Electronic Supplementary Material (ESI) for RSC Advances.

A trilateral approach for the detection of copper ions in living cells and water samples using phenothiazine incorporated fluorescent probe

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Experimental Section

Synthesis of PTZ-Et and PTZ-1-CHO

When phenothiazine (1g; 1.0 mmol) reacts with ethyl bromide in the presence of potassium tertiary butoxide (0.56g; 1.0 mmol) as a base in the DMF solvent (20 mL) under reflux for 18 h. After the reaction had finished, the mixture was cooled to room temperature and then poured into water and subjected to extraction using DCM. Purification of the product **Et-PTZ** was performed through column chromatography. The eluent used was a mixture of hexane and ethyl acetate in a 9:1 ratio.

DMF (5.1 mL) was mixed with $POCl_3$ (5.6 mL) and the resulting mixture was then cooled to 0°C for approximately 20 minutes. This caused the colorless mixture to yellow shade, indicating the formation of the Vilsmeier reagent. Afterward, the solution of the **Et-PTZ** compound dissolved in $CHCl_3$ was added dropwise to the mixture. The reaction mixture was subjected to reflux at 90 °C for 5 h. After completion reaction, the mixture was cooled down to room temperature and poured into cooled water. The mixture was neutralized using sodium carbonate to achieve a pH of seven and then extracted using DCM. Subsequent, purification of the compound **Et-PTZ-1-CHO** was performed using column chromatography with an eluent ratio of hexane: ethyl acetate (9:1). compound

