Electronic Supplementary Information (ESI)

A curcumin derived probe for colorimetric detection of azide ion in water

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General information

Chemicals and solvents were purchased from commercial suppliers and used as received. ^1H and ^{13}C NMR spectra were recorded on a Bruker Advance III HD (500 MHz) spectrometer. Chemical shifts were reported in parts per million (ppm), and the residual solvent peak was used as an internal reference: proton (chloroform δ 7.26), carbon (chloroform δ 77.16) or tetramethylsilane (TMS δ 0.00) was used as a reference. Multiplicity was indicated as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), dd (doublet of doublet), bs (broad singlet). Coupling constants were reported in Hertz (Hz). High resolution mass spectra were obtained on a Micromass/ Q-Tof. micro™ spectrometer. IR spectra were measured on Thermo Scientific Nicolet 380 instrument. For thin layer chromatography (TLC), Merck pre-coated TLC plates (Merck 60 F254) were used, and compounds were visualized with a UV light at 254 nm. Further visualization was achieved by staining with iodine. Flash chromatography separations were performed on SRL 230-400 mesh silica gel.

Synthesis of 3-(prop-2-yn-1-yl)pentane-2,4-dione (1)

Acetylacetone (1.03 mL, 10 mmol) was added to a 5ml solution of dry DMF. Then added 240 mg of NaH was added followed by 0.5 ml of propargyl bromide solution in toluene. Then the

reaction mixture was stirred at rt for 2h. After completion of the reaction confirmed by TLC, the reaction was quenched by addition of aqueous sodium bicarbonate. The organic layer was extracted by ethyl acetate 3 times, dried over sodium sulphate and vacuum evaporated to give compound 1 as yellow crude. The crude was further purified by flash chromatography using petroleum ether as eluent.^{1}H NMR (500 MHz, $CDCl_3$) : δ 3.808-3.778 (t, 1H), 2.628-2.605 (m, 2H), 2.179-2.142 (m, 6H), 1.979-1.929(m, 1H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 202.23, 190.94, 106.49, 81.68, 80.32, 70.83, 68.77, 66.57, 29.40, 23.11, 17.37.

Synthesis of CUC-P

3-(prop-2-yn-1-yl) pentane-2, 4-dione (1.38 mL, 10 mmol) was added to a solution of boric anhydride (0.35 g, 5.0 mmol) in ethyl acetate (30 mL), followed by addition of vanillin (3.04 g, 20 mmol) and tri-n-butyl borate (10.8 mL, 40 mmol). The reaction mixture was stirred at 50 °C for 5 min. Subsequently, n-butylamine (0.4 mL, 5.0 mmol) in ethyl acetate (5 mL) was added dropwise over 15 min at 50 °C and additionally stirred for 4 hours at 80 °C. Hydrochloric acid (1 N, 30 mL) was added and the mixture was stirred for another 30 min. The organic layers were separated and extracted with ethyl acetate (3 × 30 mL). The combined organic layer was washed with water. The organic layer was dried over sodium sulfate, filtered and concentrated under reduced pressure. The crude product was recrystallized from methanol to give the corresponding curcuminoids as yellow solids. The solid product was further purified by flash chromatographic technique.

^{1}H NMR (500 MHz, $CDCl_3$) : δ 7.74 (s, 1H), δ 7.69 (s, 1H), δ 7.28 (m, 1H), δ 7.188 (m, 1H),δ 7.133 (m, 1H), δ 7.11 (m, 1H), δ 7.07 (m, 1H), δ 7.04 (m, 1H), δ 7.01 (m, 1H), δ 6.96 (m, 1H),δ 4.37 (m, 2H),δ 3.92 (m, 6H),δ 2.93 (m, 1H).

^{13}C NMR (125 MHz, $CDCl_3$): δ 193.54, 182.78, 149.02, 148.15, 146.98, 145.82, 142.56, 128.03, 126.70, 124.33, 122.98, 121.11, 117.93, 115.04, 110.24, 80.97, 70.69, 69.73, 63.28, 56.12, 18.01, 16.34.

