Supporting Information

A simple and easy-to-prepare imidazole-based probe for the selective chromo-fluorogenic recognition of biothiols and of Cu(II) in HeLa Cells and in aqueous environments

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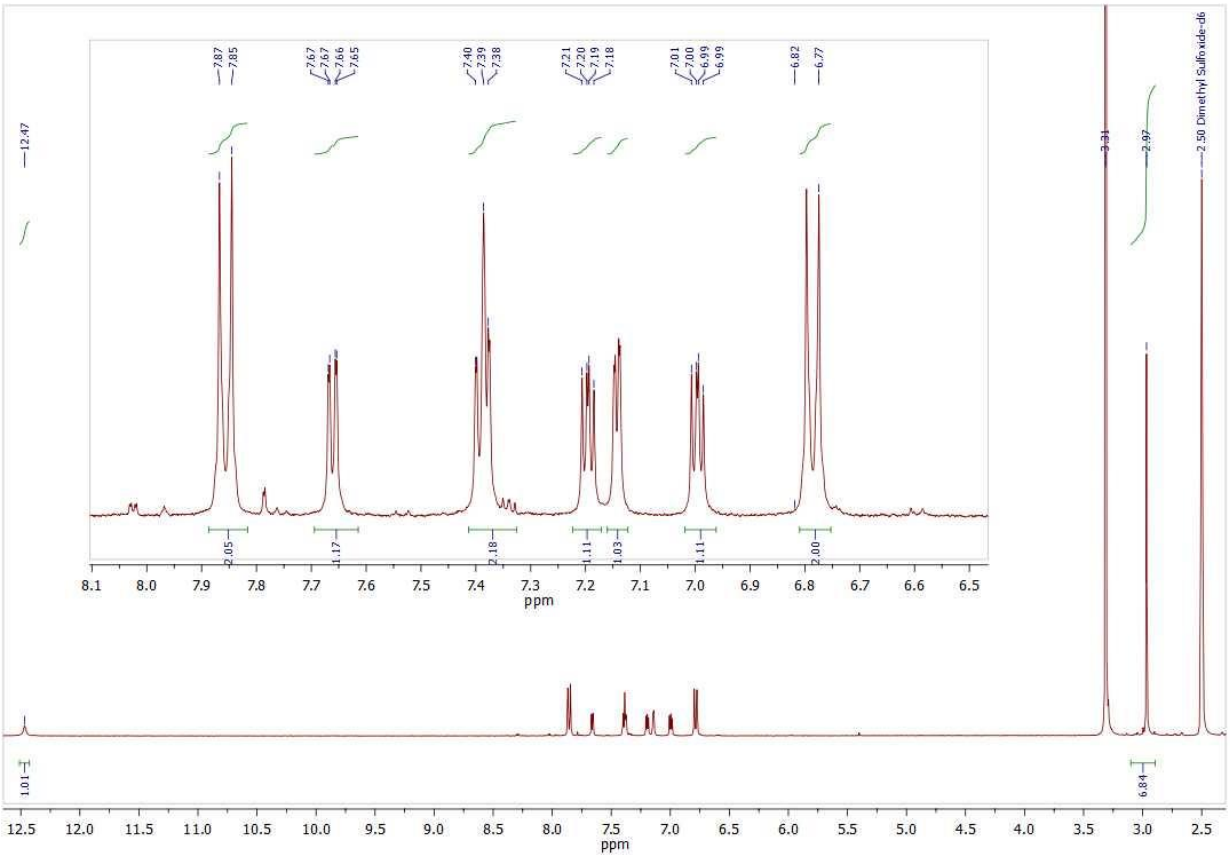

Figure S1. ^{1}H NMR spectra of probe **1** in DMSO-d_6.

