Electronic Supplementary Information(ESI)

for

A New Selective Chromogenic and Turn-On Fluorogenic Probe for Copper

(II) in Solution and Vero Cells: Recognition of Sulphide by [CuL]

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1. ¹H NMR spectrum of (2) in d₆-DMSO solution:



Figure S1: ¹H NMR of Compound **2** in d₆-DMSO (400 MHz).

2. ESI-MS Mass Spectrum of 2:



Figure S2: Mass spectrum of 2.

3. ¹H NMR of Compound (L) (CDCl₃, 400 MHz).



Figure S3: ¹H NMR of Compound L in CDCl₃ (400 MHz).

4. ¹³C NMR spectrum of L in CDCl₃ solution:



Figure S4: ¹³C NMR spectrum of L in CDCl₃ solution.

5. LCMS Mass Spectrum of L:



Figure S5: Mass spectrum of L.

6. HRMS of L in the presence of Cu(ClO₄)₂.6H₂O:



Figure S6: HRMS of L-Cu complex.

7. HRMS of L+Cu(ClO₄)₂.6H₂O+Na₂S:



Figure S7: HRMS of $L+ Cu^{2+} + S^{2-}$.

8. ORTEP diagram of (2) as obtained from the single crystal XRD:¹



Figure S8: ORTEP diagram of single crystal XRD structure of (2) at 50% ellipsoid probability.

9. Job's plot for determining the stoichiometry of sensor L and Cu²⁺ ion by fluorescence method:



Figure S9: Job's plot of sensor L with Cu^{2+} in CH₃CN-H₂O (7:3 v/v; 10 mM, HEPES buffer, pH = 7.4, ([H] = [G] = 4×10⁻⁵ M) by fluorescence method, that indicates 1:1 stoichiometry.

10. Binding properties of Cu^{2+} with L by cyclic voltammetry. We have performed CV experiments to investigate the redox properties of L and its binding behavior with Cu^{2+} in CH₃CN. Upon the addition of Cu(ClO₄)₂.6H₂O of up to 0.5 equiv, there is no appreciable change in the reversibility and/or shift of the oxidation potential. A further addition of Cu(ClO₄)₂.6H₂O of up to 1 equiv. causes a decrease in the current intensity with a reversible oxidation wave. The progressive addition of Cu(ClO₄)₂ to solution of L in CH₃CN in the

presence of n-Bu₄NPF₆ caused significant modification of the oxidation potentials in CVs, which is assigned to the Cu(II)/Cu(I) couple with $E_{1/2} = -0.276$ V [reference electrode: saturated $Hg(l)/Hg_2Cl_2(s),$ supporting electrolyte: 0.1M NaCl solution; scan rate = 50 mV S^{-1} , positive shift of oxidation potential from 0.4 V to 0.6V. The positive shifts of the oxidation potentials arise from the reduced electron density located in the iminethiazole moiety because of metal co-ordination upon binding of the N₂O₂ core with Cu²⁺ ion. Further, the presence of a $d \rightarrow d$ transition band observed at 775 nm and the appearance of the green color supports the presence of $Cu^{2+}(d^9)$ bonded species, rather than Cu^+ species. The increased absorption in the near-infrared region of the spectrum in Fig. S11, indicates formation of a new CuL complex.



Figure S10: CV diagram of L (1×10^{-4} M) in CH₃CN with the gradual addition of Cu²⁺ (1×10^{-4} M) up to 0-2 equiv. The color code represents the addition of Cu²⁺: black, 0 equiv; red, 0-0.5 equiv; green, 0.5-1 equiv; blue, 1-1.5 equiv. sky blue, 1.5-2 equiv.

11. Absorption spectrum of L with Cu²⁺ (600 nm-1000 nm):



Figure S11: UV–vis absorption titration spectra of L ($c = 4 \times 10^{-5}$ M) in aq. CH₃CN (CH₃CN/H₂O = 7:3 v/v, 10 mM HEPES buffer, pH = 7.4) upon addition of Cu²⁺ ($c = 2 \times 10^{-3}$ M), indicates d→d transition of CuL complex.

12. pH study of L:



Figure S12: Fluorescence intensity of free chemosensor L and in the presence of 3 equiv. of Cu^{2+} in aqueous CH₃CN (CH₃CN:H₂O = 7:3 v/v, 10 mM HEPES buffer) with different pH conditions.

13. Visual and fluorescence photographic image of L in presence of different metal ions:



Figure S13: The Visible color (top) and fluorescence changes (buttom) of receptor L in aq. CH_3CN ($CH_3CN:H_2O = 7:3 \text{ v/v}$, 10 mM HEPES buffer, pH = 7.4) upon addition of various metal ions.