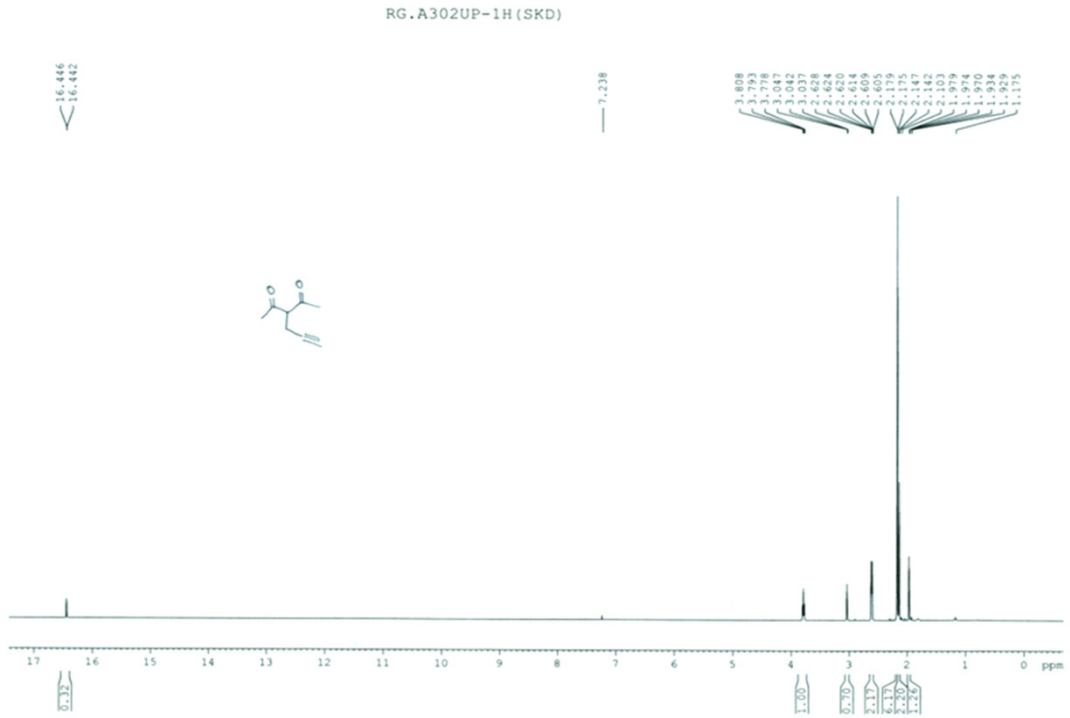


Fig. S-1[1]H NMR spectrum of **1**

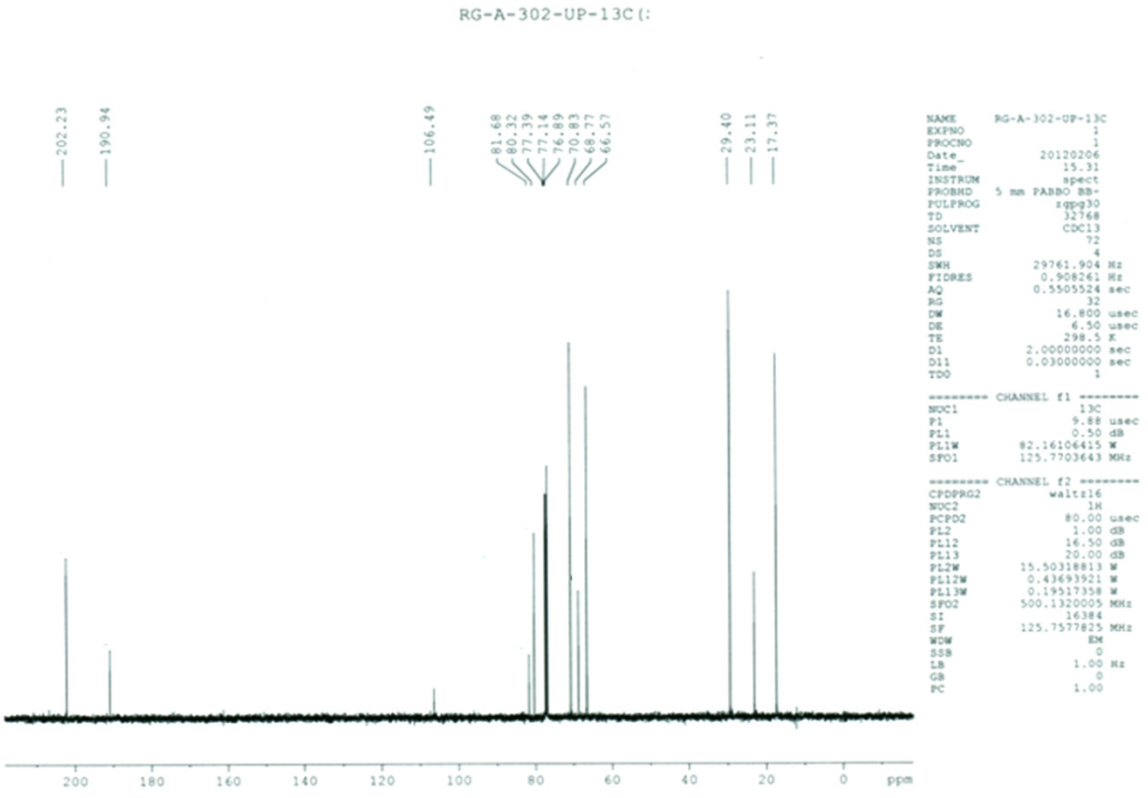


Fig. S-2[13]C NMR spectrum **1**

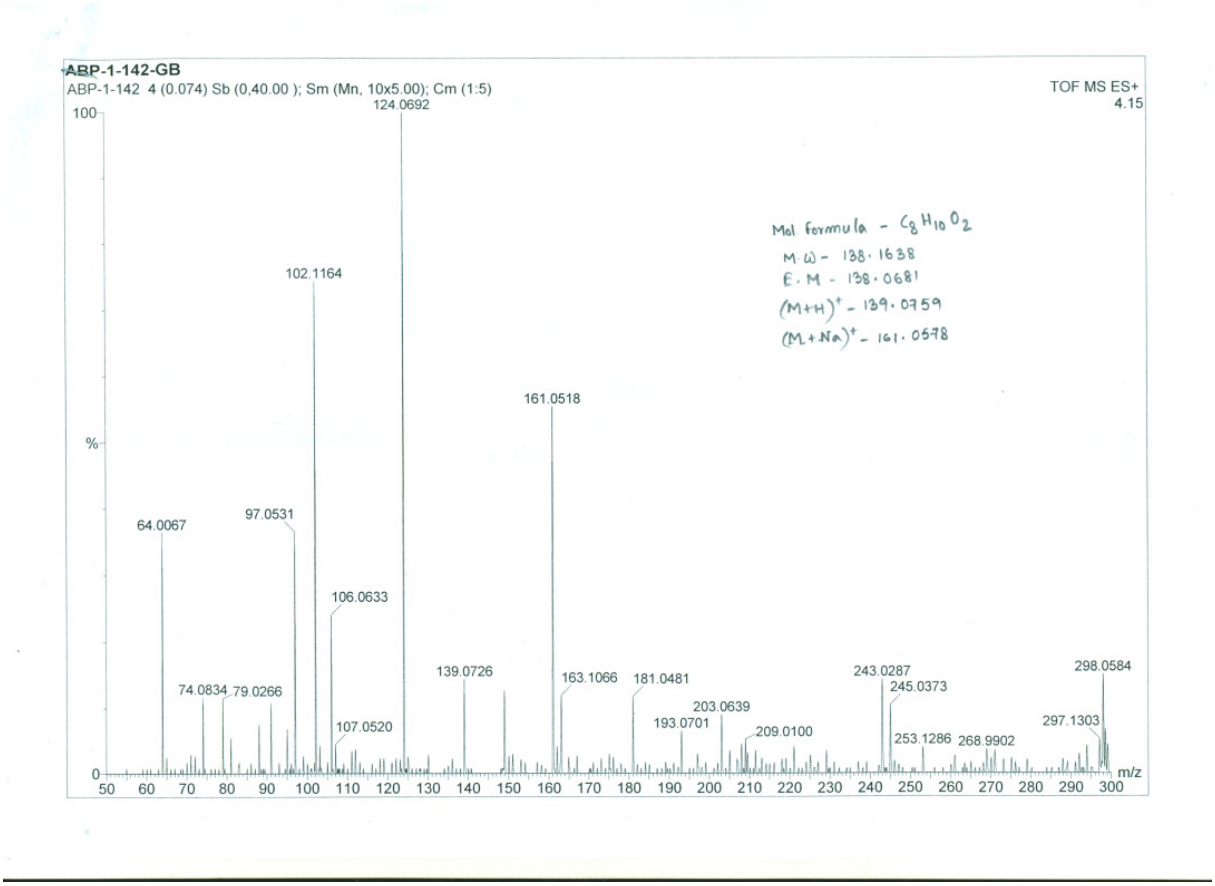


Fig. S-3HRMS spectrum **1**

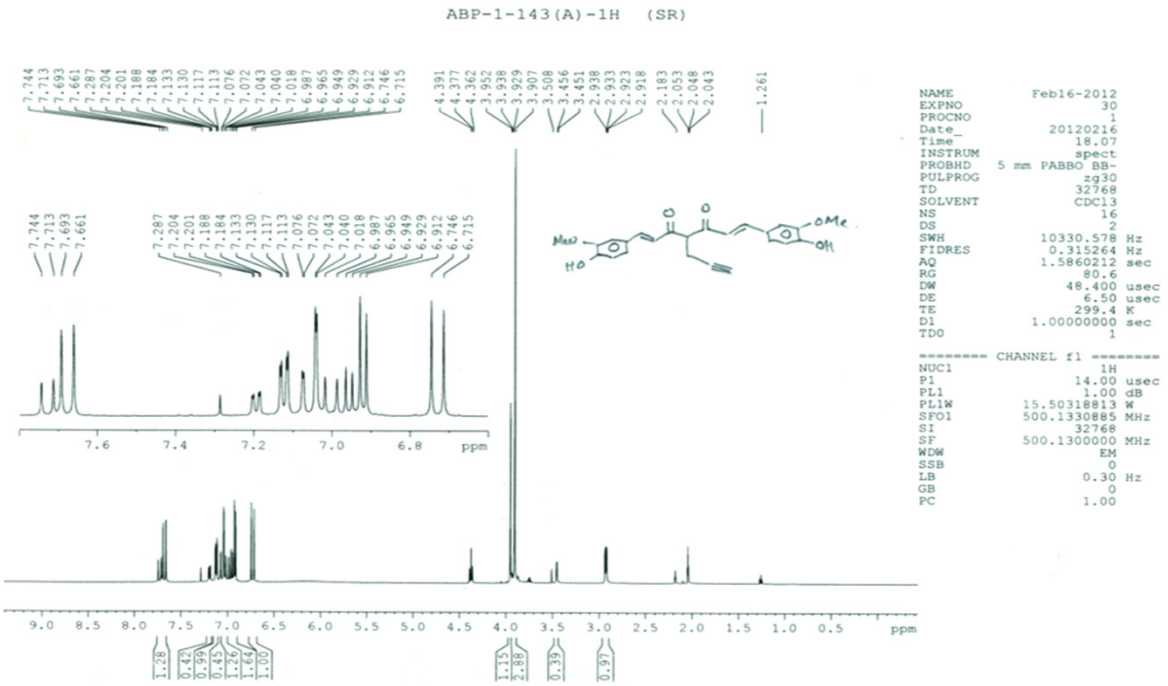


