Electronic Supporting Information

A new diformyl phenol based chemosensor selectively detects Zn^{2+} and Co^{2+} in nanomolar range in 100% aqueous medium and HCT live cells

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Contents of the Supporting Information

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1.Characterization, Structure and Crystallographic Data.

^{1}H and ^{13}C−NMR spectra: Hmpye was dissolved in d_6– DMSO and recorded with TMS as internal standard on a Bruker, AV 300 Supercon Digital NMR system.

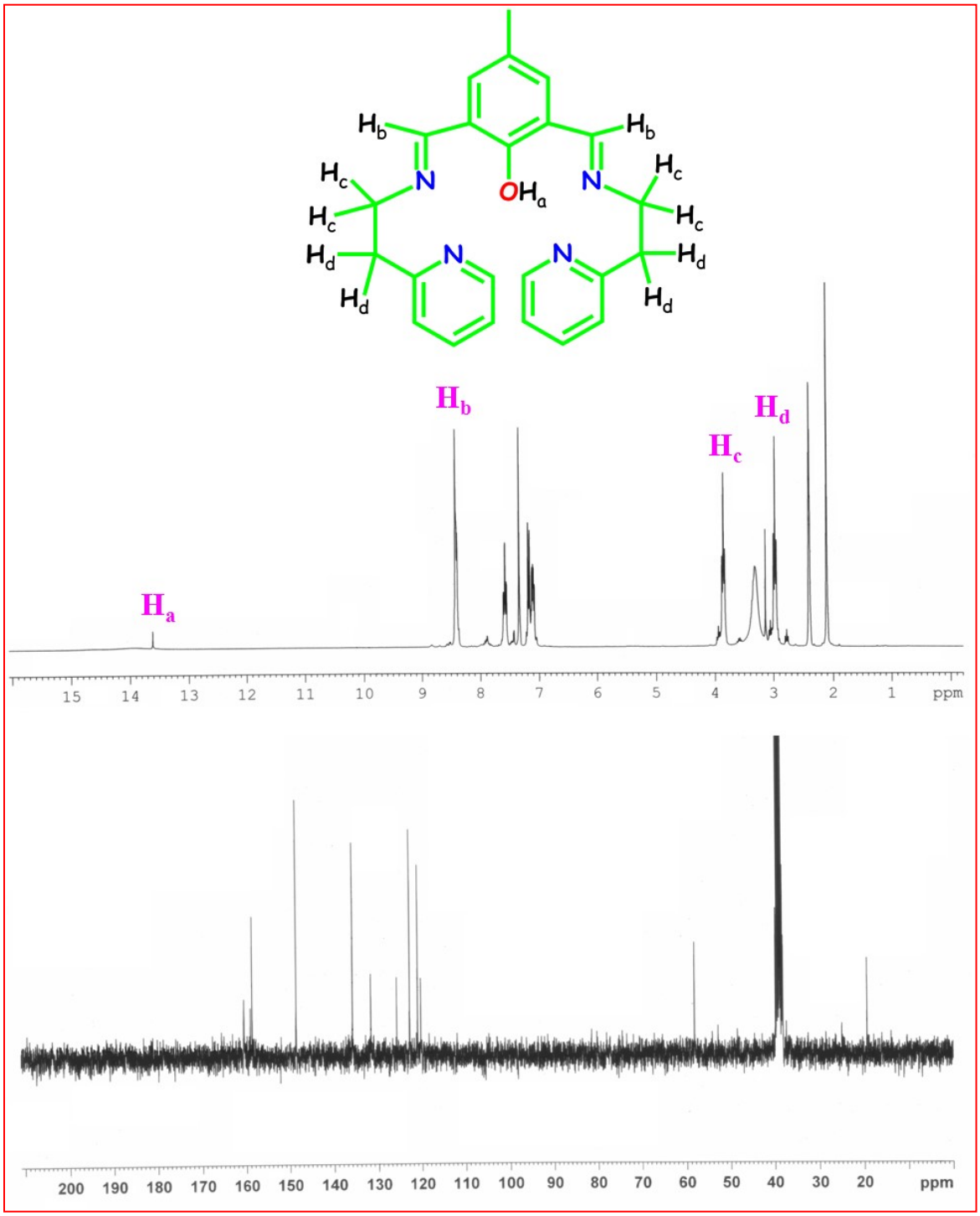
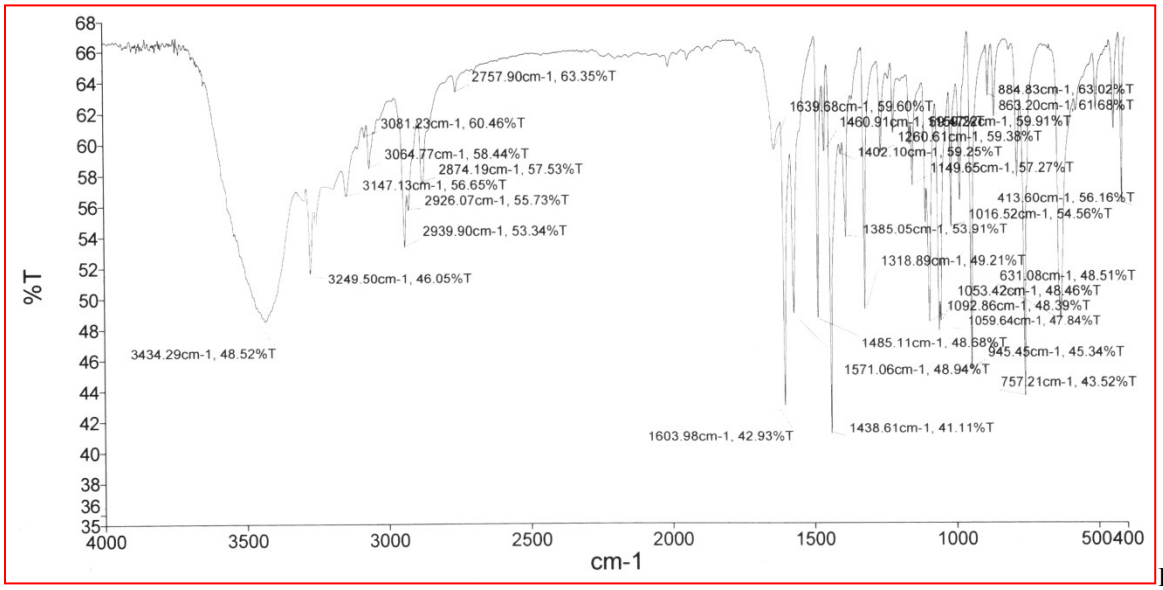


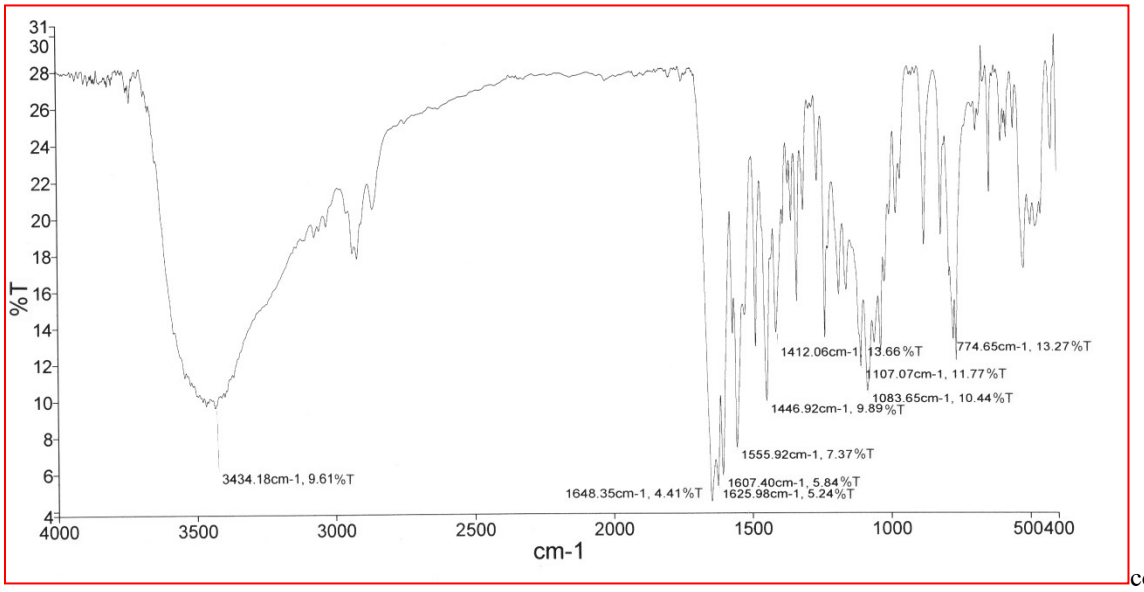
Figure S1. ^{1}H and ^{13}C−NMR spectrum of chemosensor (**Hmpye**).

1. Characterization, Structure and Crystallographic Data.

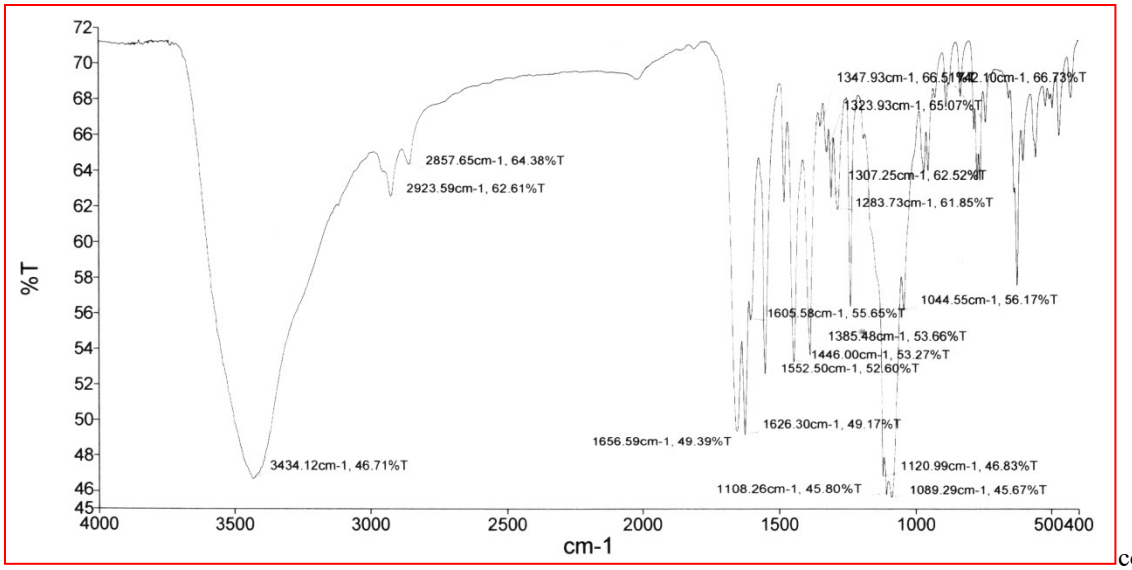
FT–IR spectroscopy: Fourier transform infrared (FT−IR) spectra were recorded with a Perkin−Elmer RXI FT−IR spectrophotometer using the reflectance technique (4000−400 cm^{-1}). Samples were prepared as KBr disks.