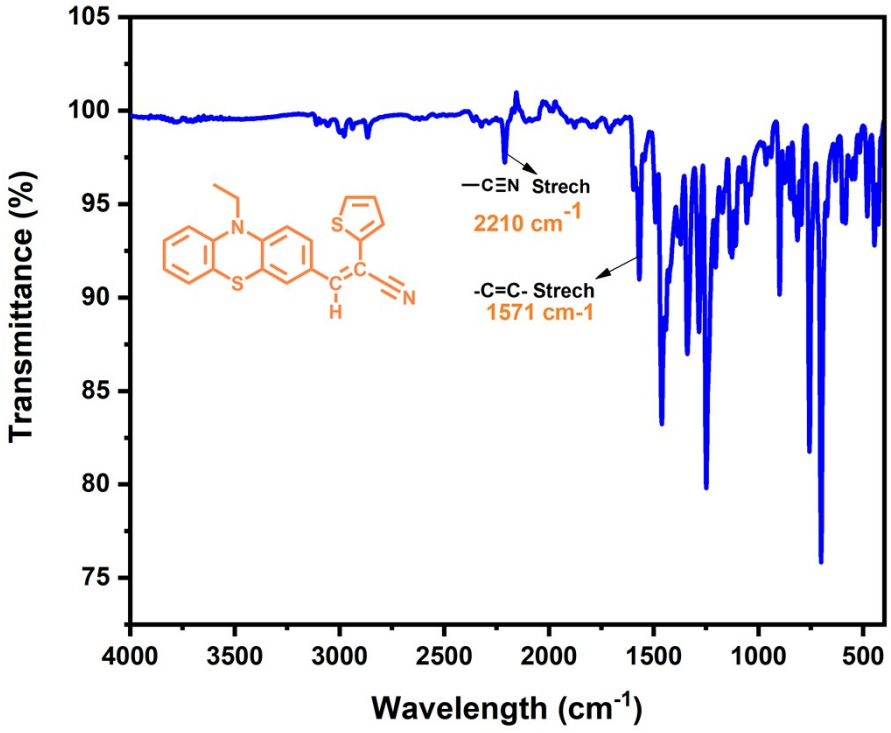


Figure S1. IR spectrum of PTZ-SCN probe

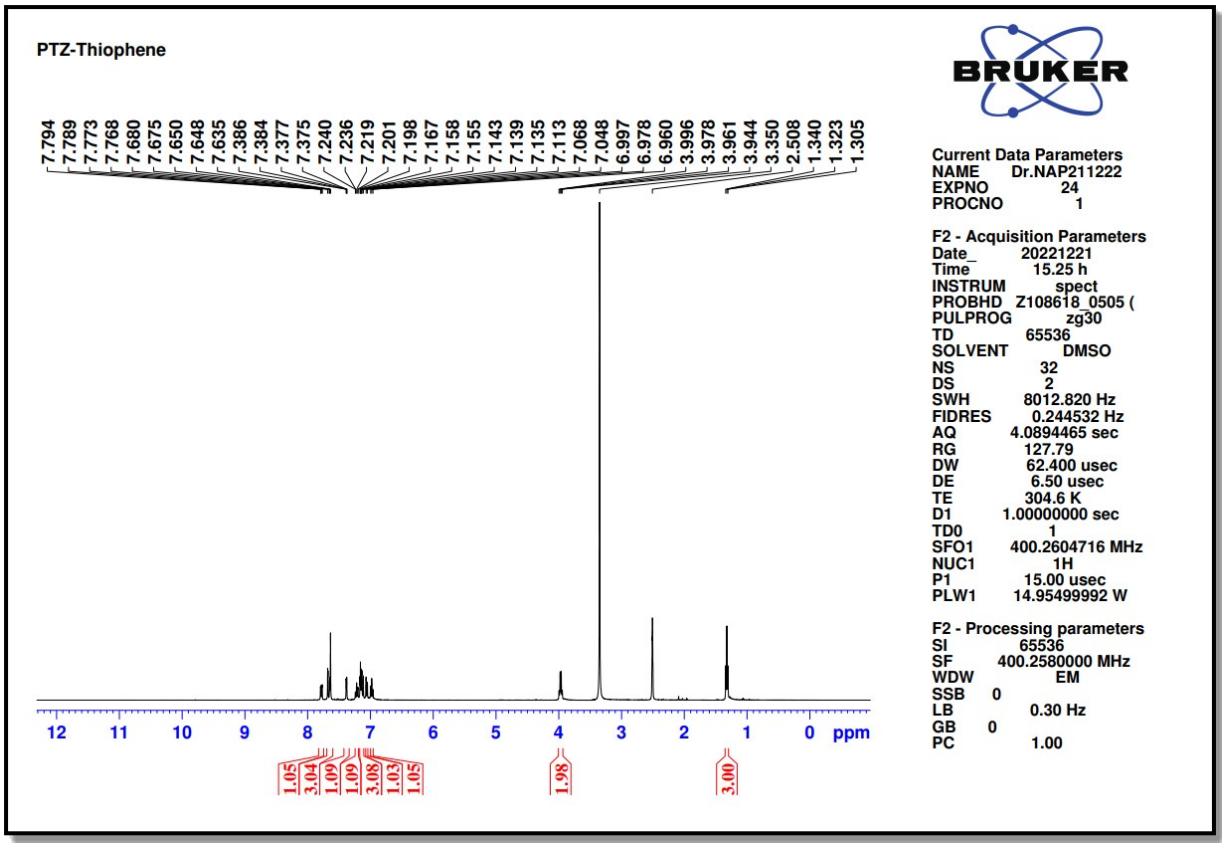


Figure S2. ^{1}H-NMR