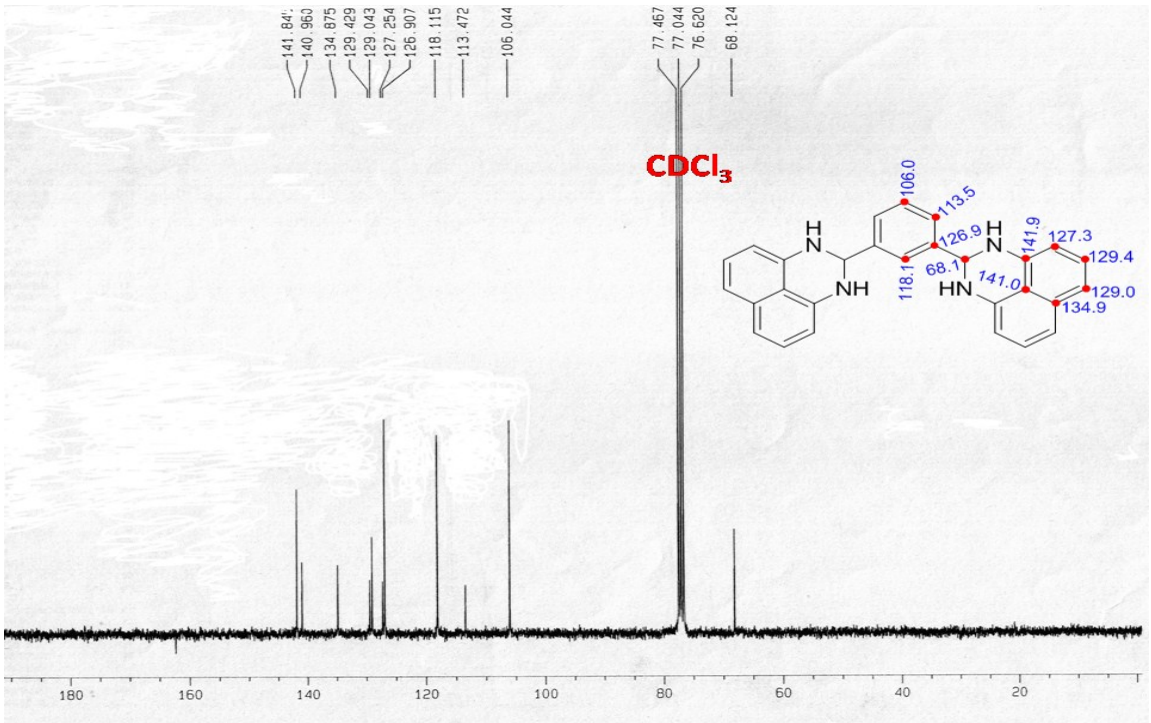

Fig. S2. ^{13}C NMR spectra of **1**

3. FT-IR spectra of 1:

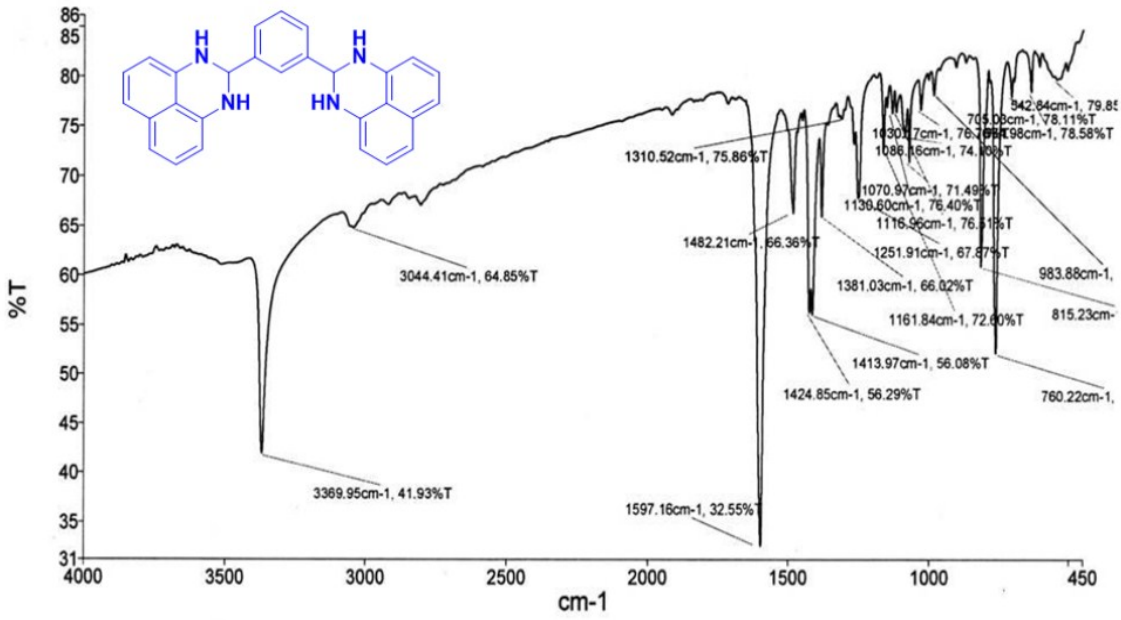

Fig. S3. FT-IR spectra of **1**

4. HRMS spectra of 1:

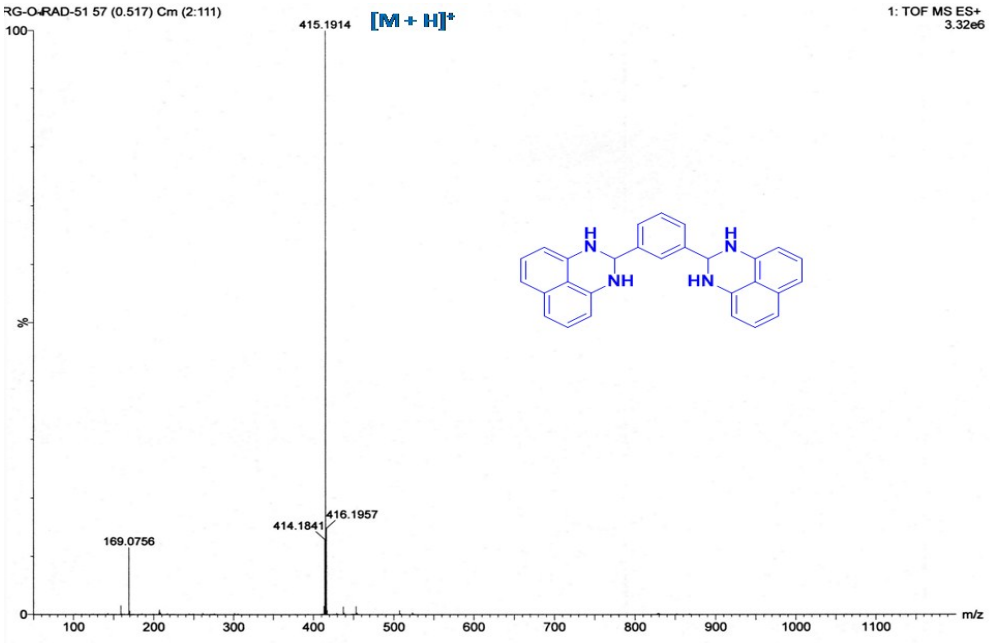

Fig. S4. High Resolution Mass Spectra of **1**.

5. Determination of quenching efficiency [1]

Quenching efficiency = $(I_0 - I_f)/I_0 \times 100$

In this equation I_0, I_f are the fluorescence intensities respectively, in the absence of and at the saturation of the interaction of analytes. Quenching efficiency of **1** for Cu^{2+} ion = 93%, and for CT-DNA quenching efficiency = 26%.

6. Determination of Binding constant