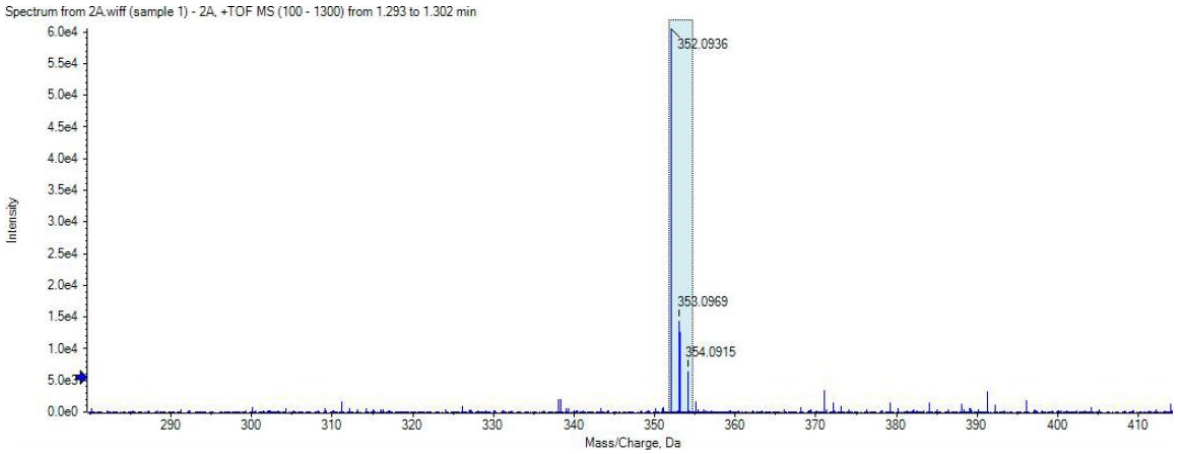

Figure S2. ESI-MS spectra of probe **1**.

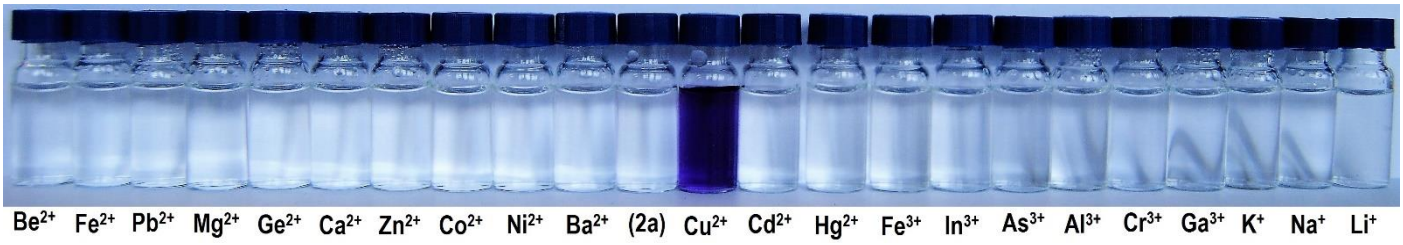

Figure S3. Colour changes observed in acetonitrile solutions of probe **1** (1.0 x 10^{-5} mol L^{-1}) upon addition of 10 equiv. of selected metal cations.

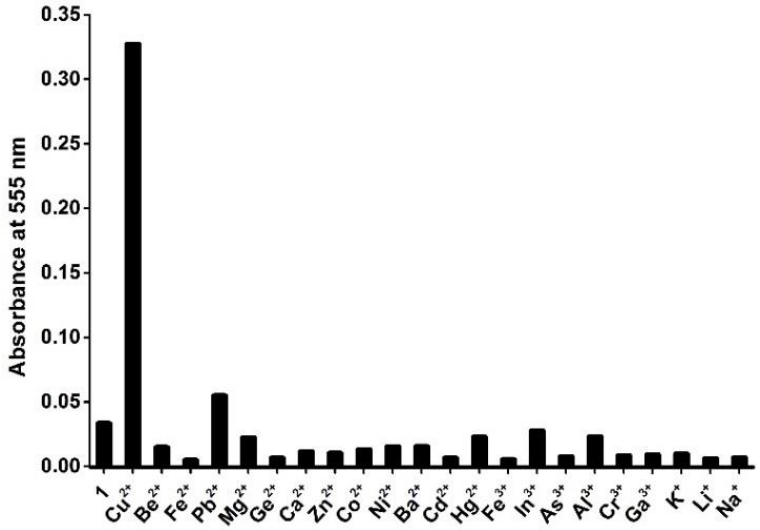

Figure S4. Absorbance at 555 nm of water (pH 7.4)-acetonitrile 90:10 v/v (1.0 x 10^{-5} mol L^{-1}) solutions of probe **1** alone and in the presence of selected metal cations (10 eq.).