Hmpye



complex**1**



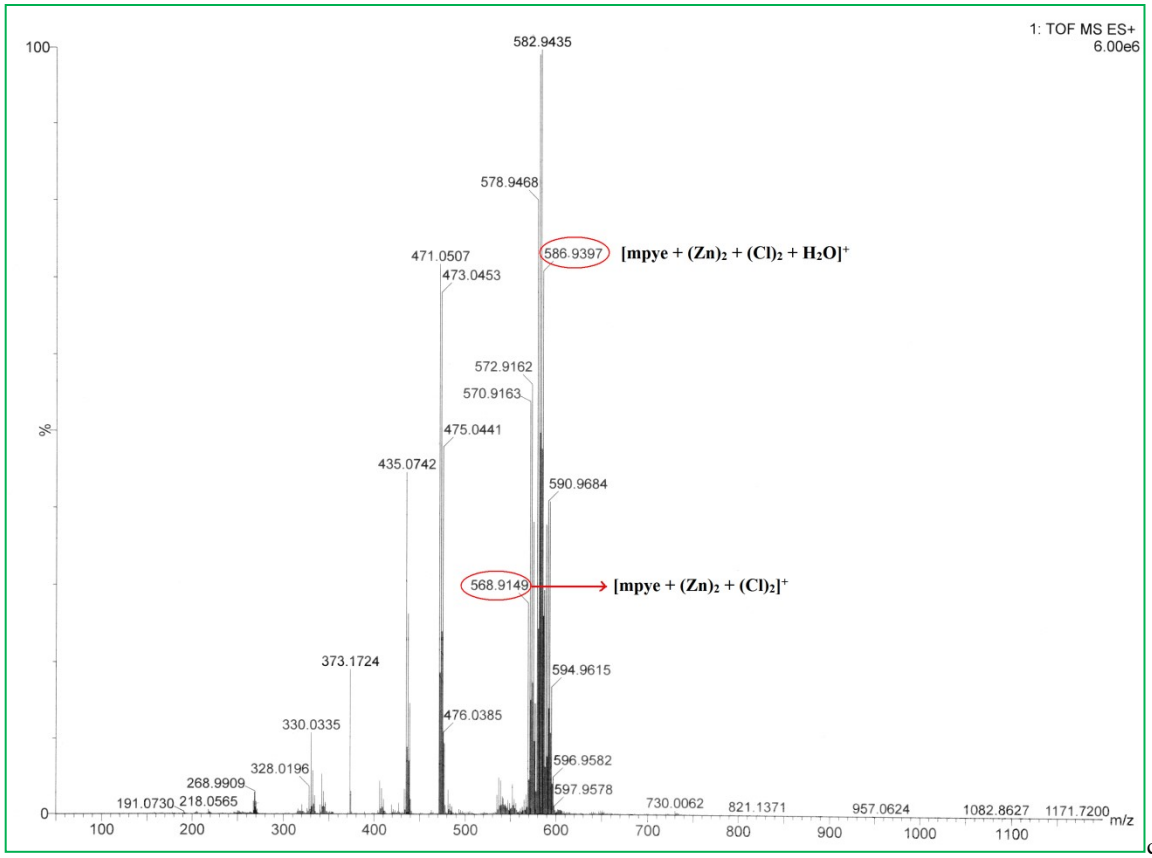
complex**2**

Figure S2. FT−IR Spectrum of **Hmpye** and in complexes **1** and **2**.

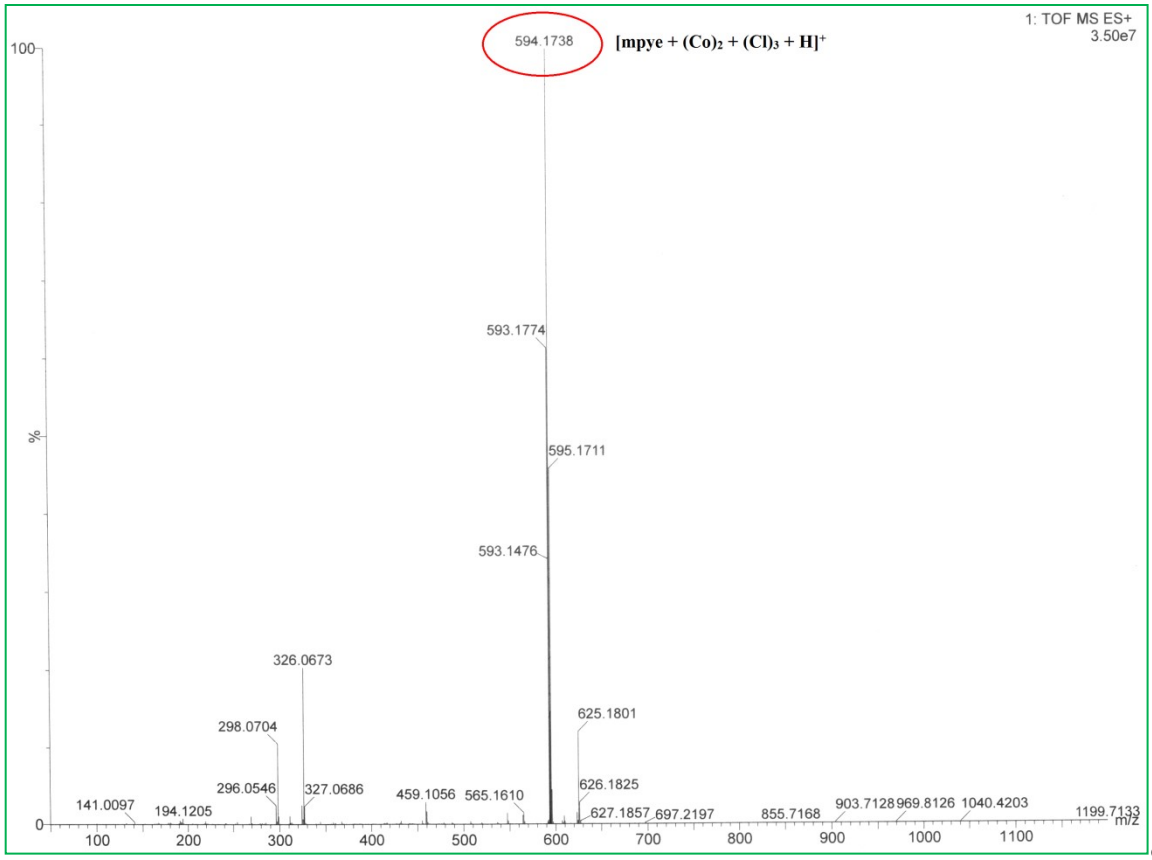
1. Characterization, Structure and Crystallographic Data.

Electrospray mass spectra (ESI-MS) were recorded on Qtof Micro YA263 mass spectrometer dissolving the samples in LC–MS quality MeOH.

Hmpye


1: TOF MS ES+
6.00e6
100
%
0
582.9435
578.9468
471.0507
473.0453
586.9397
[mpye + (Zn)2 + (Cl)2 + H2O]+
572.9162
570.9163
475.0441
435.0742
590.9684
568.9149
[mpye + (Zn)2 + (Cl)2]+
373.1724
594.9615
330.0335
476.0385
596.9582
328.0196
268.9909
597.9578
191.0730
218.0565
730.0062
821.1371
957.0624
1082.8627
1171.7200
100
200
300
400
500
600
700
800
900
1000
1100
m/z

complex**1**



complex**2**

Figure S3. ESI–MS of **Hmpye** and in complexes **1** and **2**.

Powder X−ray diffraction: (PXRD) patterns were recorded on a PANalytical, XPERT–PRO diffractometer with Cu Kα radiation (40 kV, 30 mA, λ = 1.5406 Å).

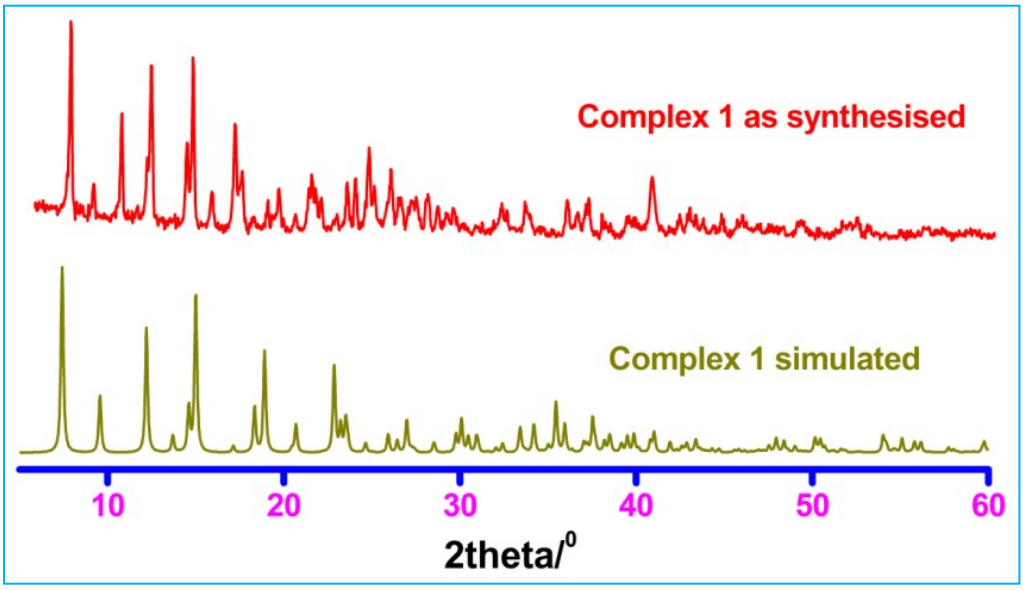
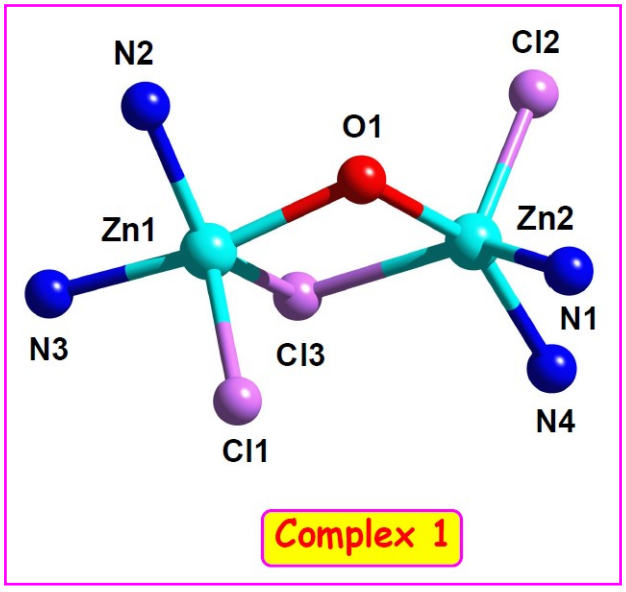


Figure S4. PXRD pattern of complex **1** vs. simulated pattern.