The binding constant of **1** toward Cu^{2+} ion is calculated using the Benesi–Hildebrand relation (eqn. 1).[2] According to this relation

$$\log\left(\frac{A - A_0}{A_f - A_0}\right) = \log[Cu^{2+}] + \log K_b \qquad (1)$$

in which A_0, A and A_f are the absorption values, in the absence of, at the intermediate and at the saturation of the interaction of Cu^{2+} ion respectively, and $[Cu^{2+}]$ represents the concentration of aqueous Cu^{2+} ion added. The binding constant (K_b= 7.95x10^7 M^{-1}) was determined by linear fitting of absorption titration curve (Fig. 5).

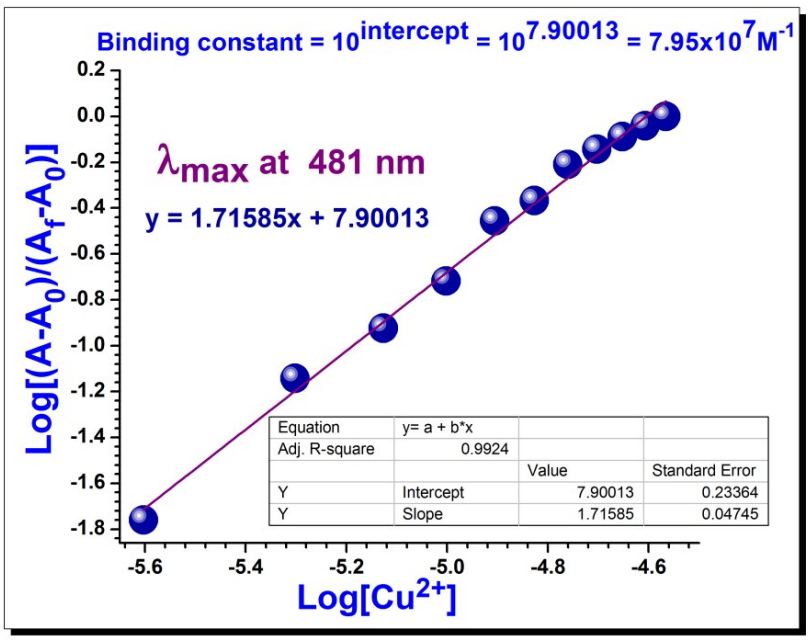

Fig. S5. Benesi–Hildebrand plots for determination of Binding constant of **1** for Cu^{2+} ion.