spectrum of PTZ-SCN probe

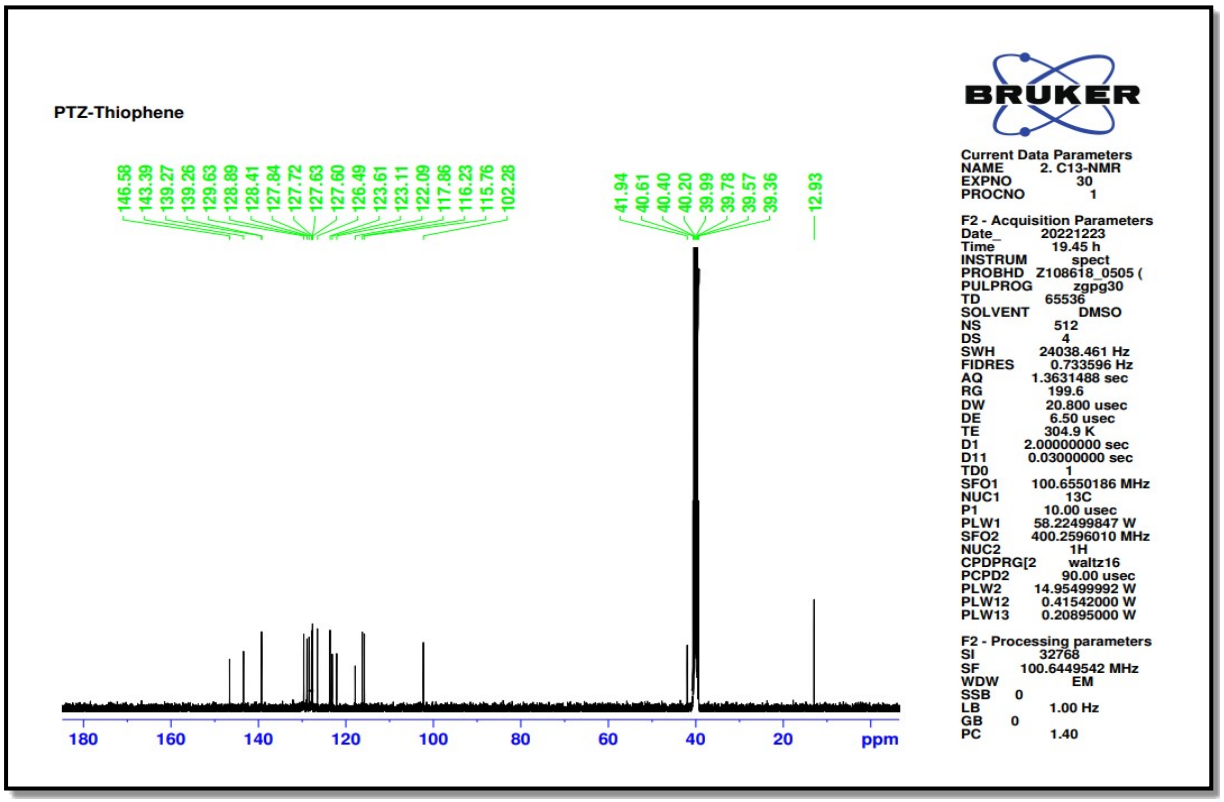


Figure S3. ^{13}C-NMR spectrum of PTZ-SCN probe

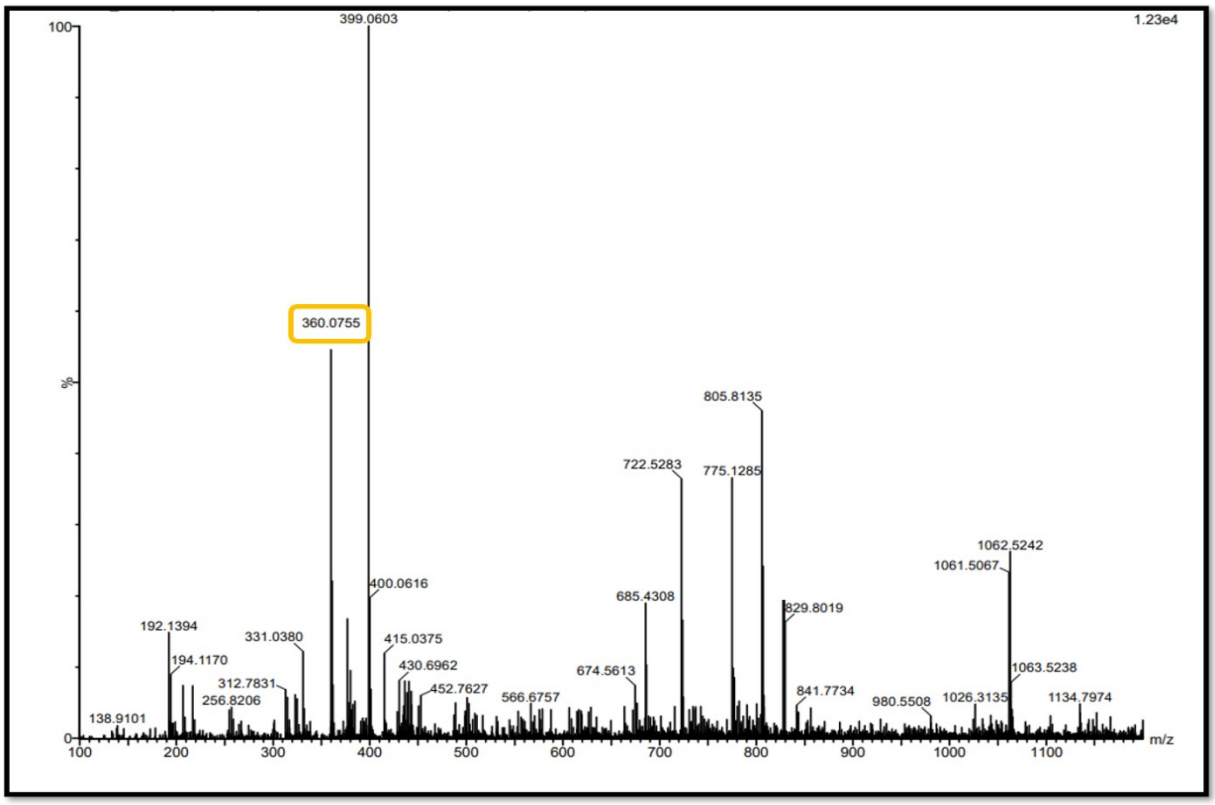