Scheme S1. Coordination modes of complex **1**.

Table S1. Single Crystal X–ray diffraction: Crystallographic data for complex **1**.

	Complex (1)
Chemical formula	$C_{45}H_{44}Cl_6N_8O_2Zn_4$
CCDC	2161072
Formula weight	547.47
Temperature/K	296(2)
λ[a]/ Å	0.71073
Crystal system	orthorhombic
Space group	*Pbcn*
***a* (Å)**	25.473(6)
***b*(Å)**	11.826(3)
***c*(Å)**	16.454(4)
***β* (°)**	90.00
Z	4
***V* (Å^3)**	4956.7(19)
***ρ* calc(g/cm^3)**	1.612
***μ*(mm^{-1})**	2.282
***F*(000)**	2432.0
θ min−max (°)	1.60 to 24.76
Reflns collected	21504
Independent reflns	4194
R(int)	0.0531
S(GOF)	0.961
***R1*, *wR2*(*I*>2*σ*(I))[b]**	0.0506[b], 0.1536[c]
***R1*, *wR2*(all data)[b]**	0.0761[b], 0.1780[c]
largest diff peak, hole/e Å^{-3}	1.644 and -0.426

[a]Graphite monochromator, [b]$R_1 = \Sigma(|F_o| - |F_c|)/\Sigma|F_o|$. [c]$wR_2 = \{\Sigma[w(|F_o|^2 - |F_c|^2)^2]/\Sigma[w(|F_o|^2)^2]\}^{1/2}$

Table S2. Bond distances (Å) and angles (^{0}) in the metal coordination spheres of complex **1**.

Complex (1)	
Zn(1)–O(1)	2.092(3)
Zn(1)–N(2)	2.057(5)
Zn(1)–N(3)	2.169(5)
Zn(2)–O(1)	2.032(4)
Zn(2)–N(1)	2.078(5)
Zn(2)–N(4)	2.110(5)
O(1)–Zn(1)–N(3)	163.82(17)
O(1)–Zn(1)–N(2)	85.50(17)
N(2)–Zn(1)–N(3)	89.79(19)
O(1)–Zn(2)–N(1)	85.43(16)
O(1)–Zn(2)–N(4)	154.26(18)
N(1)–Zn(2)–N(4)	91.88(19)

Table S3. Results of Continuous Shape Measurement analysis for the Zn(II) coordination spheres in complex **1**.

Shape analysis : Continuous Shape Measurement (CShM)[a] of coordination sphere using SHAPE v2.1.

For Zn1 and Zn2 in complex **1**

```
-------------------------------------------------------------------------------
S H A P E   v2.1         Continuous Shape Measures calculation
(c) 2013  Electronic Structure Group, Universitat de Barcelona
                  Contact:  llunell@ub.edu
-------------------------------------------------------------------------------

Zn2L structures

PP-5        1    D5h      Pentagon
vOC-5       2    C4v      Vacant octahedron
TBPY-5      3    D3h      Trigonal bipyramid
SPY-5       4    C4v      Spherical square pyramid
JTBPY-5     5    D3h      Johnson trigonal bipyramid J12

For Zn1
Structure [ML5]         PP-5         vOC-5       TBPY-5        SPY-5      JTBPY-5
Complex (1),          32.173,       2.997,      3.946,        0.805,      8.408

For Zn2
Structure [ML5]        PP-5          vOC-5       TBPY-5        SPY-5      JTBPY-5
Complex (1),         31.290,        4.437,      1.725,        2.111,      5.461
```

[a]CShM values between 0.1 and 3 usually correspond to a not negligible but still small distortion from ideal geometry.

2. Photophysical Characterization.

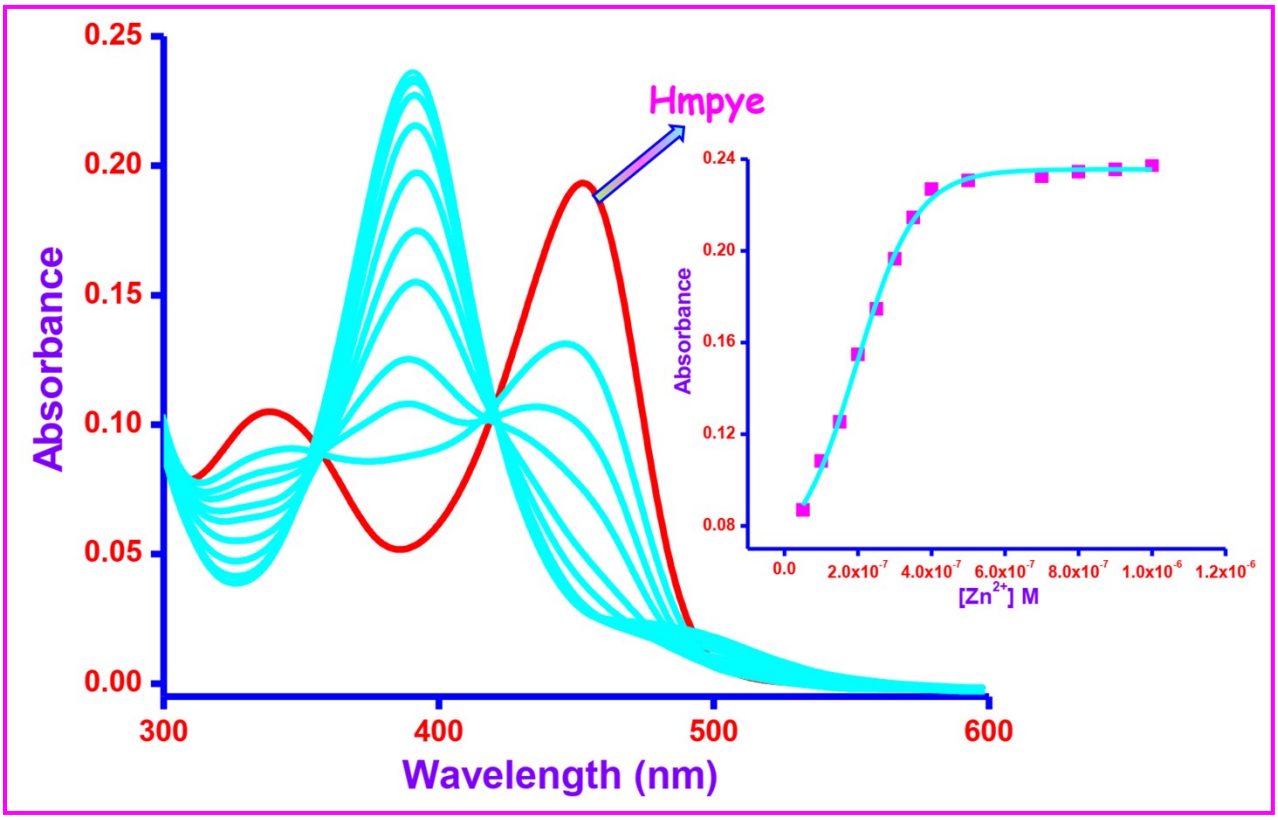


Figure S5. UV-vis spectra of **Hmpye** (5×10^{-7} M) in the presence of increasing amount of $[Zn^{2+}]$ (0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9 and 10 ($\times 10^{-7}$) M in HEPES buffer (pH = 7.4) solution. **Inset:** Absorbance spectra of **Hmpye** at 390 nm as a function of $[Zn^{2+}]$.

2. Photophysical Characterization.

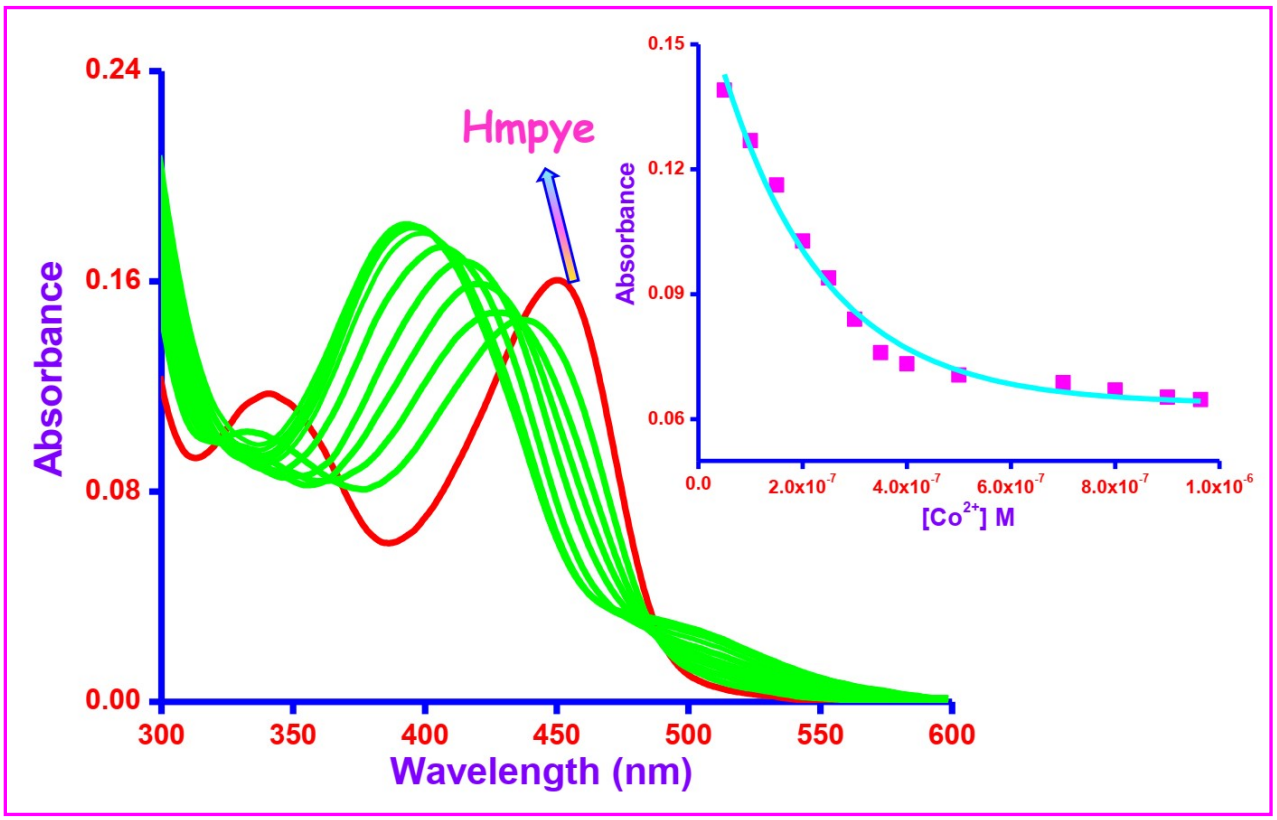


Figure S6. UV-vis spectra of **Hmpye** (5×10^{-7} M) in the presence of increasing amount of $[Co^{2+}]$ (0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5, 6, 7, 8, 9 and 10 ($\times 10^{-7}$) M in HEPES buffer (pH = 7.4) solution. **Inset:** Absorbance spectra of **Hmpye** at 450 nm as a function of $[Co^{2+}]$.