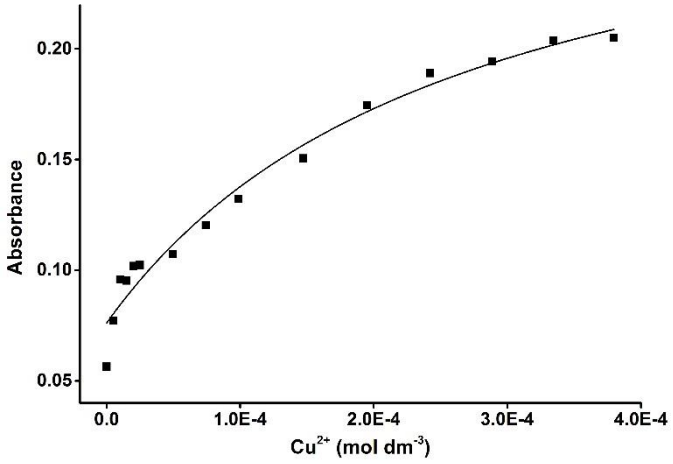

Figure S5. Absorbance at 555 nm of water (pH 7.4)-acetonitrile 90:10 v/v (1.0×10^{-5} mol L^{-1}) solutions of probe **1** upon addition of increasing quantities of Cu(II).

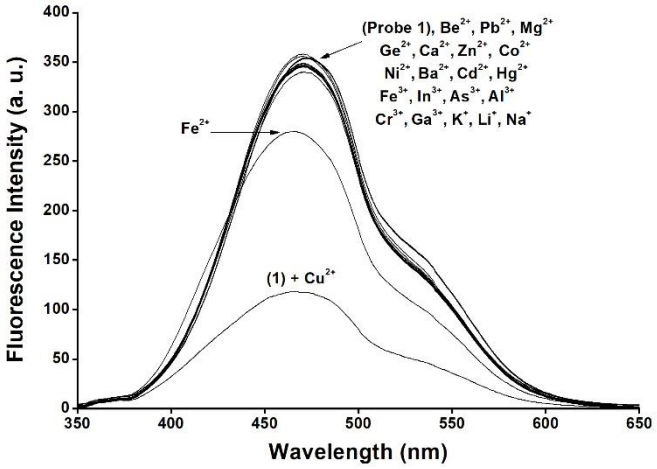

Figure S6. Fluorescence spectra (excitation at 320 nm) of probe **1** in water (pH 7.4)-acetonitrile 90:10 v/v (1.0×10^{-5} mol L^{-1}) upon addition of selected metal cations (10 eq.).

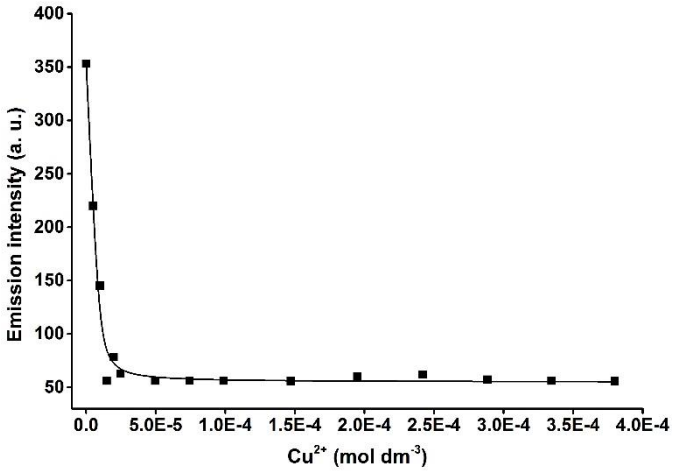

Figure S7. Fluorescence at 475 nm (excitation at 320 nm) of water (pH 7.4)-acetonitrile 90:10 v/v (1.0×10^{-5} mol L^{-1}) solutions of probe **1** upon addition of increasing quantities of Cu(II).