Fig. S-4 ^{1}H NMR spectrum of **CUC-P**

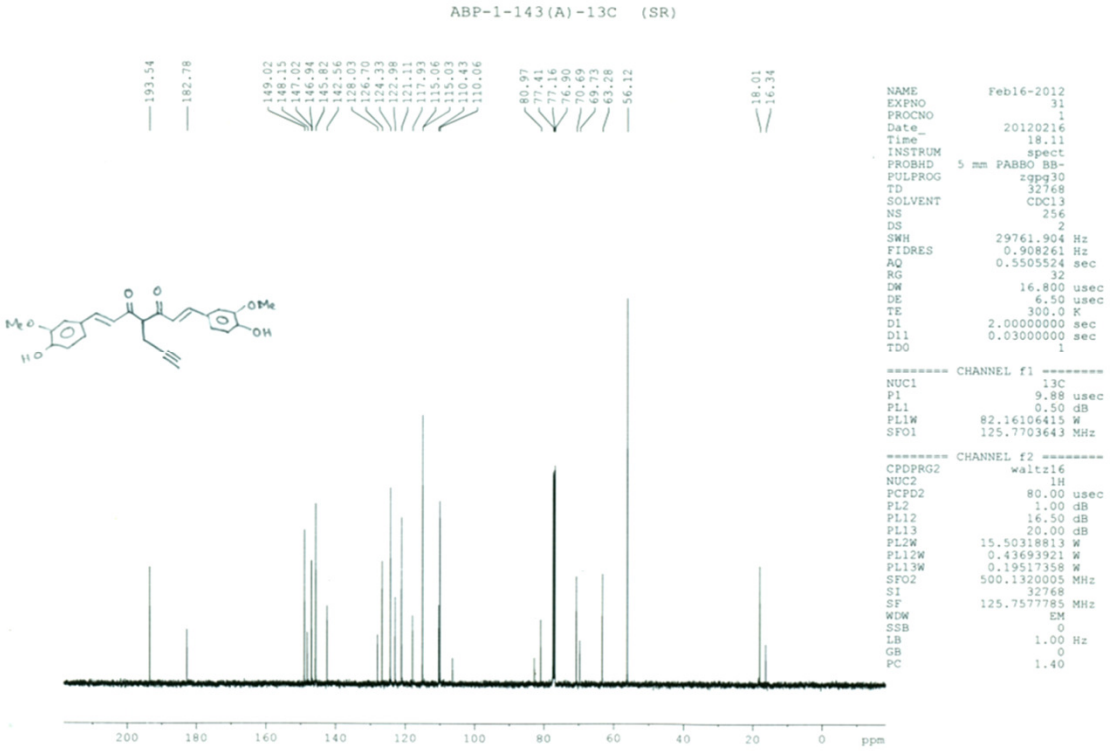


Fig. S-5 ^{13}C NMR spectrum ofCUC-P

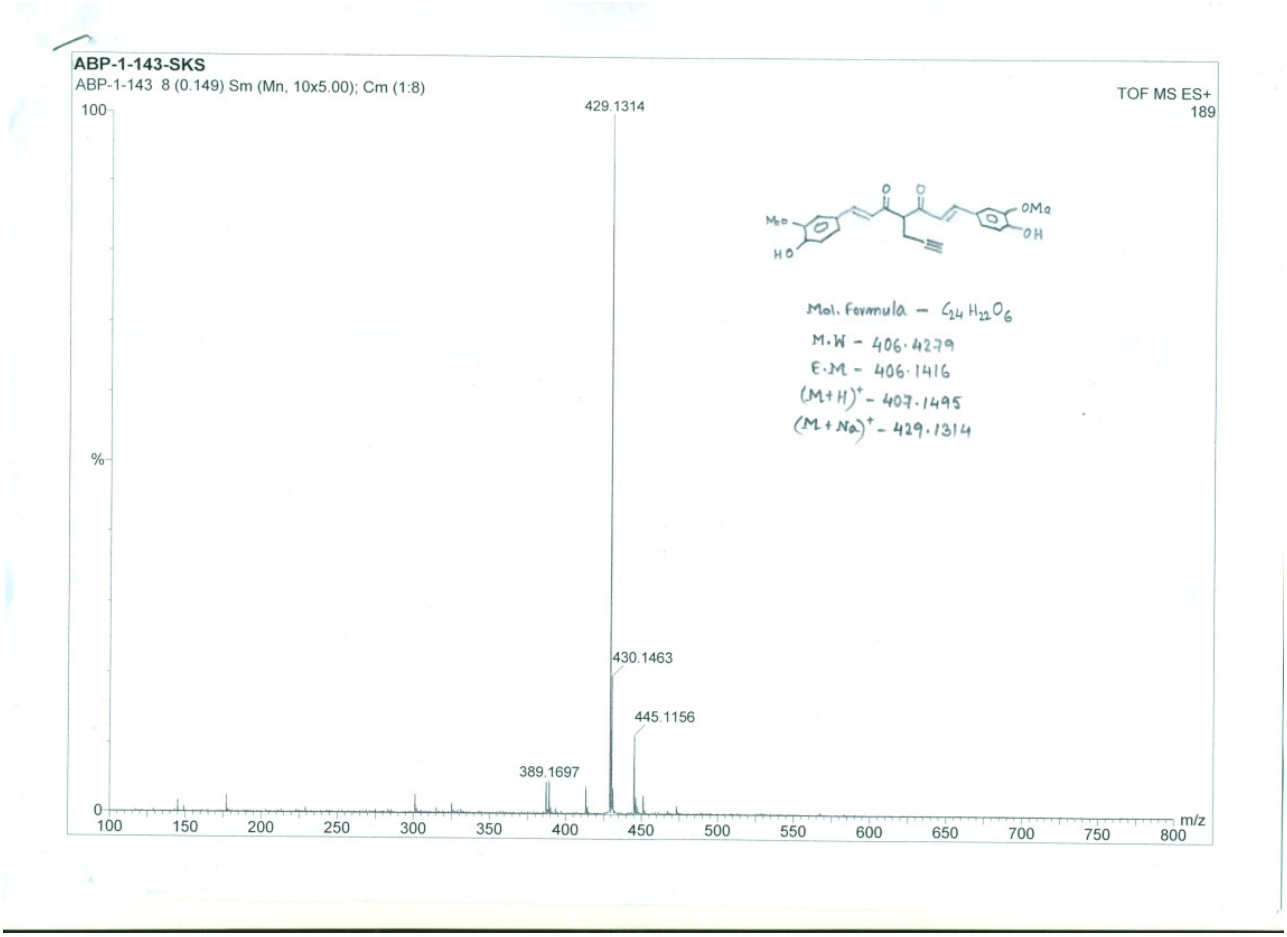


Fig. S-6 HRMS spectrum of **CUC-P**

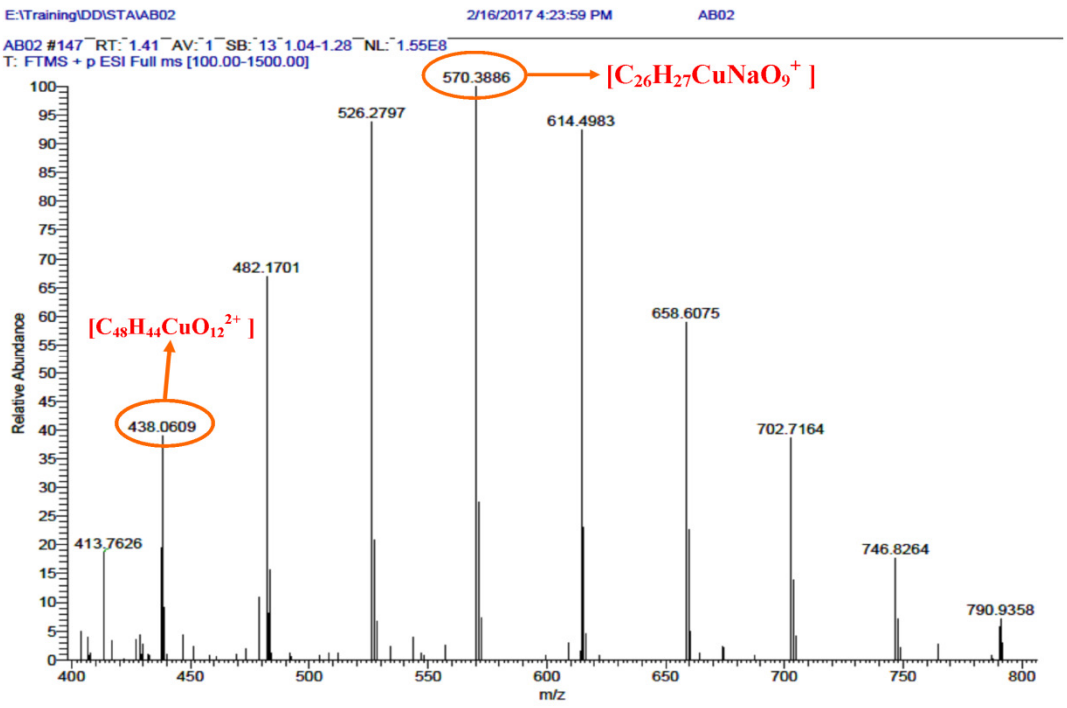


Fig. S-7 HRMS spectrum of [**CUC-P-Cu^{2+}**]