2. Photophysical Characterization.

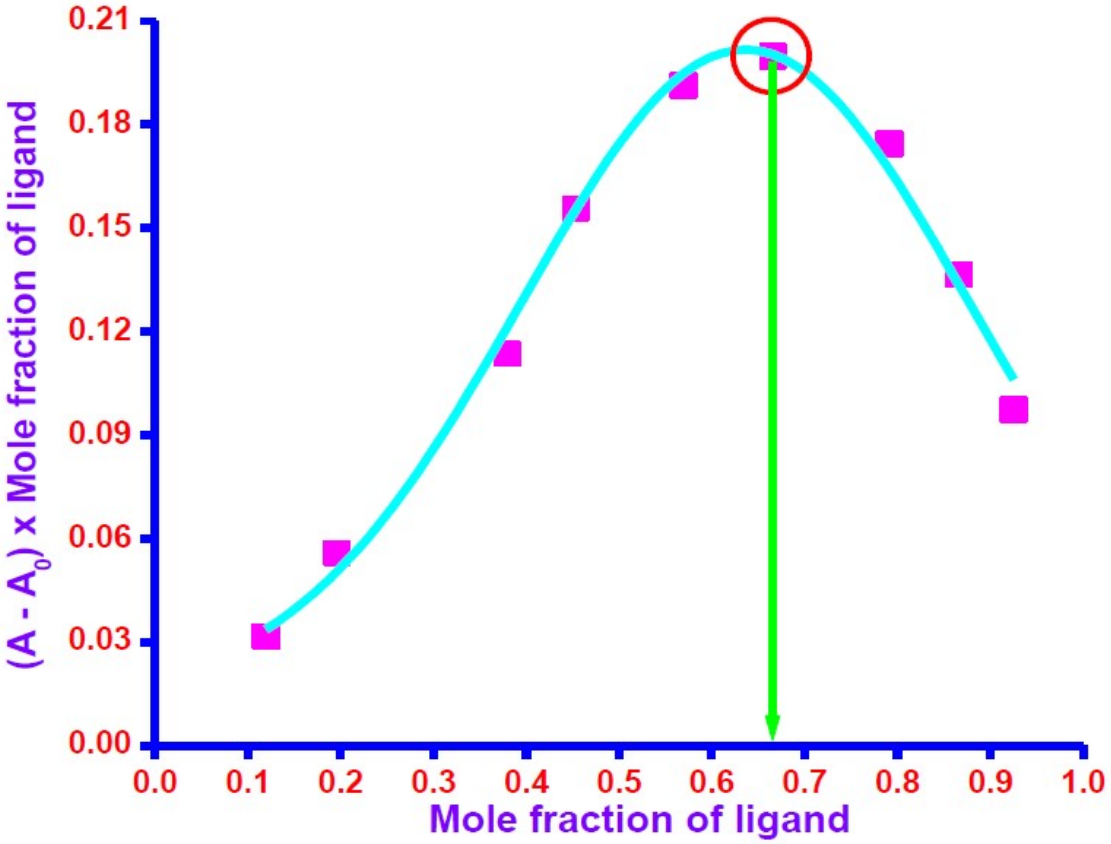


Figure S7. Job′s plot for the identification of **Hmpye-**Zn^{2+} (1:2) complex stoichiometry using absorbance values at 390 nm.

2. Photophysical Characterization.

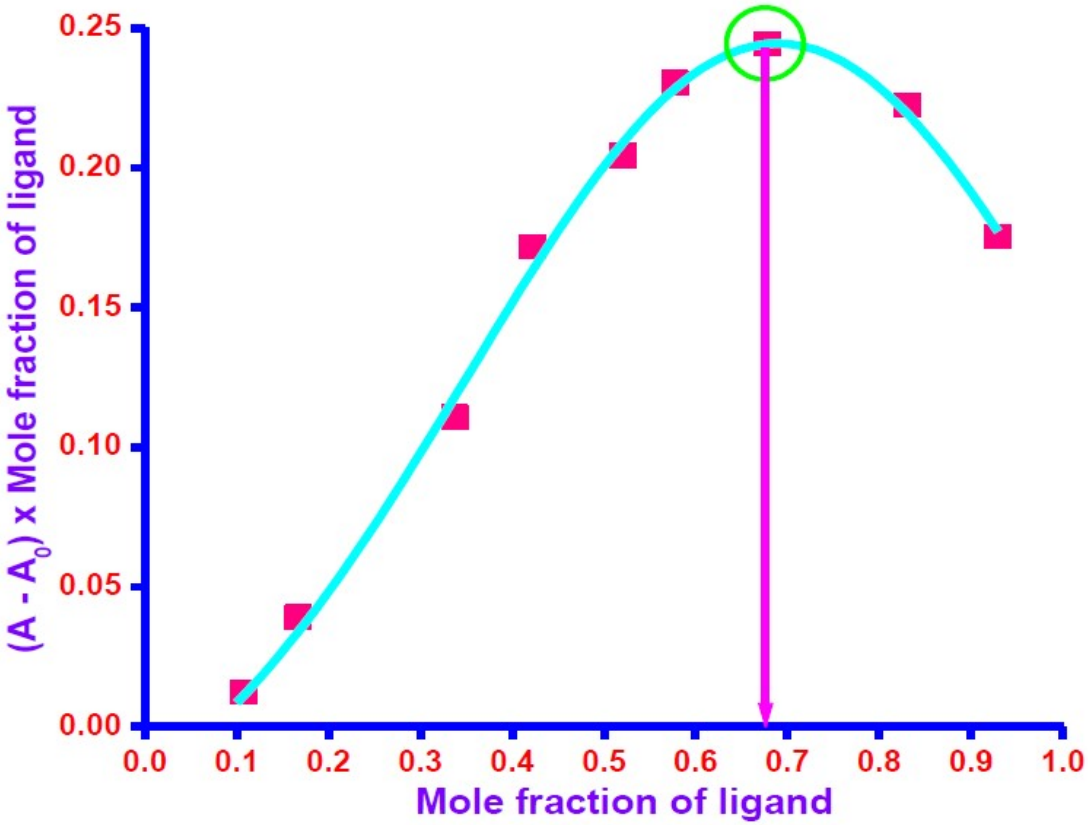


Figure S8. Job′s plot for the identification of **Hmpye**-Co^{2+} (1:2) complex stoichiometry using absorbance values at 450 nm.

2. Photophysical Characterization.

Detection limit calculation in emission spectroscopy.

The limit of detection (**LOD**) of **Hmpye**–Zn^{2+} and **Hmpye**–Co^{2+} was measured on the basis of fluorescence titration measurement. The detection limit was calculated using the following equation:

$$LOD = K \times \frac{\sigma}{S}$$

where K = 2 or 3 (we take 3 in this case), 'σ' is the standard deviation of the blank solution and 'S' is the slope between the ratio of emission intensity *versus* [Zn^{2+}] and [Co^{2+}].

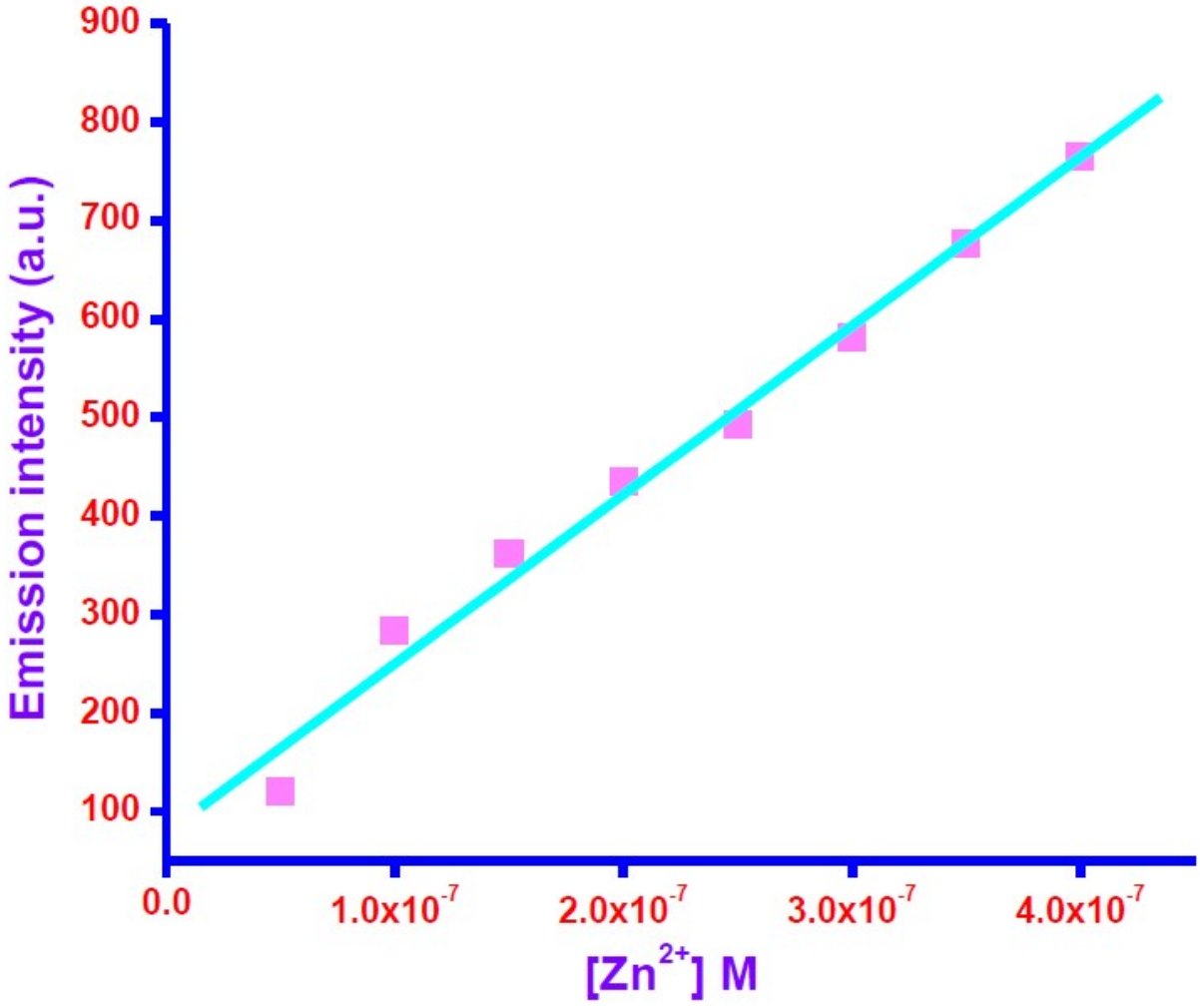


Figure S9. Determination of the detection limit of Zn^{2+} by **Hmpye** in HEPES buffer (pH = 7.4) solution at 461 nm.