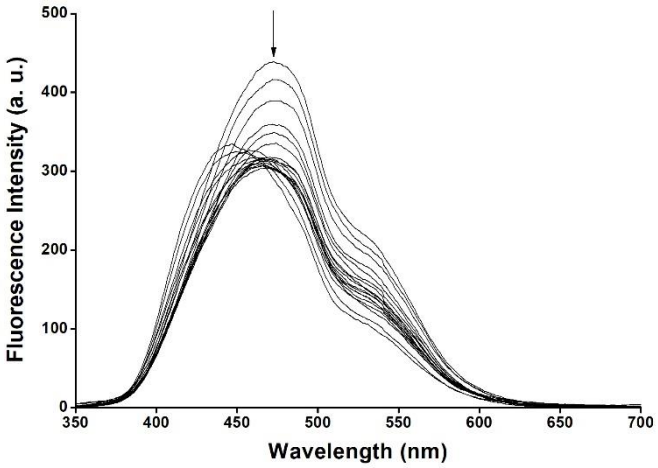

Figure S8. Emission spectra of probe **1** in water (pH 5.0)-acetonitrile 90:10 v/v (1.0×10^{-5} mol L^{-1}) solutions of probe **1** upon addition of increasing quantities of Cu(II).

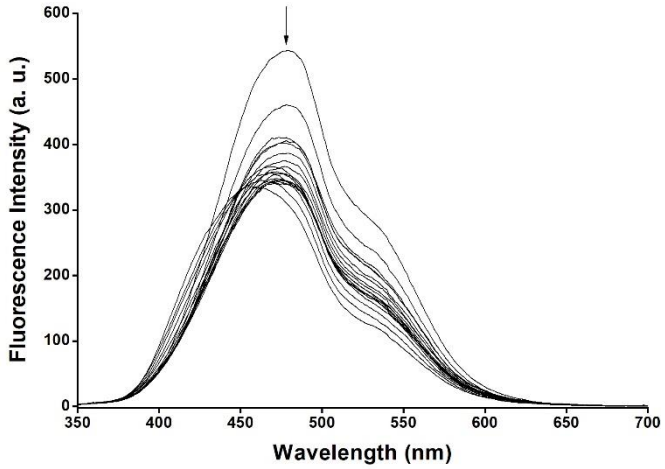

Figure S9. Emission spectra of probe **1** in water (pH 6.0)-acetonitrile 90:10 v/v (1.0×10^{-5} mol L^{-1}) solutions of probe **1** upon addition of increasing quantities of Cu(II).

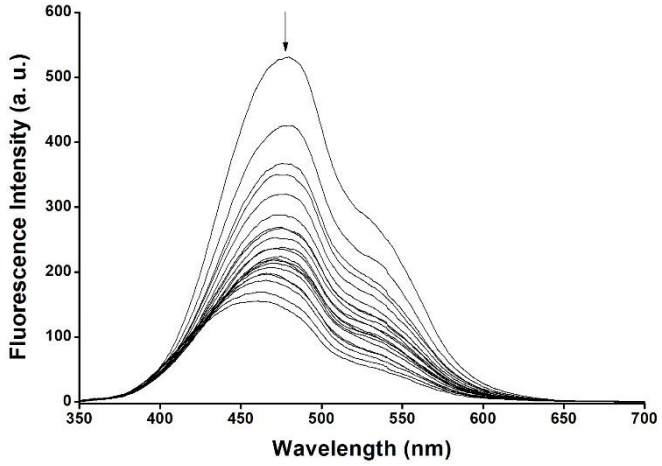

Figure S10. Emission spectra of probe **1** in water (pH 8.0)-acetonitrile 90:10 v/v (1.0×10^{-5} mol L^{-1}) solutions of probe **1** upon addition of increasing quantities of Cu(II).