14. Evaluation of the Association Constant for the Formation of $L \cdot Cu^{2+}$. Receptor L with an effective concentration of 4×10^{-5} M in CH₃CN/H₂O solution (CH₃CN:H₂O = 7:3 v/v, 10 mM HEPES buffer, pH = 7.4) was used for the emission titration studies with a Cu²⁺ solution. A stock solution of Cu(ClO₄)₂.6H₂O, having a concentration of 2×10^{-4} M in an aqueous HEPES buffer (7:3 v/v; pH = 7.4) solution, was used in UV-Absorbance method. Receptor L with an effective concentration of 1×10^{-5} M in CH₃CN/H₂O solution (CH₃CN:H₂O = 7:3 v/v, 10 mM HEPES buffer, pH = 7.4) was used for the emission titration studies with a Cu²⁺ solution. A stock solution of Cu(ClO₄)₂.6H₂O, having a concentration of 1×10^{-4} M in an aqueous HEPES buffer (7:3 v/v; pH = 7.4) solution, was used in fluorescence method. **Calculations for the Binding Constants Using Spectrophotometric Titration Data**. The association constant and stoichiometry for the formation of the respective complexes were evaluated using the Benesi–Hildebrand (B–H) plot (eq 1).³

$$1/(I - I_0) = 1/K(I_{max} - I_0)[M^{n+}] + 1/(I_{max} - I_0)$$
(1)

Here I₀, I_{max}, and I represent the emission intensity of free L, the maximum emission intensity observed in the presence of added metal ion at 488nm for Cu²⁺ (λ_{ext} = 446 nm), and the emission intensity at a certain concentration of the metal ion added, respectively.



14A. Association constant calculation graph (Absorption method):

Figure S14A: Bensei-Hildebrand plot obtained from absorption method (Excitation calculated from 446 nm) studies. Binding constant ($K_a = 2.28 \times 10^4 \text{ M}^{-1}$) curve of sensor L with Cu²⁺ determined by UV-vis spectroscopy method.

14B. Association constant calculation graph (Fluorescence method):



Figure S14B: Bensei-Hildebrand plot obtained from the Fluorescence (emmission calculated from 488 nm) studies. Binding constant ($K_a = 3.33 \times 10^4 \text{ M}^{-1}$) curve of sensor L with Cu²⁺ determined by fluorescence method.

15. Detection limit calculation graph (Fluorescence method):

(A) The detection limit (DL) of L for Cu^{2+} was determined from the following equation⁴: DL = K* Sb1/S, Where K = 2 or 3 (we take 3 in this case); Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve.



Figure S15A: Calibration curve for fluorescence titration of L with Cu²⁺.

From the graph we get slope (S) = 9×10^{10} , Standard deviation (Sb1 = 46111.96).

Thus using the formula we get the detection limit = $1.53 \mu M = 1.53 \times 10^{-6} M$.

(B) The detection limit (DL) of CuL for S²⁻ was determined from the following equation:

 $DL = K^* Sb1/S$, Where K = 2 or 3 (we take 3 in this case); Sb1 is the standard deviation of the blank solution; S is the slope of the calibration curve.



Figure S15B: Calibration curve for fluorescence titration of CuL with S².

From the graph we get slope (S) = 1×10^{10} , Standard deviation (Sb1 = 38088.00952)

Thus using the formula we get the detection limit = $11.4 \mu M = 1.14 \times 10^{-5} M$.

16. Fluorescence spectra showing reversibility of Cu²⁺ co-ordination to receptor (L), CN⁻ in CH₃CN:



Figure S16: Change in fluorescence spectra of L-Cu²⁺ complex (c = 1×10^{-5} M) in CH₃CN-H₂O solution (CH₃CN:H₂O = 7:3 v/v, 10 mM HEPES buffer, pH = 7.4) upon addition of KCN (c = 4×10^{-4} M).

17. DFT study:



Figure S17A: Optimized structure of L by DFT/B3LYP/6-31+G(d) method.



Figure S17B: Optimized structure of L-Cu²⁺ complex by DFT/UB3LYP method.



Figure S17C: Spin density plot of L-Cu²⁺ calculated by DFT/UB3LYP method.

Table S1. Calculated selected bond angles (°) and bond lengths (A°) data of L and complex**CuL** (B3LYP/BS1 level of theory).

Matric data(Bond	L	CuL
angles)(°)		
N ₃₆ -C ₃₃ -C ₃₇ -C ₃₈	45.91	25.94
C ₃₂ -C ₃₃ -C ₃₇ -C ₃₉	42.73	29.53
S ₃₅ -C ₃₁ -N ₂₉ -C ₂₈	172.49	4.27
N ₃₆ -C ₃₁ -N ₂₉ -C ₂₈	-7.88	161.33
N ₂₉ -C ₂₈ -C ₈ -C ₉	-0.26	170.78
N ₂₉ -C ₂₈ -C ₈ -C ₉	179.66	-8.44
N ₂₉ -Cu-N ₃₆		65.59
N ₃₆ -Cu-O ₄₃		79.30
O ₂₇ -Cu-O ₄₃		120.97
O ₂₇ -Cu-N ₃₆		157.63
N ₂₉ -Cu-O ₄₃		143.27
Matric data (Bond lengths)	CuL (Aº)	
O ₂₇ –Cu	1.870	
O ₄₃ –Cu	2.168	
N ₂₉ -Cu	2.037	
N ₃₆ -Cu	2.013	1

λ (nm)	E (eV)	Osc. Strength (f)	Key excitations
440.7	2.8133	0.1445	(90%)HOMO→LUMO
413.7	2.9972	1.2987	(88%)HOMO→LUMO+1
325.6	3.8082	0.2294	(90%)HOMO-2→LUMO
275.6	4.4985	0.1091	(72%)HOMO-4→LUMO

Table S2. Selected vertical electronic transitions of L calculated by TDDFT method.