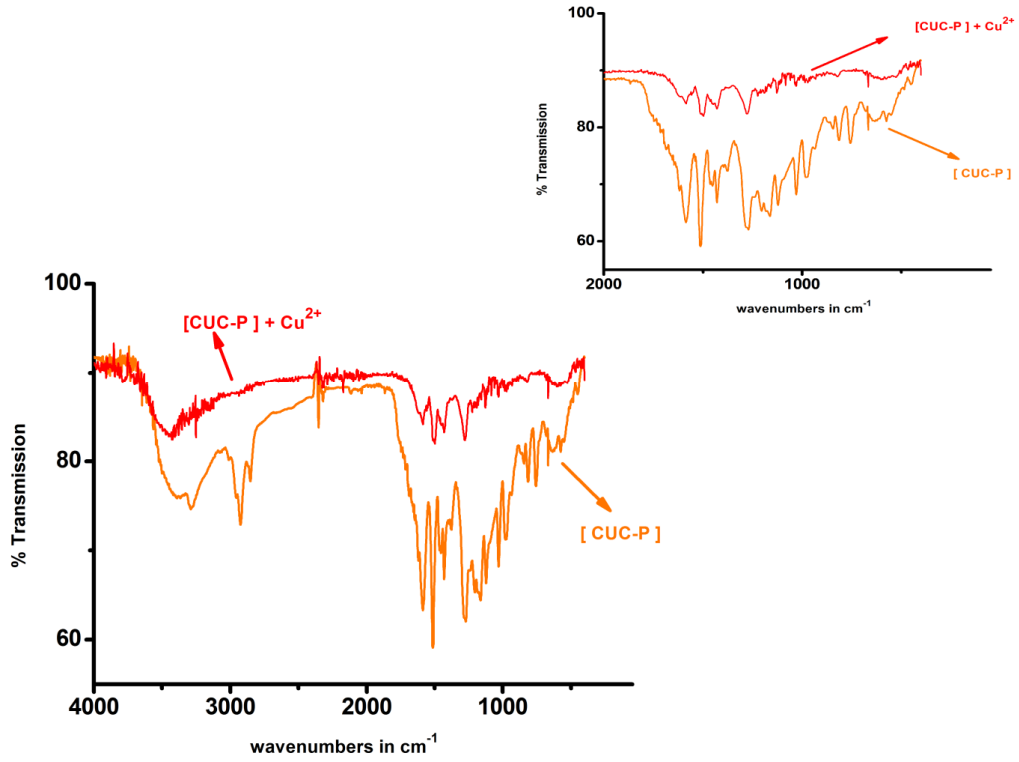


Fig. S-8 IR of **CUC-P** and **[CUC-P]-Cu** complex

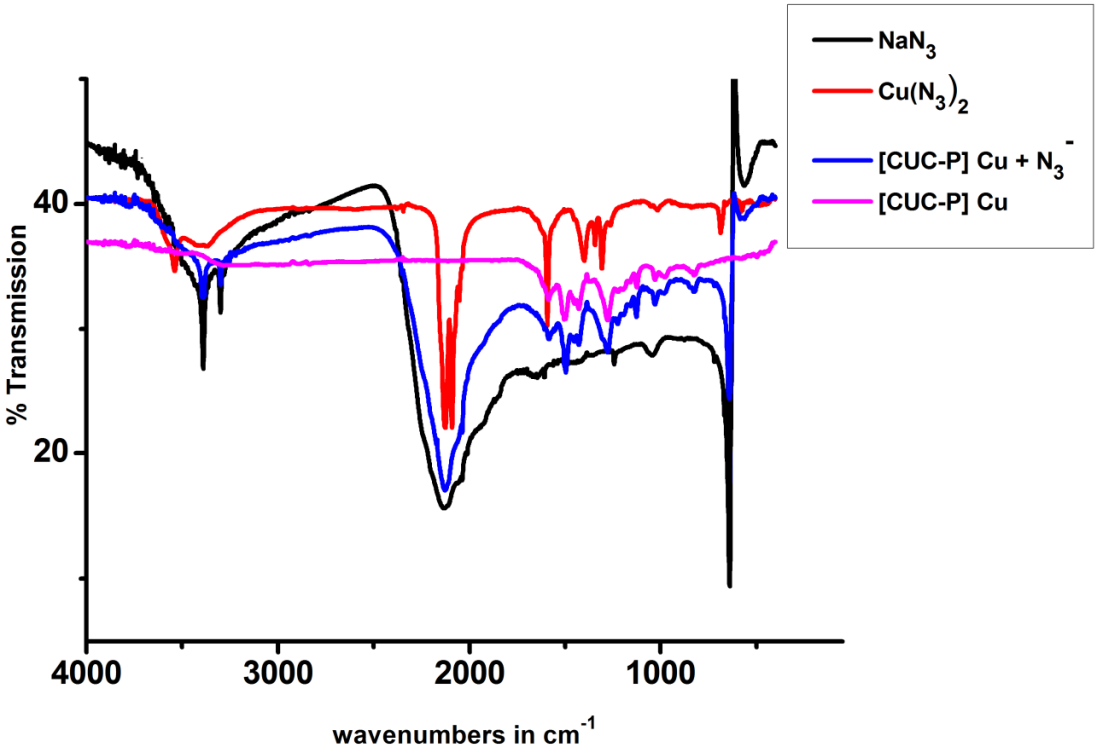


Fig. S-9 Change in IR spectrum of **[CUC-P]-Cu** complex upon addition of 1 equivalent of N $\mathbf{N_3^-}$ ion