Figure S4. HRMS spectrum of PTZ-SCN probe

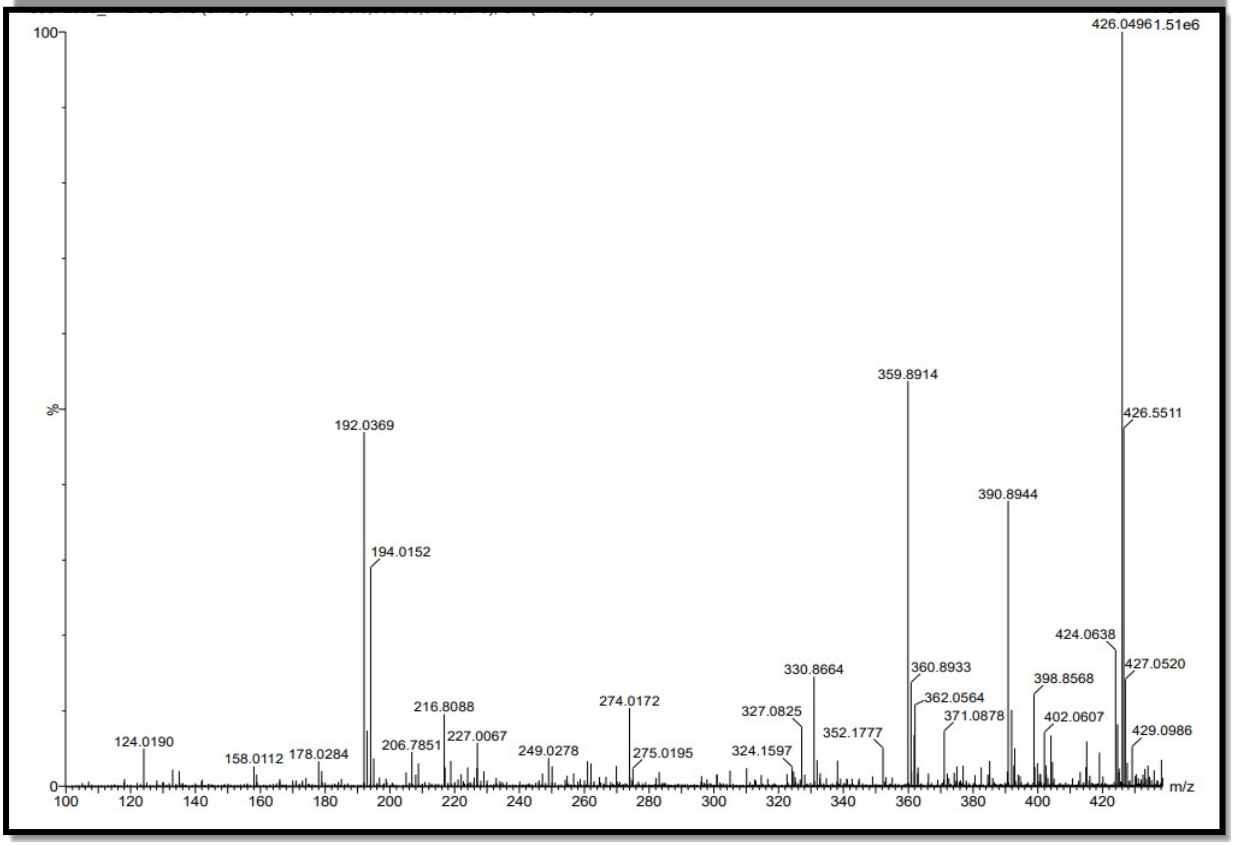


Figure S5. HRMS spectrum of PTZ-SCN-Cu^{2+} complex