Table S3. Selected vertical electronic transitions of L-Cu²⁺ calculated by TDDFT method

λ (nm)	E (eV)	Osc. Strength (Key excitations	Character
		<i>f</i>)		
741.7	1.6717	0.0146	(37%) HOMO- $5(\beta) \rightarrow$ LUMO (β)	d-d
			(35%)HOMO-4 (β) \rightarrow	
			$LUMO(\beta)$	
447.1	2.7729	0.9960	(67%)HOMO(β) \rightarrow	ILCT
			LUMO+2(β)	
433.1	2.8628	0.1020	(63%)HOMO-1(β) \rightarrow	ILCT
			LUMO+1(β)	
354.9	3.4932	0.3191	(72%)HOMO-2 $(β)$ →	ILCT
			LUMO+1(β)	

ILCT: Intra-ligand charge transfer transition

18. X-ray crystallography study:

X-ray diffraction data of single crystals of L (with dimensions of $0.241 \times 0.351 \times 0.601$ mm) was collected using Bruker APEX II Duo CCD area-detector diffractometer operating at 50kV and 30mA using Mo K α radiation ($\lambda = 0.71073$ Å). Data collection and reduction were performed using the APEX2 and SAINT software. The SADABS software was used for absorption correction. L was solved by direct method and refinement was carried out by the full-matrix least-squares technique on F² using SHELXTL package.⁵ All non-hydrogen atoms were refined anisotropically whereas hydrogen atoms were refined isotropically. The crystallographic data and hydrogen bonds geometry for L is presented in Table 1. Crystallographic data for L have been deposited with the Cambridge Crystallographic Data Center CCDC **1011197**.

Crystal data	
CCDC number	1011197
Emperical formula	$C_{12}H_8N_2O_2S$
<i>M</i> _r	244.26
Crystal system, space group	Monoclinic, $P2_1/n$
Temperature (K)	294
<i>a</i> , <i>b</i> , <i>c</i> (Å)	4.8213 (4), 14.2939 (12), 15.8175 (14)
β(°)	93.8920 (18)
V (Å ³)	1087.55 (16)
Ζ	4
Radiation type	Μο Κα
μ (mm ⁻¹)	0.29
Crystal size (mm)	0.241×0.351×0.601
Data collection	
Diffractometer	Bruker APEX II DUO CCD area-detector
	diffractometer
Absorption correction	Multi-scan
	(SADABS; Bruker, 2009)
T _{min} , T _{max}	0.847, 0.934
Independent and	2114, 1889
observed $[I > 2\sigma(I)]$ reflections	

Table S4: Experimental details:

θ range (deg)	2.6–25.9
hkl range	$h = 5 \rightarrow 5, k = 17 \rightarrow 16, l = 19 \rightarrow 19$
Refinement	
$\mathbf{R}_{1},\mathbf{w}\mathbf{R}_{2}(\boldsymbol{\%})$	0.036, 0.119
Completeness (%)	99.1
GOF (F ²)	1.09
H-atom treatment	isotropically

References:

- V. N. Nesterov, Y. T. Struchkov, S. N. Kovalenko, Y. A. Sharanin and I. A. Zhuravel', (1995) Izv. Akad. Nauk SSSR, Ser. Khim. (Russ.) (*Russ. Chem. Bull*), 492.
- 2. H. Saravani, A. R. Rezvani, G. Mansouri, A. R. S. Rad, H. R. Khavasi and H. Hadadzadeh, *Inorg. Chim. Acta*, 2007, **360**, 2829.
- (a) H. A. Benesi and J. H. Hildebrand, J. Am. Chem. Soc., 1949, 71, 2703; (b) C. Yang, L. Liu, T. -W. Mu and Q. -X. Guo, Anal. Sci., 2000, 16, 537; (c) Y. Shiraishi, S. Sumiya, Y. Kohno and T. Hirai, J. Org. Chem., 2008, 73, 8571.
- (a) L. Long, D. Zhang, X. Li, J. Zhang, C. Zhang and L. Zhou, *Anal. Chim. Acta*, 2013, 775, 100; (b) M. Zhu, M. Yuan, X. Liu, J. Xu, J. Lv, C. Huang, H. Liu, Y. Li, S. Wang and D. Zhu, *Org. Lett.*, 2008, 10, 1481. (c) C. Kar, M. D. Adhikari, A. Ramesh and G. Das, *Inorg. Chem.*, 2013, 52, 743. (d) M. Shortreed, R. Kopelman, M. Kuhn and B. Hoyland, *Anal. Chem.*, 1996, 68, 1414.
- 5. G. M. Sheldrick, Acta Cryst., 2008, A64, 112.