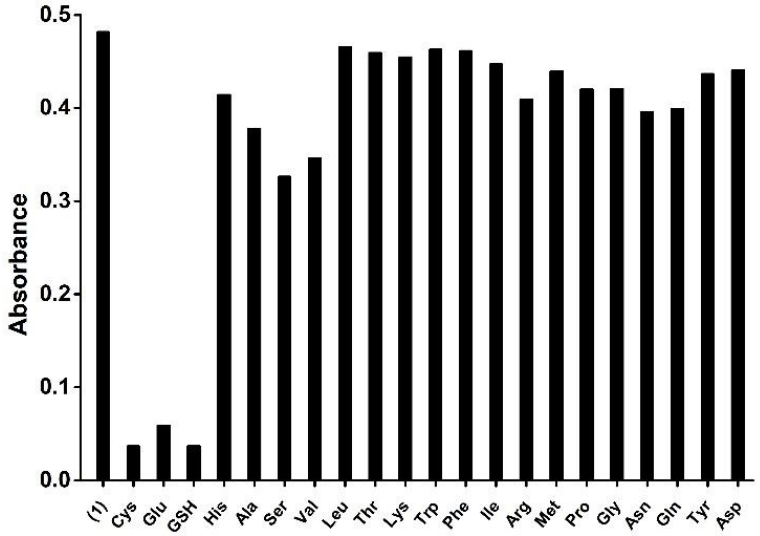

Figure S11. Absorbance at 555 nm of water (pH 7.4)-acetonitrile 90:10 v/v solutions of **1**·Cu(II) complex (6.2 x 10^{-6} mol L^{-1}) in the presence of Cys, Hcy and GSH (0.3 eq.) and selected amino acids (0.3 eq.).

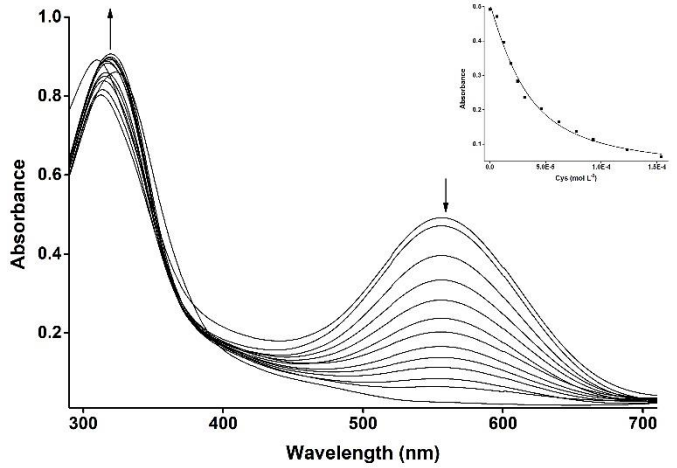

Figure S12. UV/Vis. titration profile of **1**·Cu(II) complex (6.2 x 10^{-6} mol L^{-1}) in water (pH 7.4)-acetonitrile 90:10 v/v upon addition of Cys (0 - 1.0 equivalents). Inset: Absorbance at 555 nm vs Cys concentration.

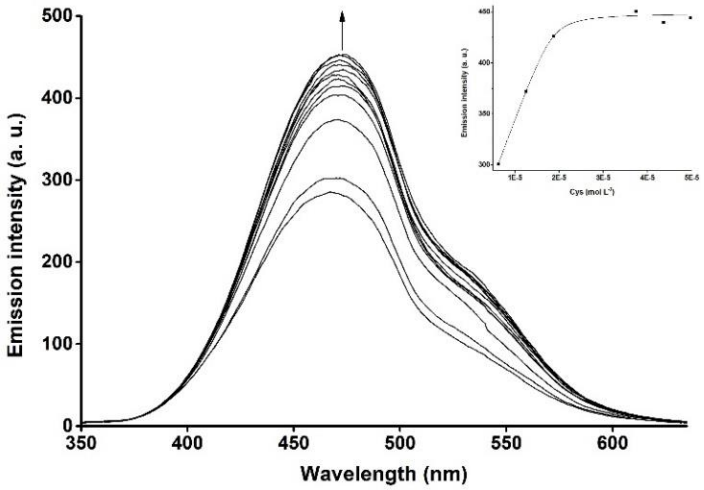

Figure S13. Fluorescence spectral changes of water (pH 7.4)-acetonitrile 90:10 v/v solutions of **1**·Cu(II) complex (6.2 x 10^{-6} mol L^{-1}) upon addition of increasing quantities of Cys (excitation at 324 nm). Inset emission at 475 nm vs. Cys concentration.