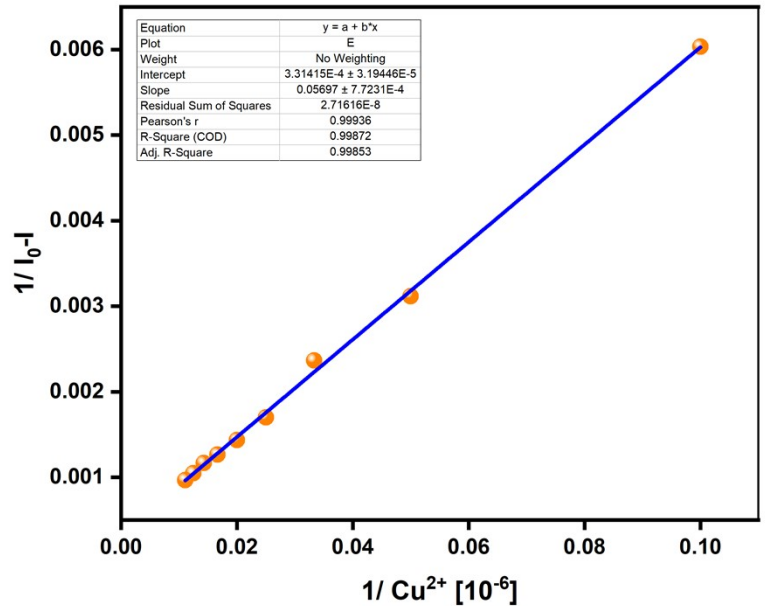


Figure S6 Benesi-Hildebrand plot of PTZ-SCN probe with different concentrations of cupric ion in emission studies