2. Photophysical Characterization.

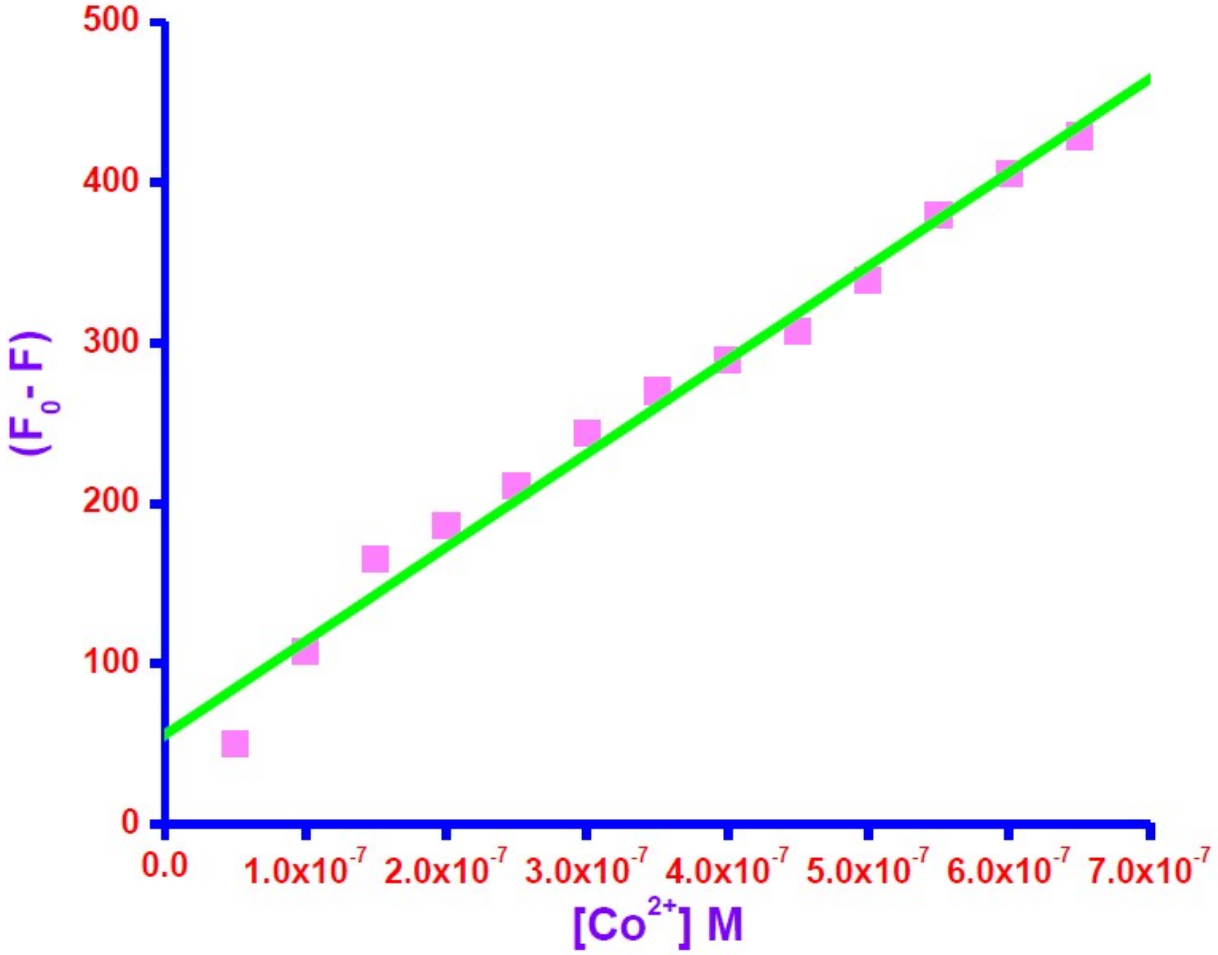


Figure S10. Determination of the detection limit of Co^{2+} by **Hmpye** in HEPES buffer (pH = 7.4) solution at 503 nm.

2. Photophysical Characterization.

Benesi–Hildebrand plot in emission spectroscopy.

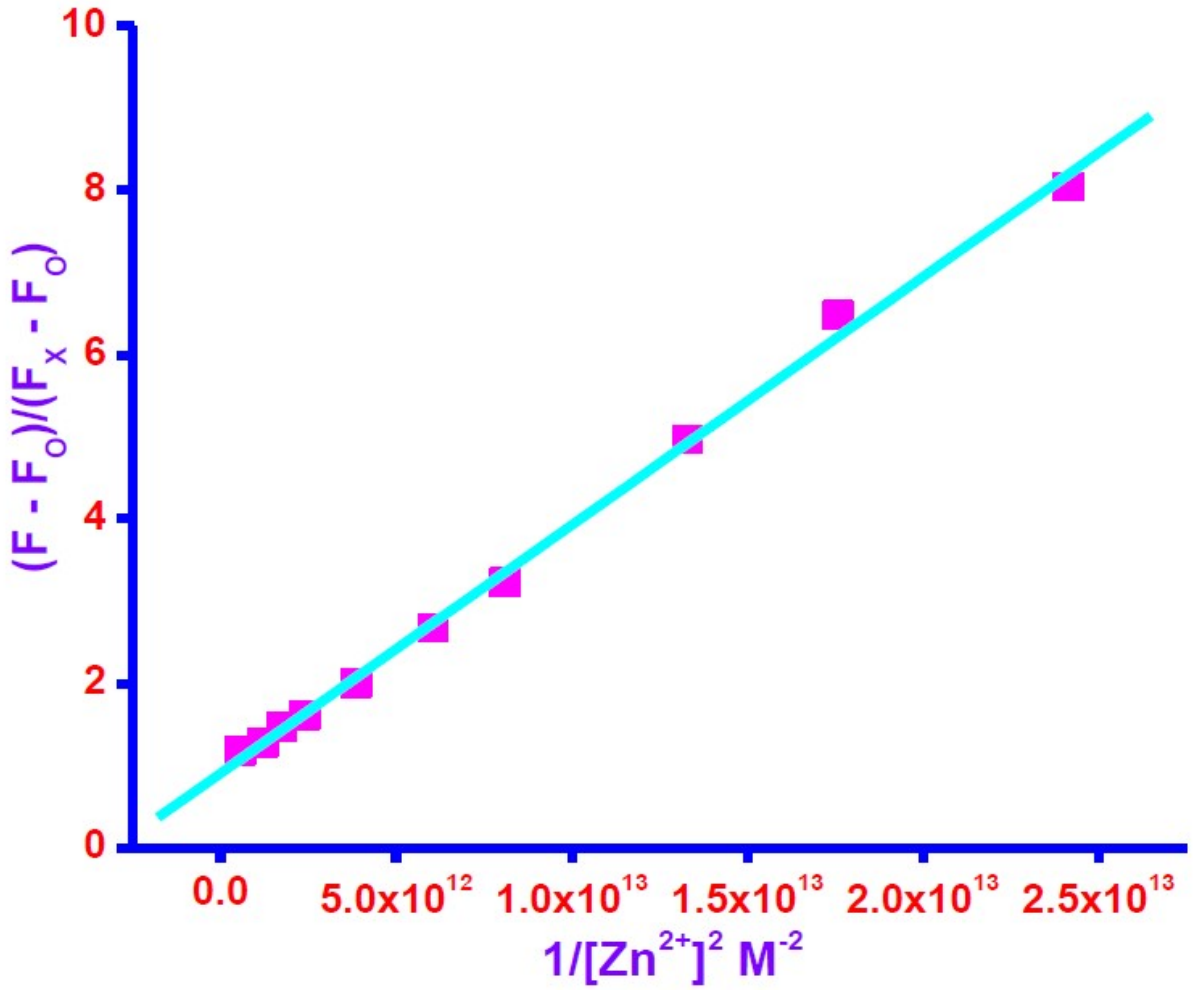


Figure S11. Benesi–Hildebrand plot $(F - F_0)/(F_x - F_0)$ vs. $1/[Zn^{2+}]^2$ for complexation between **Hmpye** and Zn^{2+} derived from emission titration curve at 461 nm.