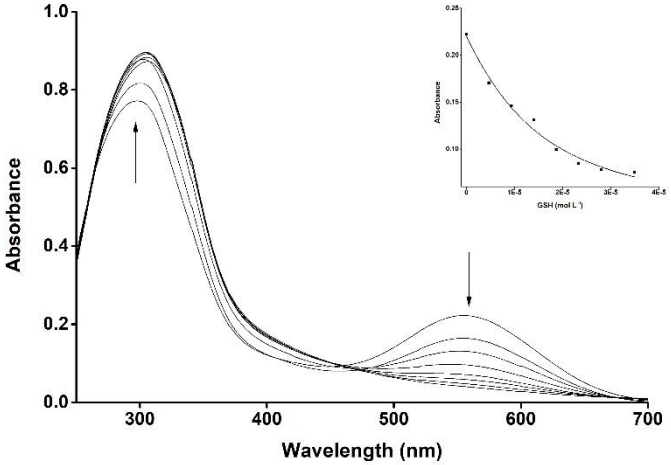

Figure S14. UV/Vis. titration profile of **1**·Cu(II) complex (6.2 x 10^{-6} mol L^{-1}) in water (pH 7.4)-acetonitrile 90:10 v/v upon addition of GSH (0 – 0.8 equivalents). Inset: Absorbance at 555 nm vs GSH concentration.

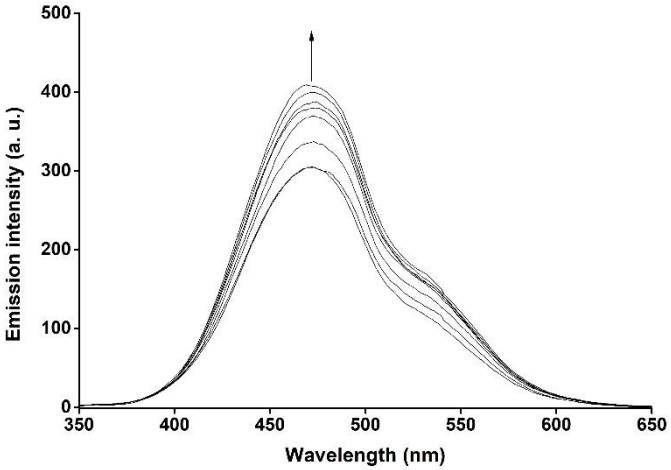

Figure S15. Fluorescence spectral changes of water (pH 7.4)-acetonitrile 90:10 v/v solutions of **1**·Cu(II) complex (6.2 x 10^{-6} mol L^{-1}) upon addition of increasing quantities of GSH (excitation at 324 nm).

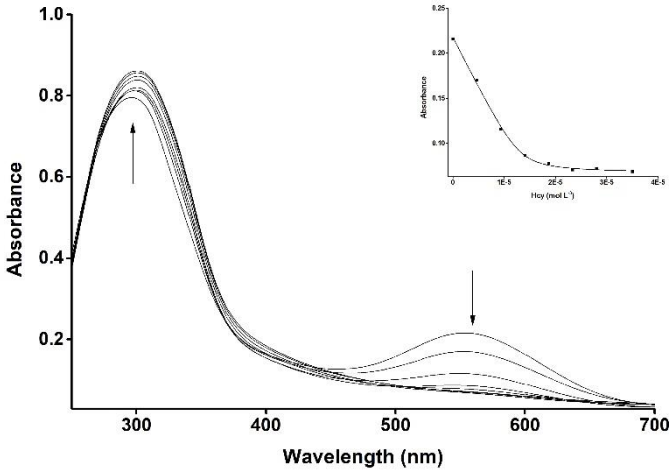

Figure S16. UV/Vis. titration profile of **1**·Cu(II) complex (6.2 x 10^{-6} mol L^{-1}) in water (pH 7.4)-acetonitrile 90:10 v/v upon addition of Hcy (0 – 0.8 equivalents). Inset: Absorbance at 555 nm vs Hcy concentration.