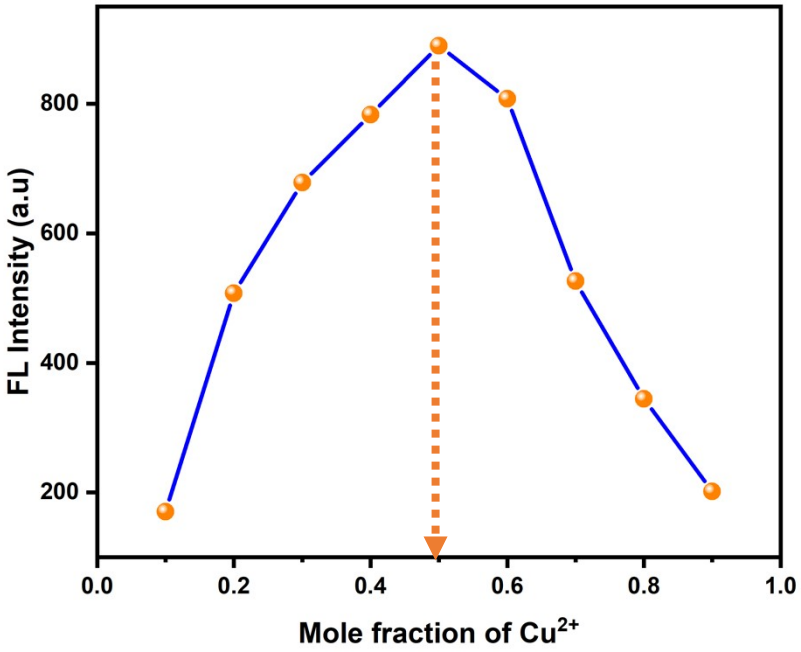


Figure S7. Job's plot of PTZ-SCN on emission spectrum

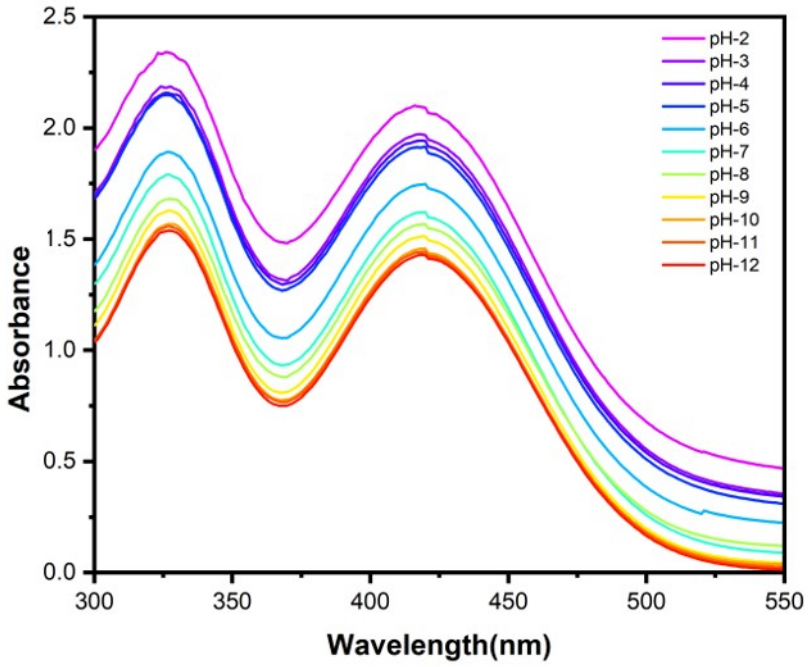


Figure S8. pH effect of PTZ-SCN on absorption spectrum

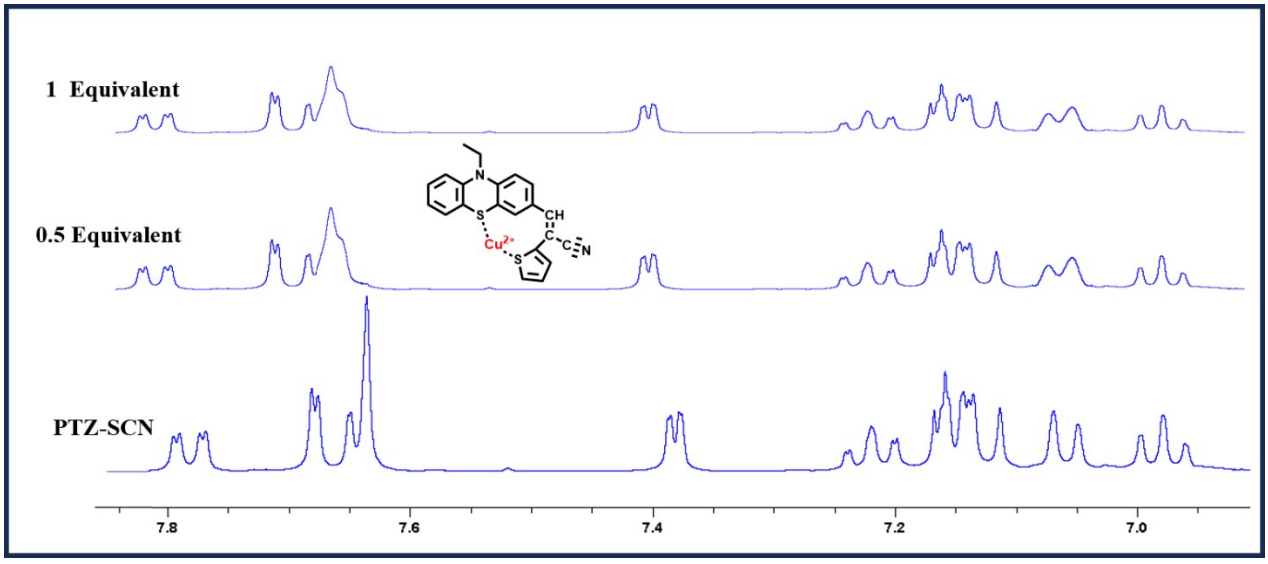


Figure S9. ^{1}H NMR spectra of **PTZ-SCN** probe in the presence and absence of Cu^{2+} ions (0.5, and 1 equivalent).