2. Photophysical Characterization.

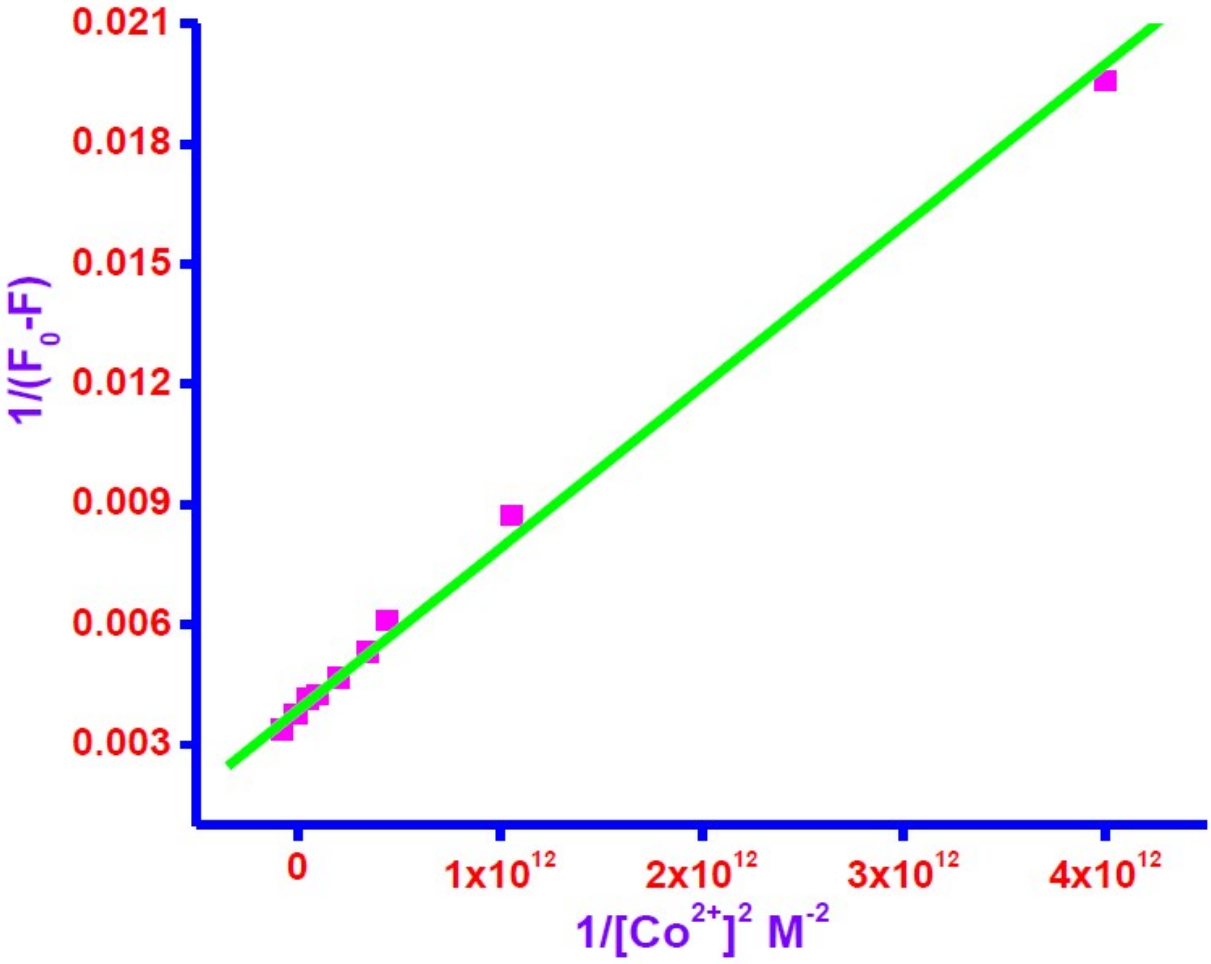


Figure S12. Benesi−Hildebrand plot *1/(F₀ − F)* vs. *1/[Co²⁺]* for complexation between **Hmpye** and Co^{2+} derived from emission titration curve at 503 nm.

2. Photophysical Characterization.

Reversibility studies in emission spectroscopy.

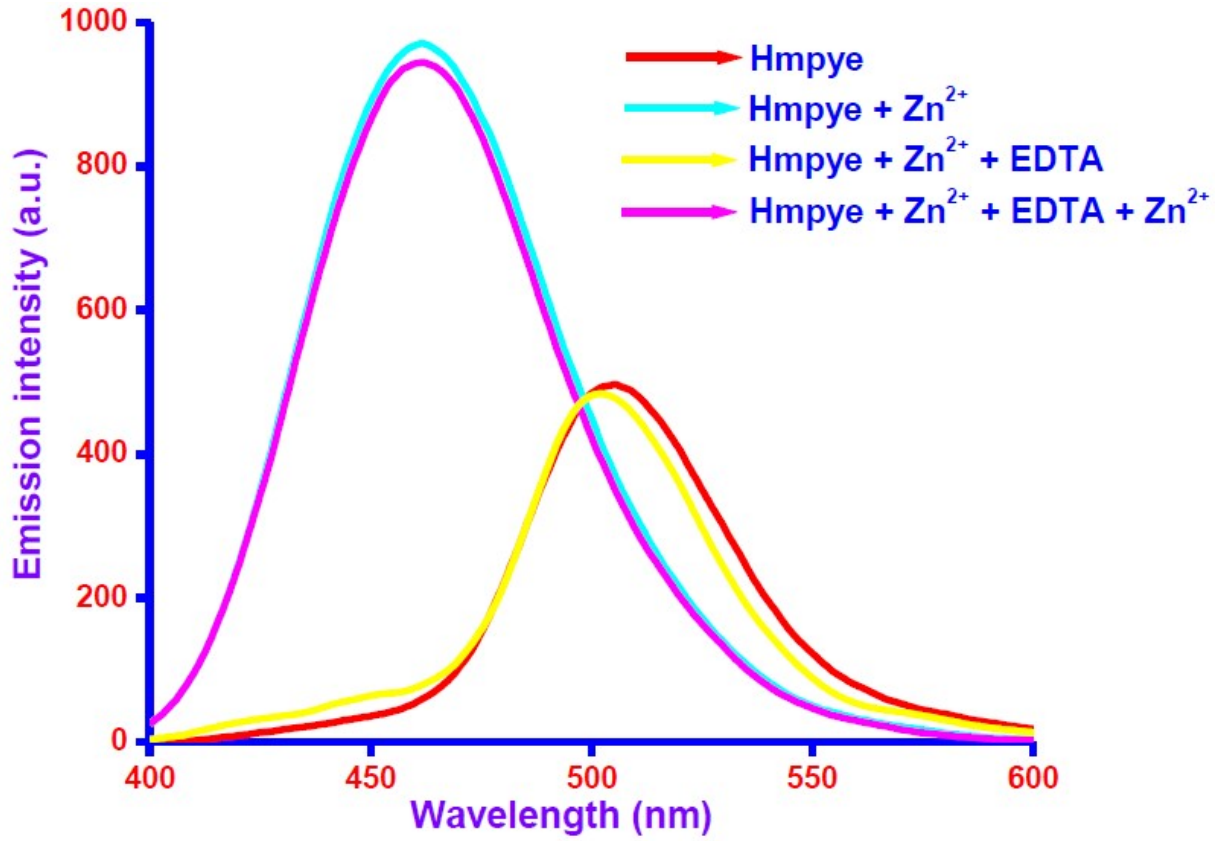


Figure S13. Fluorescence emission spectra of **Hmpye** in the presence of Zn^{2+} ion followed by addition of EDTA (λ_{ex}= 340 nm, λ_{em}= 461 nm).

2. Photophysical Characterization.

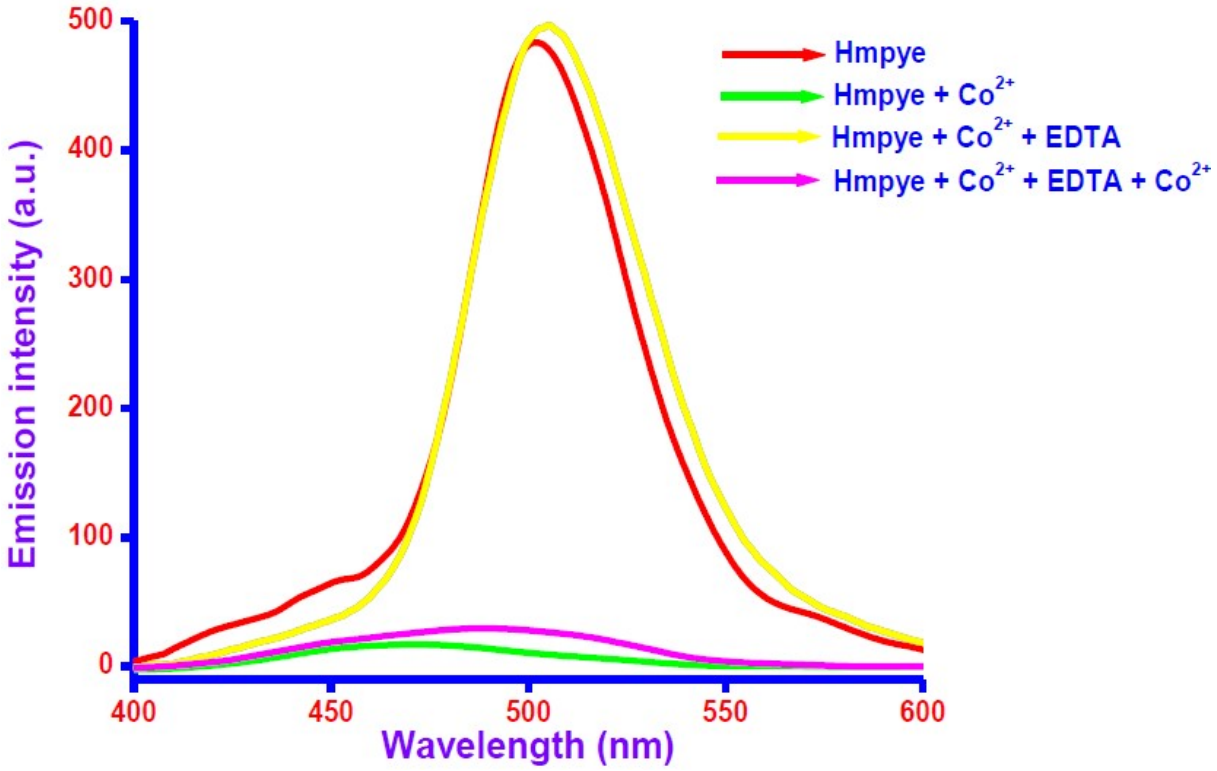


Figure S14. Fluorescence emission spectra of **Hmpye** in the presence of Co^{2+} ion followed by addition of EDTA (λ_{ex}= 340 nm, λ_{em}= 503 nm).

2. Photophysical Characterization.

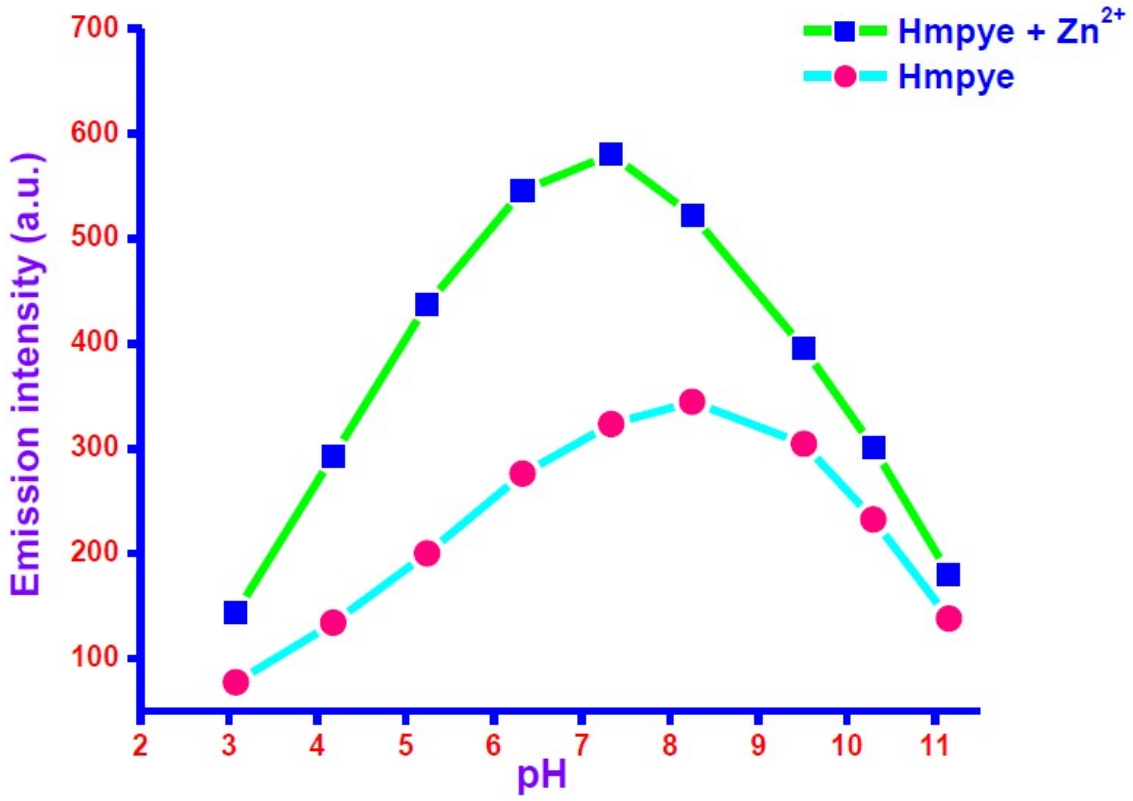


Figure S15. Emission intensity of probe **Hmpye** (5×10^{-7} M) in absence and in presence of Zn^{2+} as a function of pH values in aqueous solution at 461 nm.