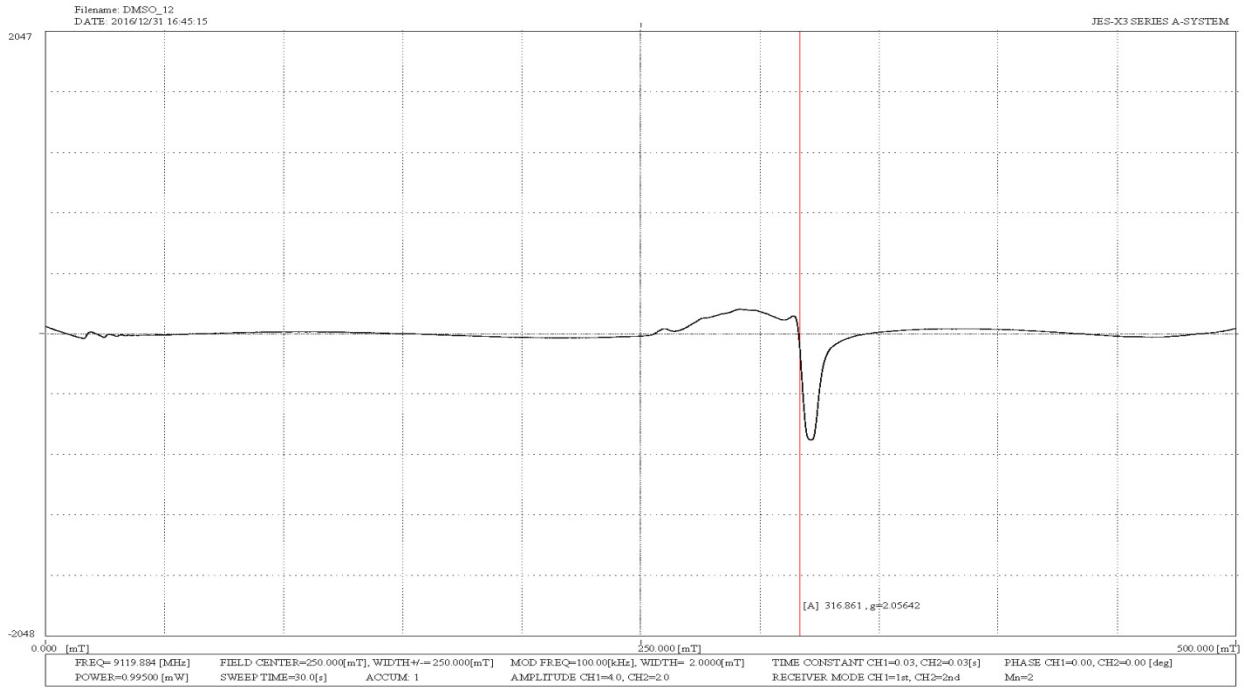


Fig. S-10 EPR of copper complex at room temperature

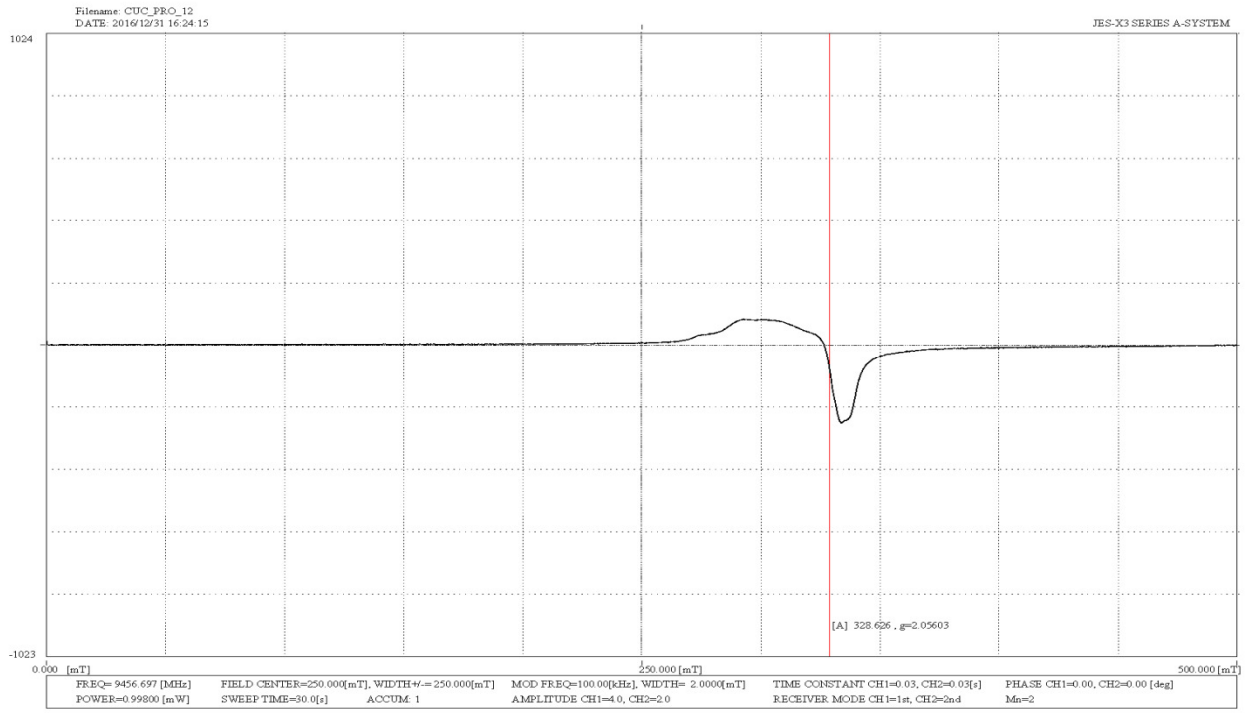


Fig. S-11 EPR of copper complex at 77K

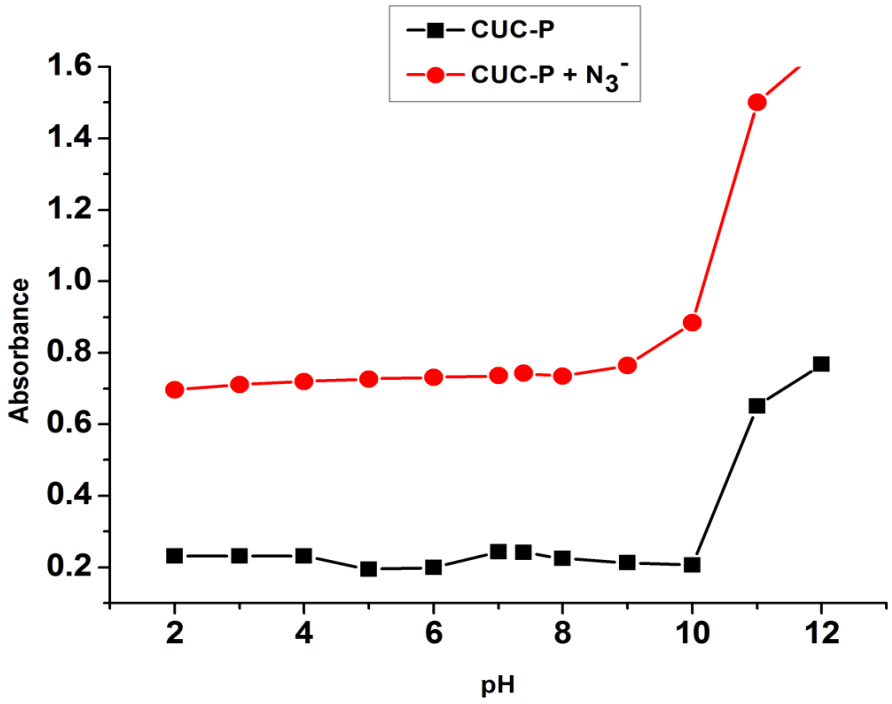


Fig. S-12 Effect of pH on absorbance of **CUC-P** upon addition of N_3^-

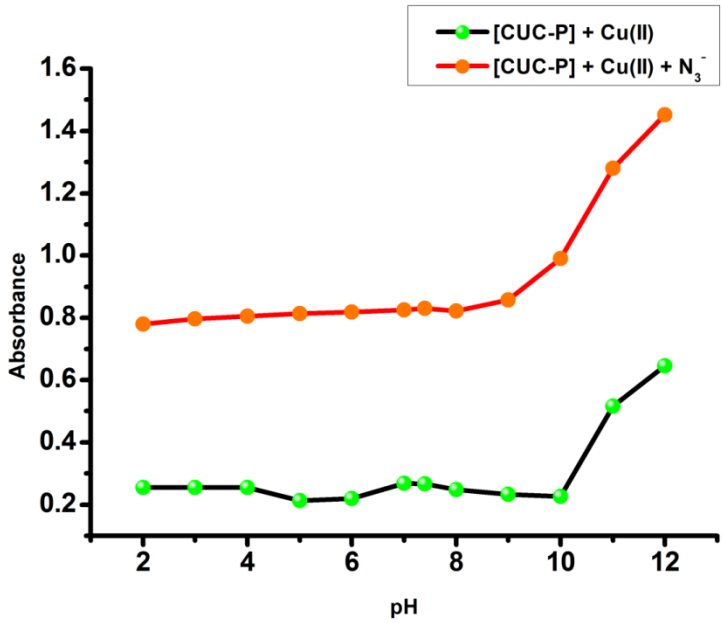


Fig. S-13 Effect of pH on absorbance of [**CUC-P]-Cu (II)]** upon addition of N_3^-