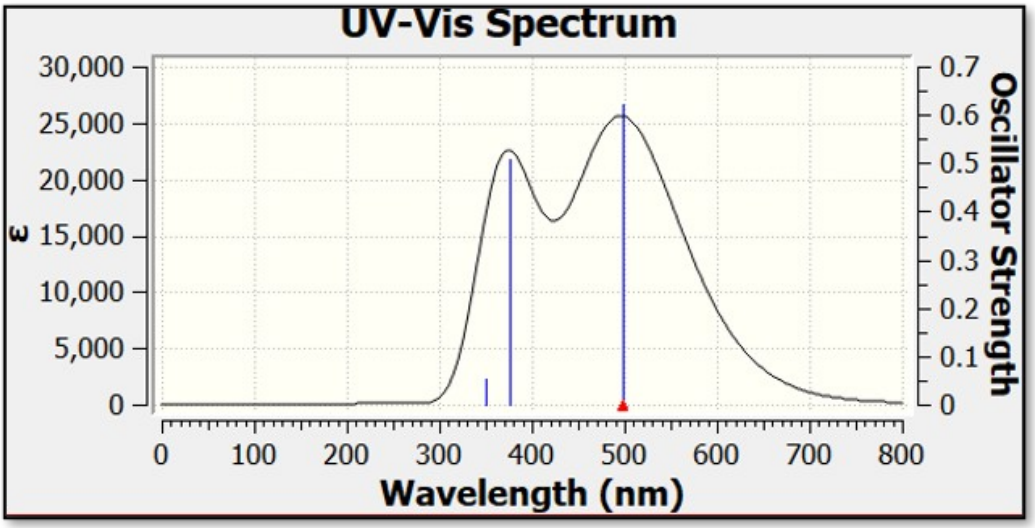


Figure S10. Theoretical UV-visible spectrum of **PTZ-SCN**

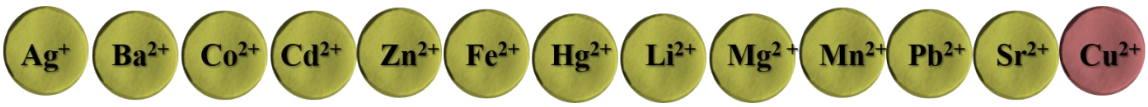


Figure S11. Test strip images of **PTZ-SCN** ions coated in filter paper identification of cupric ion.

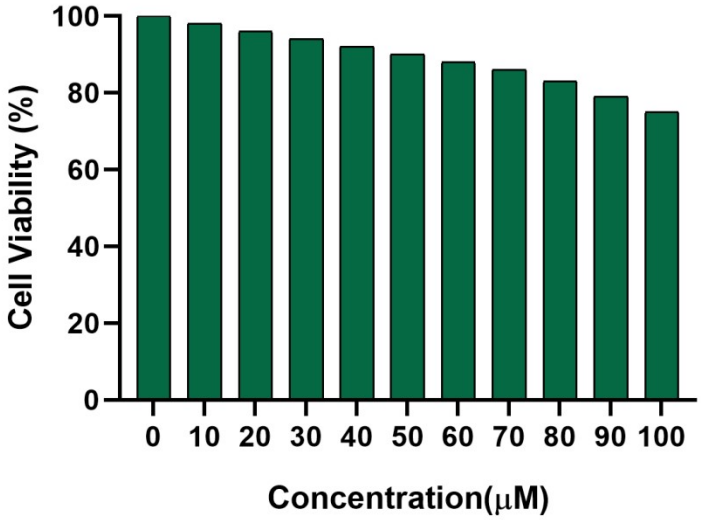


Figure S12. The cytotoxicity of **PTZ-SCN**$^{-}$ **Cu**$^{2+}$ across different concentrations

Table S1. Theoretically computed HOMO, LUMO energy gap and dipole moment of **PTZ-SCN** and **PTZ-SCN-Cu^{2+}**complex at B3LYP/6-31G** level of theory.