2. Photophysical Characterization.

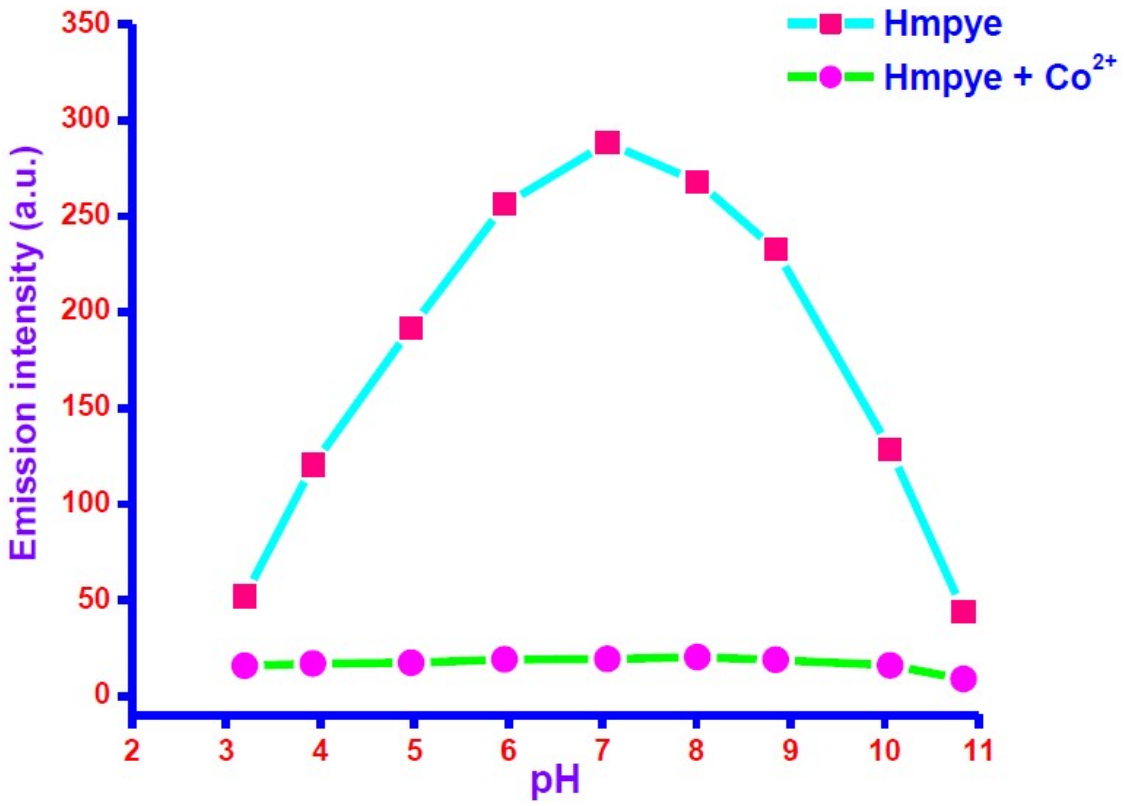


Figure S16. Emission intensity of probe **Hmpye** (5×10^{-7} M) in absence and in presence of Co^{2+} as a function of pH values in aqueous solution at 503 nm.

2. Photophysical Characterization.

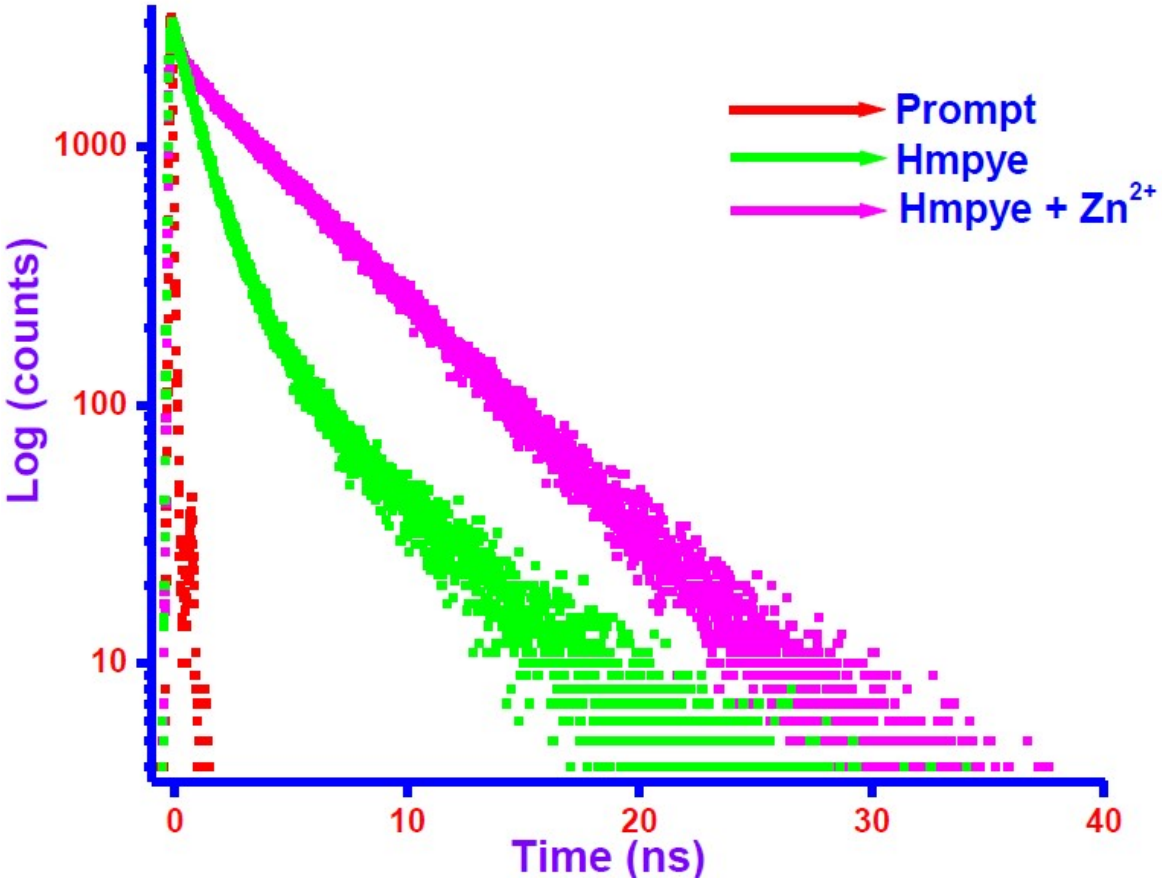


Figure S17. Time-resolved fluorescence decay of **Hmpye** in the absence and presence of added Zn^{2+} at 375 nm.

2. Photophysical Characterization.

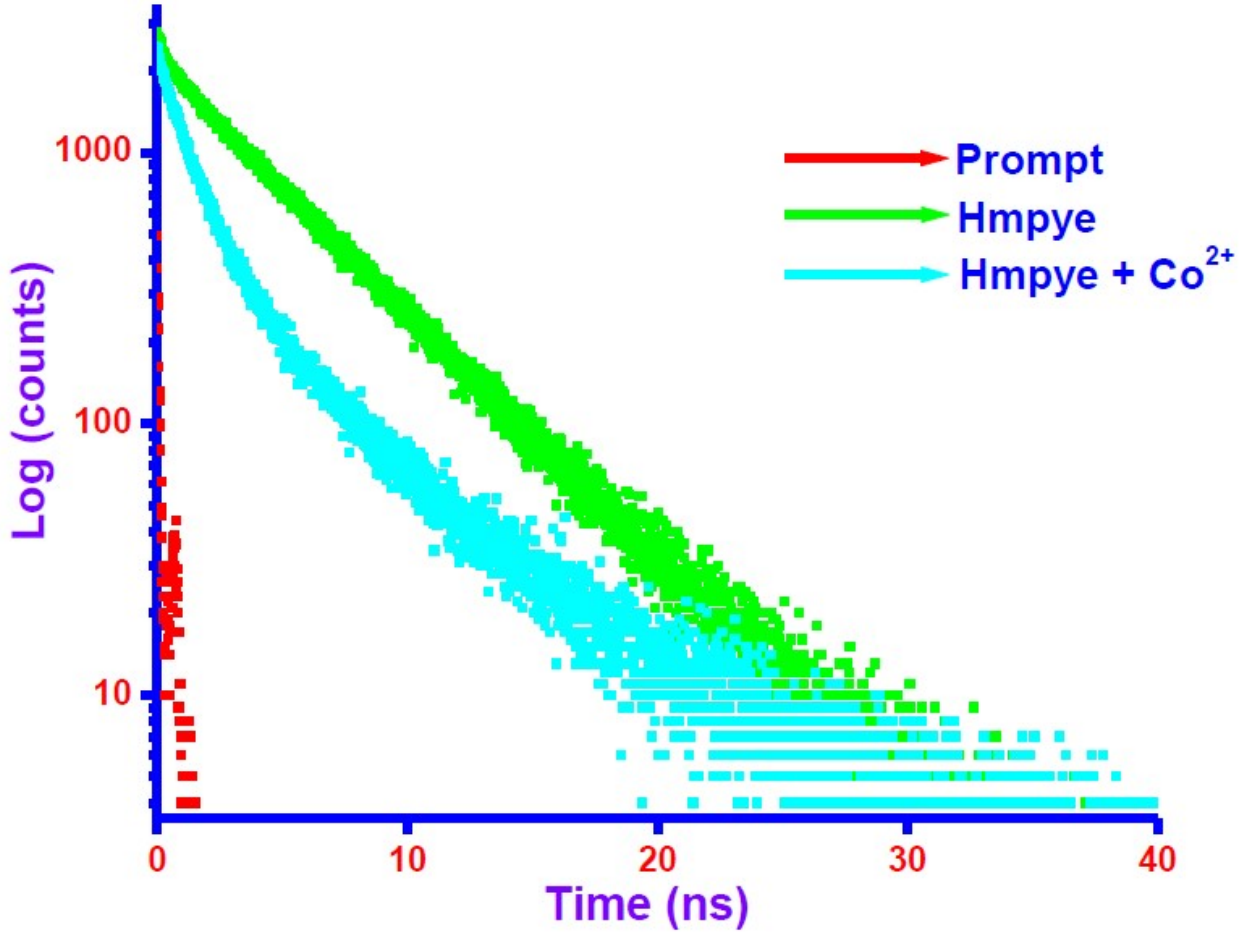
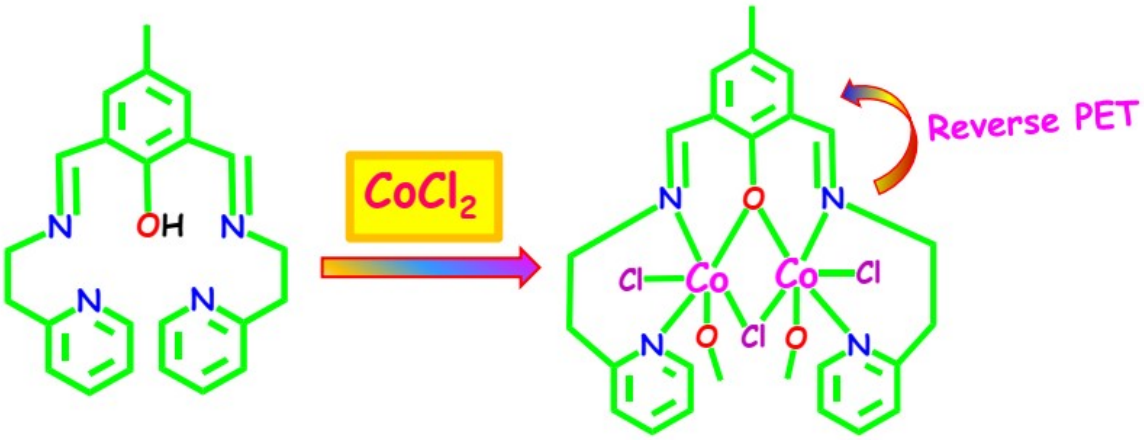