7. Determination of Detection limits[3]

Detection limit (LOD) of **1** for Cu^{2+} was determined using the equation LOD= 3σ /m, where σ = standard deviation of the blank (6.25 μM solution of **1** in acetonitrile only, O.D at 481 nm), and m= slope of the curve obtained from titration of **1** with Cu^{2+} (Fig. 6).

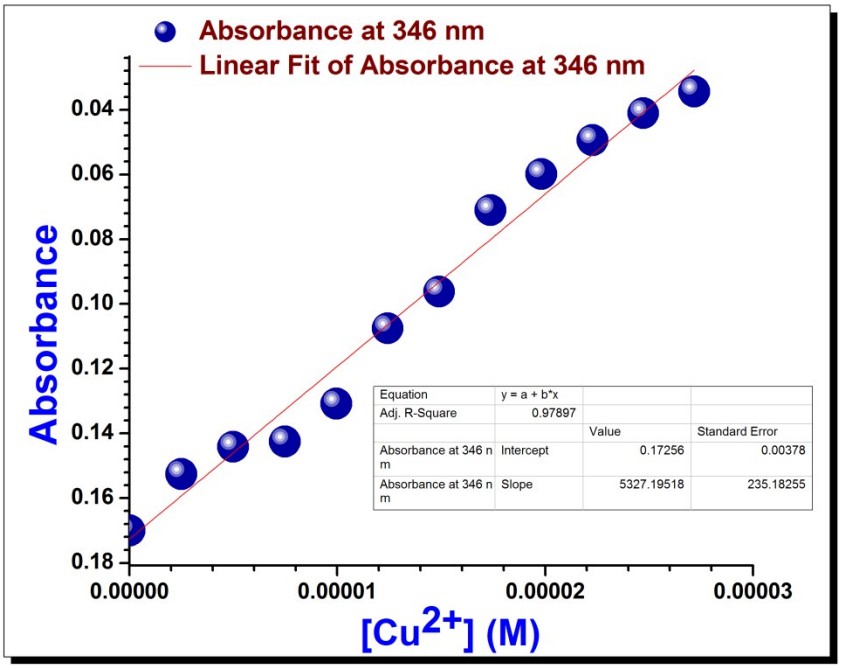

Fig. S6. Absorbance changes of **1** at 346 nm as a function of aqueous Cu^{2+} ion concentration.

σ = 0.00011 (from experimental data) , and m = 5327.2 (from graph, Fig. 6.). Using the formula, we got LOD = 3σ /m = 61.9 $x10^{-9}$ (M).

8. Life time measurement of 1 in presence of Cu^{2+} [4]

We have calculated the radiative rate constant (k_r) and the total non-radiative rate constant (k_{nr}) of **1** and (1+ Cu^{2+}) using the following equations:

$$\tau^{-1} = k_r + k_{nr} \quad (2)$$

$$k_r = \Phi/\tau \quad (3)$$

where τ, k_r, k_{nr} and Φ are the mean fluorescence lifetime, radiative rate constant and nonradiative rate constant, fluorescence quantum yield respectively.

Table S1: Time resolved fluorescence decay parameters of **1** in aqueous buffer in the presence of Cu^{2+} ion

Solution	**τ_1 (ns)**	**a_1**	**τ_2 (ns)**	**a_2**	**τ_{avg} (ns)**	**χ^2**	**Φ**	**k_r (ns^{-1})**	**k_{nr} (ns^{-1})**
[**1**]	0.91166	-	-	-	0.91166	1.06355	0.1	0.10969	0.98717
[**1**] + Cu^{2+}	0.78001	0.75596	0.20166	0.24403	0.63887	1.21665	0.0058	0.00908	1.55619

9. Measurement of antioxidant activity[5]

Table S2: Comparison of free radical scavenging activity between **1** and L-ascorbic acid by DPPH assay.

Concentration (µM)	**Absorption of 1 at 517 nm**	**% inhibition by 1**	**Absorption of L-ascorbic acid at 517 nm**	**% inhibition by L-ascorbic acid**
0	0.55965	0	0.38838	0
0.39063	0.47757	14.66631	0.39449	-1.5732
0.78125	0.55594	0.66291	0.36262	6.63268
1.5625	0.39317	29.74716	0.35971	7.38195
3.125	0.23264	58.43116	0.34942	10.03141
6.25	0.19167	65.75181	0.30559	21.31675
12.5	0.07977	85.74645	0.22929	40.96246
25	0.0189	96.62289	0.06611	82.97801
50	0.01581	97.17502	0.02857	92.6438
100	0.0318	94.31788	0.03229	91.68598
	IC_{50} = 2.3		IC_{50} = 13.9	

10. Notes and References

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