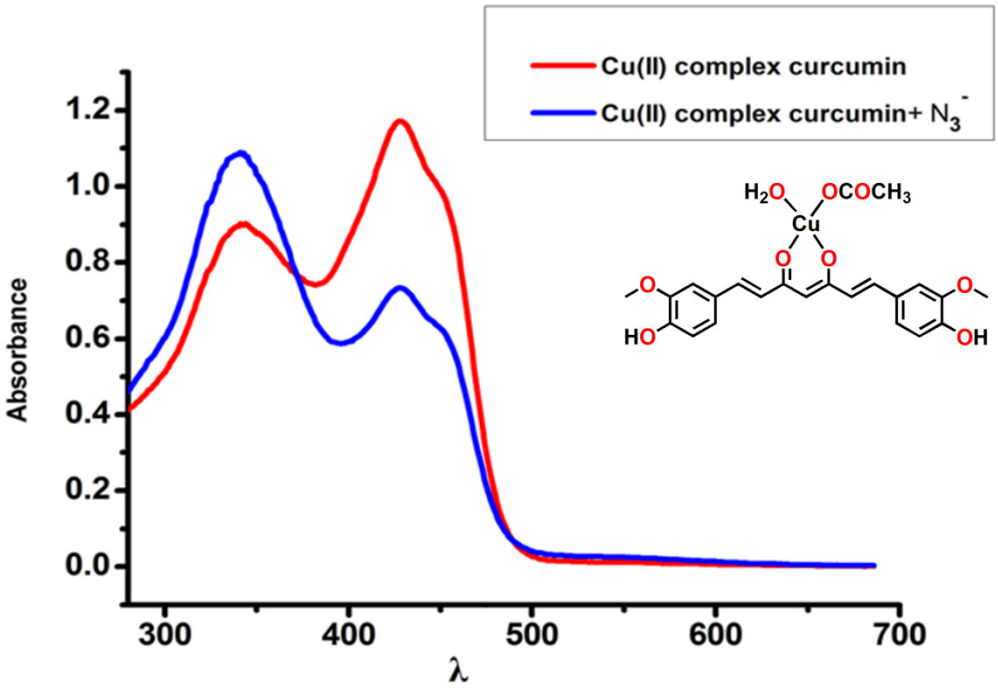


Fig. S-14 Change in the absorbance spectra of **Curcumin-Cu(II) complex**(20 μM) upon gradual addition of 20 μM N_3^- ion in 3: 7 DMSO-HEPES buffer.

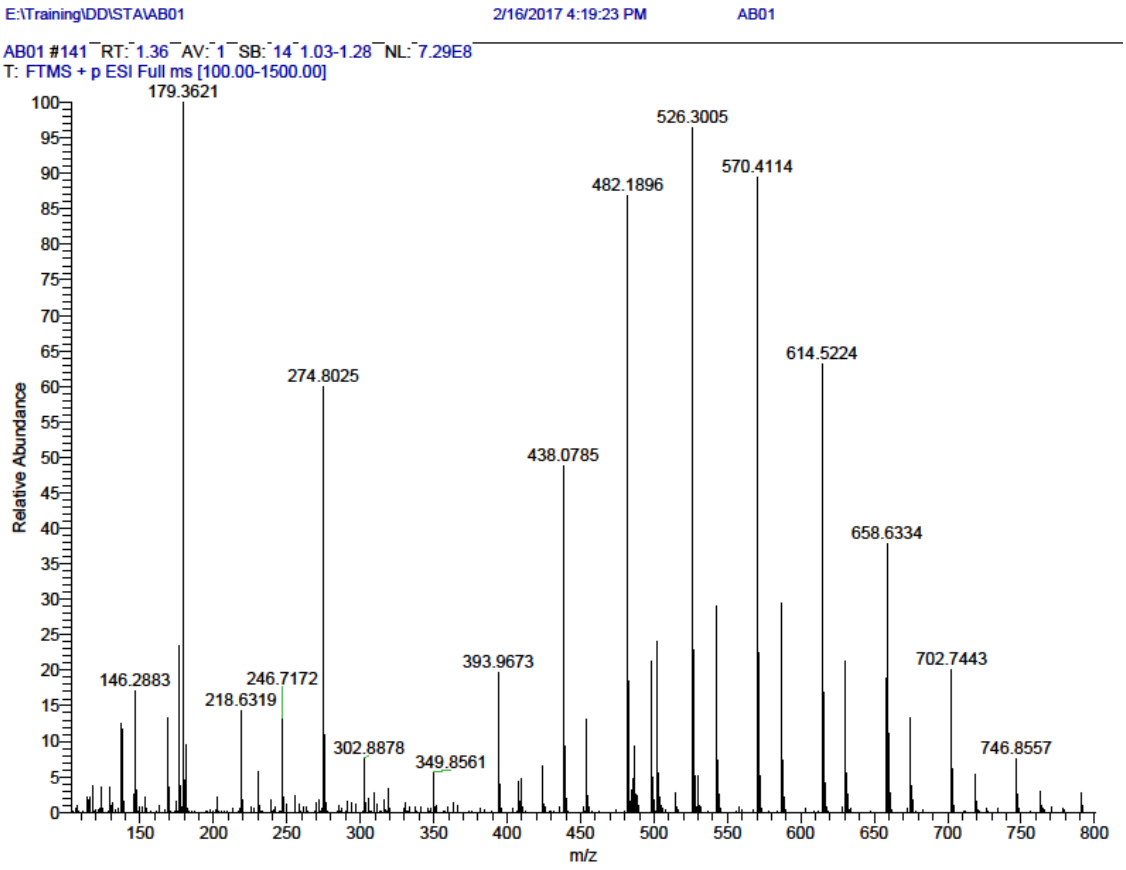


Fig. S-15 HRMS spectrum of [**CUC-P-Cu**$^{2+}$] + **N**$_3^-$

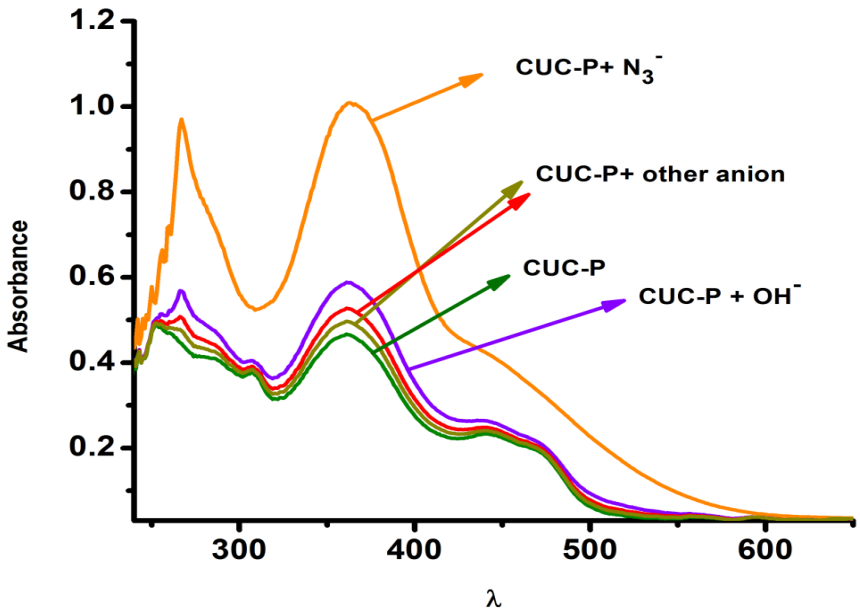


Fig. S-16 Change in the absorbance spectra of **CUC-P** (20 µM) upon gradual addition of 20 µM all common anions in 3: 7 DMSO-HEPES buffer.