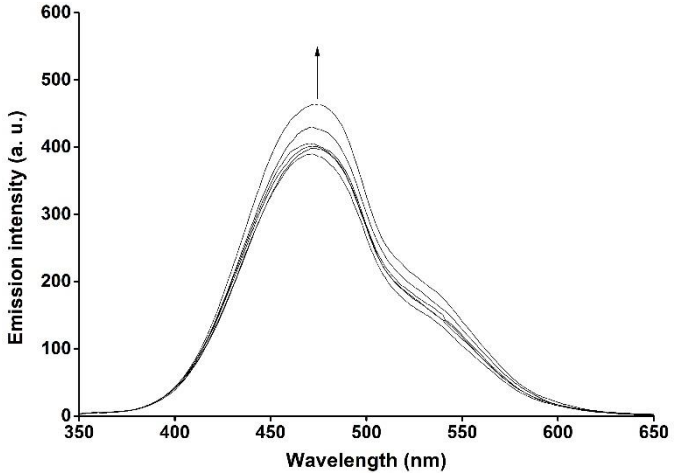

Figure S17. Fluorescence spectral changes of water (pH 7.4)-acetonitrile 90:10 v/v solutions of **1**·Cu(II) complex (6.2 x 10^{-6} mol L^{-1}) upon addition of increasing quantities of Hcy (excitation at 324 nm).

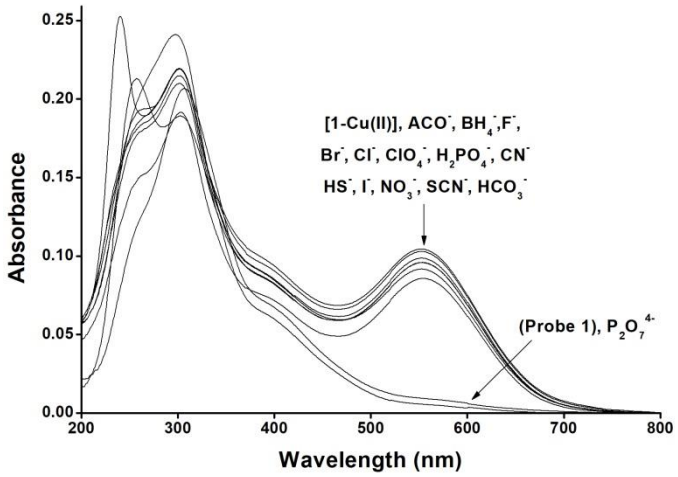

Figure S18. UV-visible changes of **1**-Cu(II) (3.2 x 10^{-6} mol L^{-1}) in water (pH 7.4)-acetonitrile 90:10 v/v in the presence of selected anions (0.2 eq.).

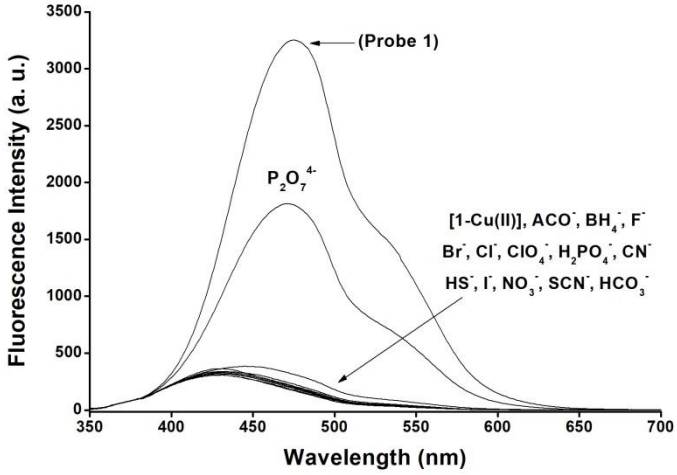

Figure S19. Changes in the emission band of **1**-Cu(II) complex (3.2 x 10^{-6} mol L^{-1}) in water (pH 7.4)-acetonitrile 90:10 v/v upon addition of selected anions (0.2 eq.).