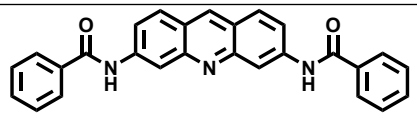
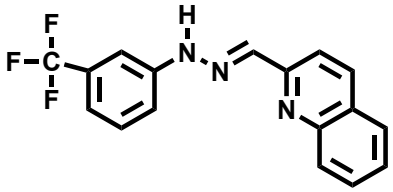
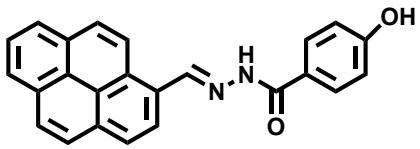
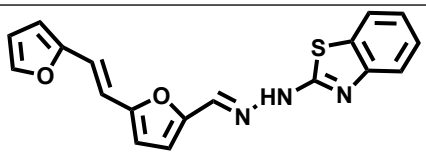
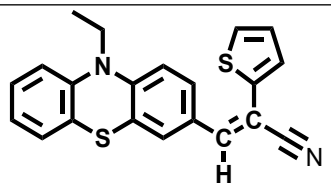
Compound	**[a]E_{HOMO} (eV)**	**[a]E_{LUMO} (eV)**	**[a]Energy gap(eV)**	***[a]μ_{total} (Debye)***	**[b]Major contributions/ Oscillation strength (*f*)**
PTZ-SCN	5.3753	2.5126	2.8626	5.8716	H-1→L (93.7%) /0.68547 H-2 →L (64.5%) / 0.17970
PTZ-SCN-Cu^{2+} Complex	5.5279	3.1491	2.3788	4.0905	H-4→L (68%)/0.58319 H-3→L (32%)/0.12769 H-2→L (95%)/0.21810

[a]Theoretically calculated HOMO, LUMO, band gap and dipole moment values, [b]Oscillation strength and major contributions B3LYP/6-31G** level of theory.

Sample	Spiked amount of Cu^{2+} ions (µM)	Found Amount of Cu^{2+} ions Mean ±SD (µM)	Recovery (%)
Lake water			
1	5	4.87±0.07	97.4
2	10	9.71±0.15	97.1
3	15	14.64±0.51	97.6
4	20	18.95±0.64	94.7
Tap water			
1	5	4.72 ±0.08	94.4
2	10	9.52 ±0.107	95.2
3	15	14.45 ±0.458	96.3
4	20	19.22±0.005	96.1
Distilled water			
1	5	4.75±0.08	95
2	10	9.74±0.11	97.4
3	15	14.76 ±0.44	98.4
4	20	19.55±0.12	97.75

Table S2 Analysis of cupric ions in various water samples with the **PTZ-SCN** probe.

Table S3 Comparative Analysis of **PTZ-SCN** chemosensor for Cu^{2+} detection

Probe	*Solvent system*	*LOD*	*Binding Ratio*	*Applications*	*Ref*
	DMSO: HEPES	1.2×10^{-7} M	2:2	Bioimaging	[1]
	DMF: HEPES	0.02μM	2:1	Real water sample	[2]
	DMSO: Water	2.19×10^{-7}M	1:1	Test strip and Real water sample	[3]
	THF: water	2.16×10^{-7} M	1:1	Test strip, and Real water sample	[4]
	**CH_3CN: HEPES**	**$1.04X\ 10^{-8}$ M**	**1:1**	**The test strip, Real water sample, and bioimaging cells**	**This paper**

References

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2 M. S. Kim, S. Y. Lee, J. M. Jung and C. Kim, *Photochem. Photobiol. Sci.*, 2017, **16**, 1677–1689.

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2023, **157**, 111412.