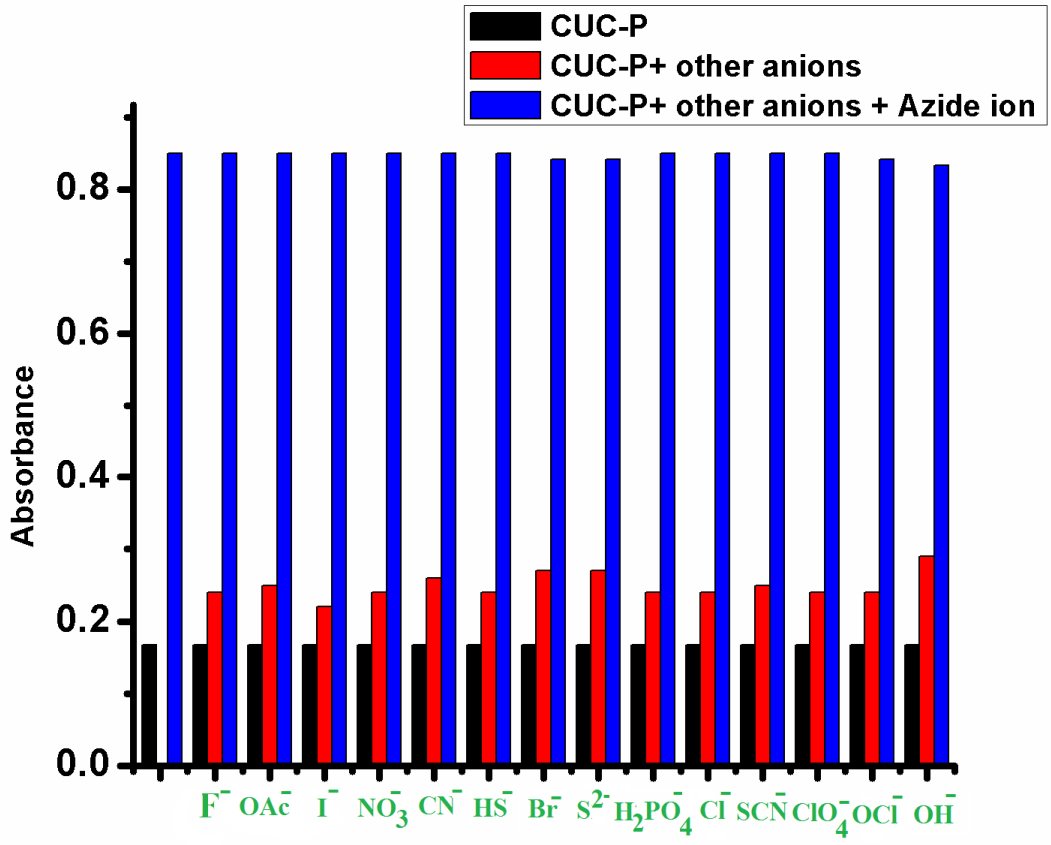


Fig. S-17 Change in the absorbance intensity of **CUC-P** (20 μM) upon gradual addition of 20 μM all common anions in 3: 7 DMSO-HEPES buffer in presence of 20 μM $\mathbf{N_3^-}$ ion.

Calculation of detection limit:

Equation used for calculating detection limit (DL) by fluorescence method

DL = CL × CT

CL = Conc. of probe; CT = Conc. of N_3^- at which fluorescence quenched.

Thus;

DL = $20 \times 10^{-6} \times 0.05 \times 10^{-6} = 1 \times 10^{-6}$ M

Equation used for calculating detection limit (DL) by absorbance method

DL = CL × CT

CL = Conc. of probe; CT = min. Conc. of N_3^- at which absorbance value changes.

Thus;

DL = $20 \times 10^{-6} \times 0.5 \times 10^{-6} = 10 \times 10^{-6}$ M

Calculation of quantum yield:

Fluorescence quantum yields were determined from the spectrum integrated fluorescence by using quinine sulfate as a reference in 0.1 M $HClO_4$ excited at its 347.5 nm absorbance < 0.01; $\Phi_{Ref} = 0.60$. [1] The calculated quantum yields were corrected for differences in peak absorbance and in refractive index of the solvents (obtained from the product specification).

$$QY_{unkown} = QY_{Sandard}\ (FA_{unkown}/\ FA_{Sandard})(A_{Sandard}/A_{unkown})(\lambda_{ex\ Sandard}/\ \lambda_{ex\ unkown})(\eta_{unkown}^2)/\ (\eta_{Sandard}^2)$$

where QY = quantum yield; FA = integrated area under the corrected emission spectrum (in Ep units); A = absorbance at the excitation wavelength; λ_{ex} = the excitation wavelength; η = the refractive index of the solution; and the subscripts u and s refer to the unknown and the standard, respectively.

	Integrated peak area of standard/sample in DMSO	Absorbance
CUC-P	1000	0.031
	1405	0.044
	1900	0.062

Refractive index of 3:7 DMSO: water is 1.3754 [2]

Refractive index Quinine sulphate in 0.1 M $HClO_4$ is 1.33

sample	Integrated emission intensity	Absorbance At 340	Refractive index	Quantum yield
Quinine sulphate	40893	0.03835	1.33	0.54

CUC-P	1000	0.0325	1.37	0.0164

Quantum yield of CUC-P =0.0164

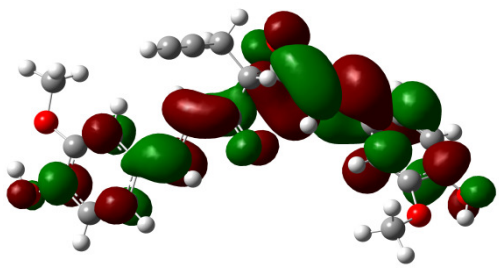
Table S-1 Comparison table of known azide probes

Probe type	**Solvent System**	**LOD**	**Reference**
Fluorescence Turn on	Aqueous medium	2.6 x 10^{-4} g/L = 3.99μM	*Chem. Commun.,* 2010, **46**, 1754–1756
Fluorescence Turn on	Ethanol : water (4 : 1, v/v, in 0.1 M HEPES buffer solution at pH 7.4)	1.0 x 10^{-7} M	***Analyst***, 2012,**137**, 1544-1546
Fluorescence Turn on	1:1 (v/v) mixture of CH_3CN and HEPES buffer solution (pH 7.1, 20 mM)	1.6×10^{-5} M	*RSC Adv.,* 2015,**5**, 4623-4627
Fluorescence Turn on	H_2O/THF, 10:90, v/v)	4.87×10^{-7} M	*Sens. Actuator B-Chem.* 2016, **224**, 73–80
Fluorescence Turn on	50 mM HEPES buffer and dioxane	10 μM	*Bioorg Med Chem Lett.* 2016, **26**, 1651–

	(1: 1) at pH 7.4		1654.
Fluorescence Turn on	1:9 (v/v) tris buffer (pH 8.0): acetonitrile.	0.4 µM	*Sens. Actuator B-Chem.,* 2014, **192**, 9–14.
FRET based probe	DMSO	-	*Sens. Actuator B-Chem.,* 2017, **239**, 1076–1086.
Colorimetric probe and fluorescent probe	DMSO-HEPES buffer (3: 7, v/v);	10 µM by colorimetric method and 1 µM by fluorescence method	Present work

Table S-2

Frontier molecular orbitals (MOs) of **CUC-P (keto form)** and the energy levels of the MOs are shown (in a.u). Calculations are based on ground state geometry by DFT at the B3LYP/ 3-21G/level using Gaussian 09

Frontier orbital	**Energy (a.u.)**	**Energy optimised geometry**
LUMO+1	-0.0593	

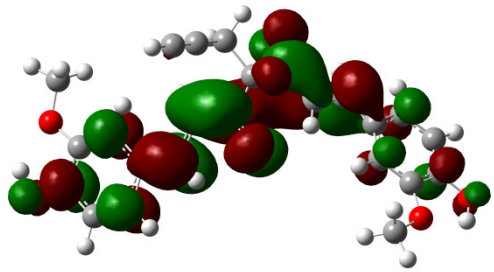
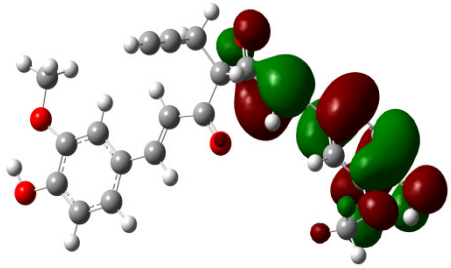
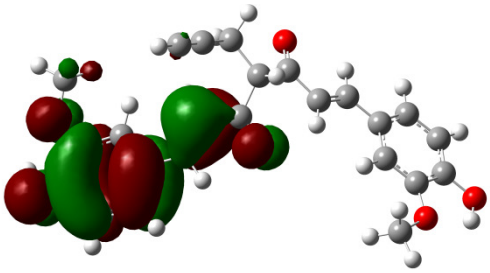
LUMO	-0.0725	
HOMO	-0.2054	
HOMO-1	-0.2089	

Table S-3

Frontier molecular orbitals (MOs) of **[CUC-P]-Cu (II)** complex and the energy levels of the MOs are shown (in a.u). Calculations are based on ground state geometry by DFT at the B3LYP/ LANL2DZ /level using Gaussian 09