Table S1. Analytical features of recently chromo-fluorogenic probes for biothiols detection recently published.

Probe	Mechanism	Media	Response	Limit of detection (μM)	Reference
Imidazole derivative-Cu(II) complex	Demetallation	Water-ACN 9:1	Cys, Hcy, GSH	6.5 (Cys); 5.0 (Hcy); 10.2 (GSH)	This paper
Fluorescein derivative-Cu(II) complex	Demetallation	Hepes	Cys, Hcy, GSH	0.12 (Cys); 0.036 (Hcy); 0.024 (GSH)	41
Naphthol derivative-Cu(II) complex	Demetallation	Water-DMSO 7:3	Cys (Hcy and GSH not tested)	2.9	42
Hydroxynaphthalene derivative-Cu(II) complex	Demetallation	Tris-DMSO 1:1	Cys	10.77	43
Anthracenyl derivative-Cu(II) complex	Demetallation	Hepes-ACN 3:7	Cys	0.019	44
Hydorxyjulolidine derivative-Cu(II) complex	Demetallation	Tris-DMF 5:1	Cys	3.6	45
Hydorxyjulolidine derivative-Cu(II) complex	Demetallation	Tris-DMSO 2:8	Cys	7.82	46
BODIPY derivative	Michael addition	Hepes-ACN 8:2	Cys	Not reported	28
Coumarin derivative	Michael addition	Water-MeOH 1:1	Cys, Hcy, GSH	0.192 (Cys); 0.158 (Hcy); 0.155 (GSH)	29
Triphenylamine derivative	Michael addition	Hepes-MeOH 1:1	Cys, Hcy, GSH	0.13 (Cys); 0.12 (Hcy); 0.085 (GSH)	30
Coumarin derivative-Cu(II) complex	Cu(II)/Cu(I) conversion	Hepes	Cys, Hcy, GSH	Not reported (Cys); not reported (Hcy); 15 (GSH)	31
Cyanine derivative	Aldehyde-thiazoline conversion	PBS	Cys, Hcy	0.008 (Cys); 0.008 (Hcy)	32
BODIPY derivative	Aldehyde-thiazoline conversion	Water-ACN 2:8	Cys, Hcy	Not reported (Cys); 2 (Hcy)	33
Binaphthyl derivative	Aldehyde-thiazoline conversion	Hepes-EtOH 2:98	Hcy	54	34
Pyronin derivative containing 4-methoxythiophenol	Nucleophilic aromatic substitution	PBS	Cys, Hcy, GSH	Not reported (Cys); not reported (Hcy); not reported (GSH)	35
Cyanine derivative containing 4-methoxythiophenol	Nucleophilic aromatic substitution	Hepes-DMSO 4:1	Cys	1.26	36
Fluorescein derivative	Hydrolysis of an acrylate ester	PBS-DMSO 7:3	Cys	0.084	37
Iminocoumarin derivative	Hydrolysis of a 2,4-dinitrobenzenesulfonyl moiety	PBS	Cys, Hcy, GSH	5.0 (Cys); 10.0 (Hcy); 5.0 (GSH)	38

Fluorescence quantum yield measurements

The fluorescence quantum yield of pyrene in cyclohexane (φ_f = 0.32) was used as a reference to measure the fluorescence quantum yields of probe **1** and complex [**1**·Cu(II)]. The following equation was used to calculate the fluorescence quantum yield:

$$\varphi_s = \varphi_f \frac{I_s A_f \eta_s^2}{I_f A_s \eta_r^2}$$

Here φ_f is the fluorescence quantum yield of reference. I stand for the integrated area under the emission curves. The subscripts s and r stand for sample and reference, respectively. A is the absorbance at a particular excitation wavelength. η is the refractive index of the medium. The absorbance of the dye at the excitation wavelength was always kept ~0.1. The steady state absorption and emission spectra were fitted by the log normal line shape function. Consequently the fluorescence quantum yield for probe **1** is φ = 0.26 and for [**1**·Cu(II)] complex is φ = 0.07.