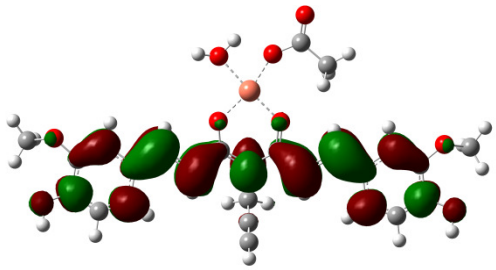
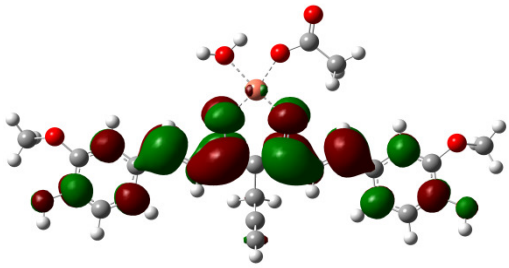
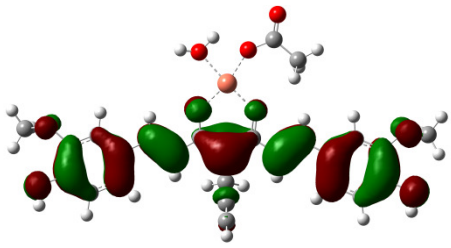
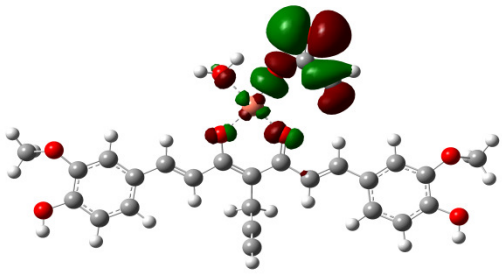
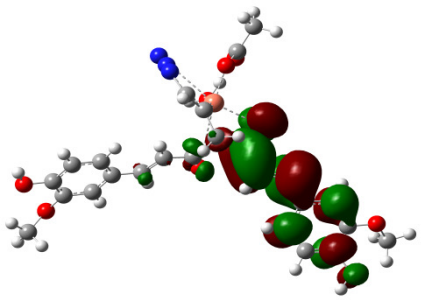
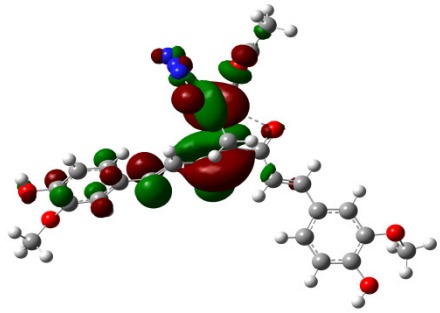
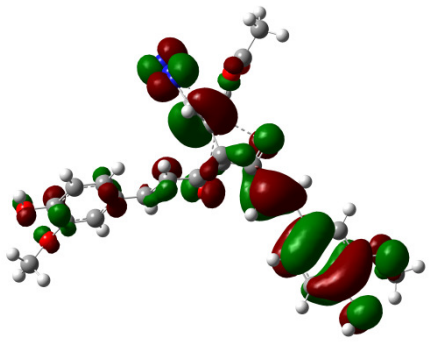
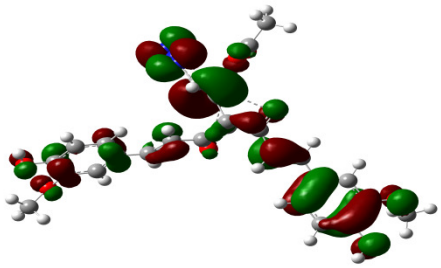
Frontier orbital	Energy (a.u.)	Energy optimised geometry
LUMO+1	-0.0617	
LUMO	-0.1021	
HOMO	-0.2175	
HOMO-1	-0.2241	

Table S-4

Frontier molecular orbitals (MOs) of **[CUC-P]-Cu (II) complex + N_3^-** and the energy levels of the MOs are shown (in a.u). Calculations are based on ground state geometry by DFT at the RB3LYP/ LANL2DZ/level using Gaussian 09

Frontier orbital	Energy (a.u.)	Energy optimised geometry
LUMO+1	-0.0907	
LUMO	-0.1227	
HOMO	-0.2271	

HOMO-1	-0.2304	

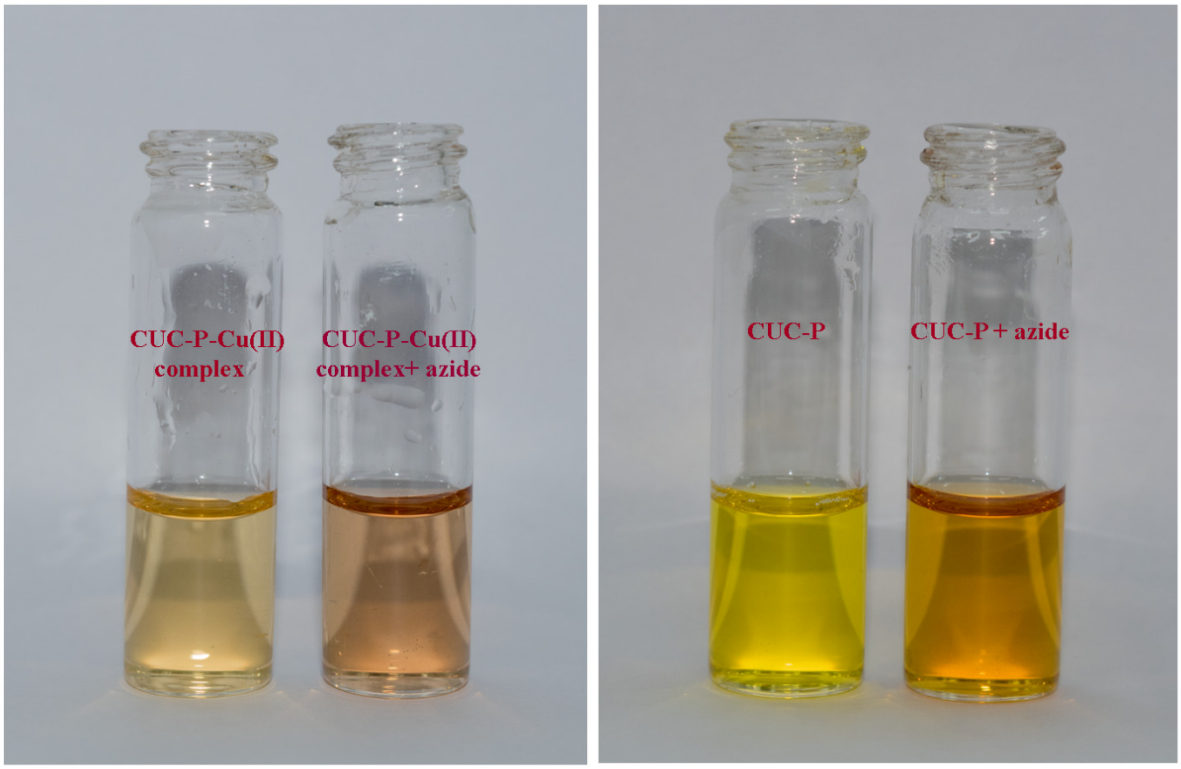


Fig. S-18 The colour change observed in visible light of both the **CUC-P** and [**CUC-P-Cu (II)]** complex upon addition of N_3^- ion in solution phase.

Reference:

1. (a) R. Velapoldi and H.H. Tønnesen, Corrected emission spectra and quantum yields for a series of fluorescent compounds in the visible spectral region, *J. Fluoresc.* 14 (2004), 465–472. (b) L. Nardo, R. Paderno, A. Andreoni , M. Másson , T. Haukvik and H. H. Tønnesen,

Role of H-bond formation in the photoreactivity of curcumin Spectroscopy 22 (2008) 187–198 187. DOI 10.3233/SPE-2008-0335.

2. R. G. LeBel, D. A. I. Goring, Density, Viscosity, Refractive Index, and Hygroscopicity of Mixtures of Water and Dimethyl Sulfoxide. J. Chem. Eng. Data, 1962, 7 (1), pp 100–101 DOI: 10.1021/je60012a032.