Figure S18. Time-resolved fluorescence decay of **Hmpye** in the absence and presence of added Co^{2+} at 375 nm.

Table S4. Fluorescence lifetime measurement of chemosensor (**Hmpye**) and the presence of Zn^{2+} and Co^{2+} ion in solution.

	φ_f	τ_{av} (ns)	$K_r(\times 10^9)$ S^{-1}	$K_{nr}(\times 10^9)$ S^{-1}	$\chi 2$
Hmpye	0.325	3.56	0.091	0.189	1.011
Hmpye + Zn^{2+}	0.610	4.89	0.125	0.079	0.995
Hmpye + Co^{2+}	0.025	2.13	0.012	0.457	1.155



Scheme S2. Schematic illustration of proposed mechanism for the fluorescence changes of chemosensor (**Hmpye**) upon addition of Co^{2+}.

3. Cell Study.

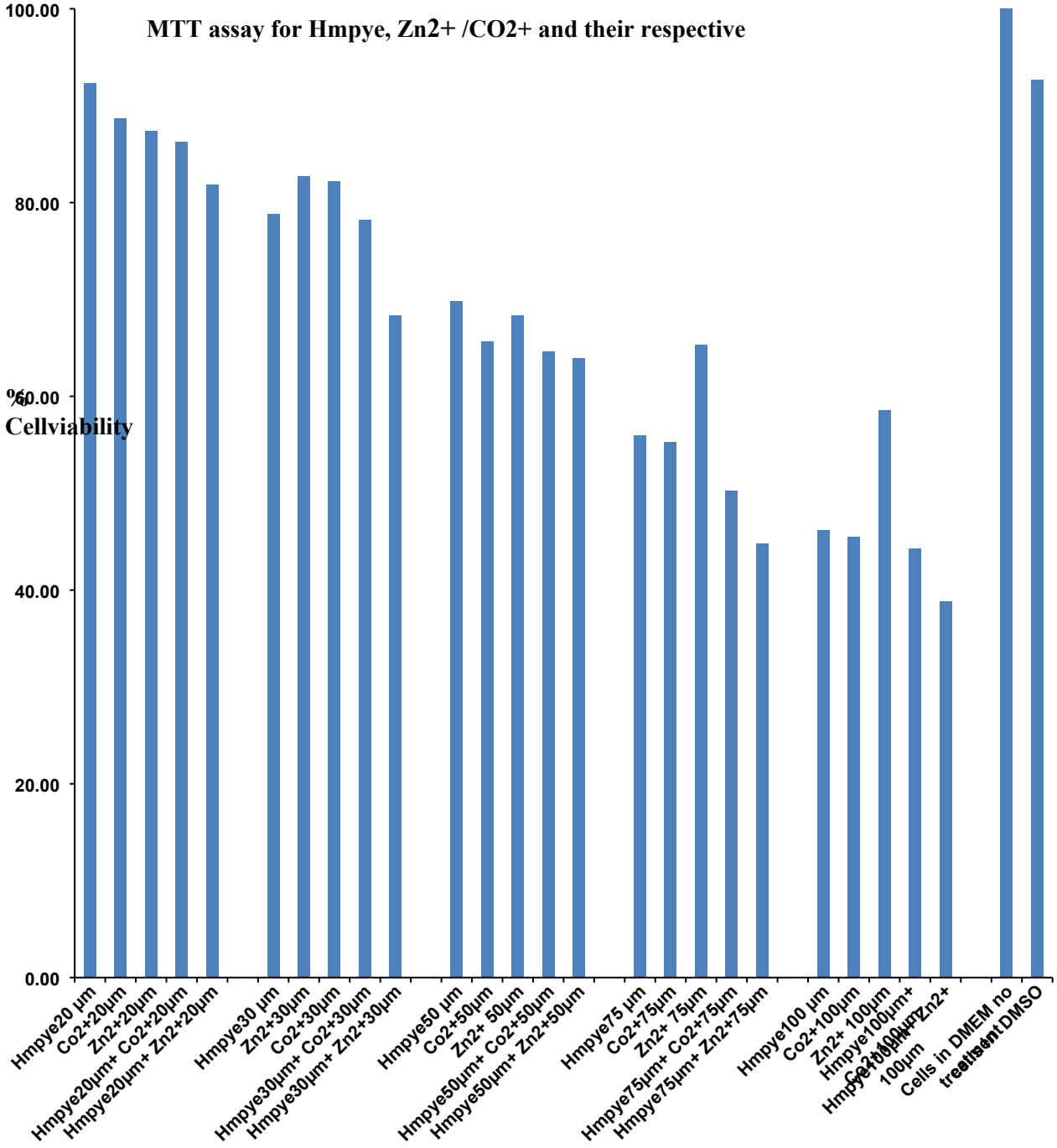
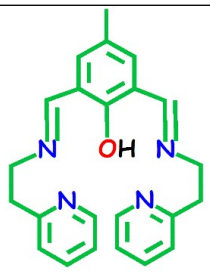
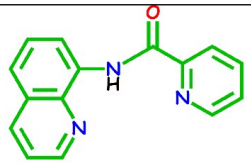
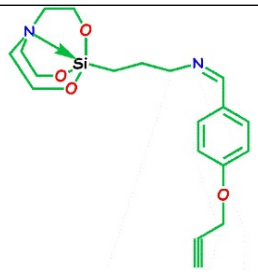
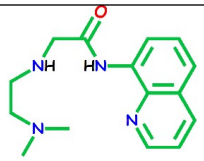
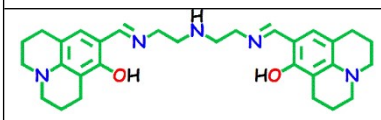
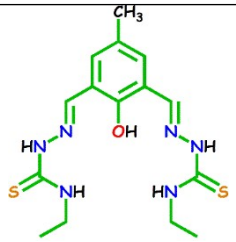
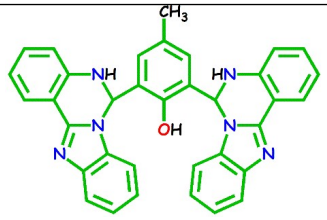
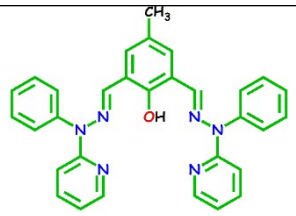
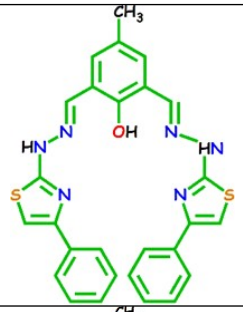
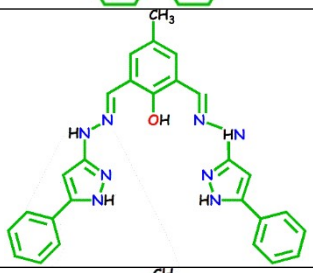
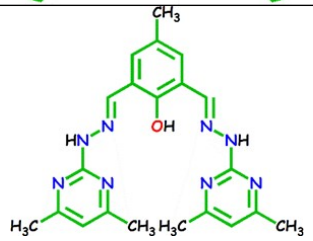
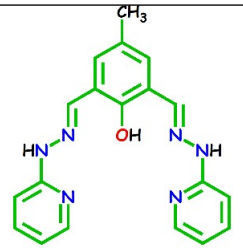
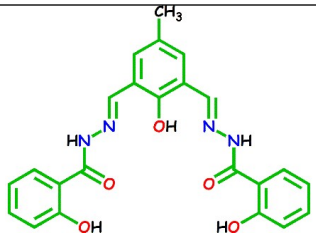

MTT assay for Hmpye, Zn2+ /CO2+ and their respective
% Cellviability
100.00
80.00
60.00
40.00
20.00
0.00
Hmpye20 µm
Co2+20µm
Zn2+20µm
Hmpye20µm+ Co2+20µm
Hmpye20µm+ Zn2+20µm
Hmpye30 µm
Zn2+30µm
Co2+30µm
Hmpye30µm+ Co2+30µm
Hmpye30µm+ Zn2+30µm
Hmpye50 µm
Co2+50µm
Zn2+ 50µm
Hmpye50µm+ Co2+50µm
Hmpye50µm+ Zn2+50µm
Hmpye75 µm
Co2+75µm
Zn2+ 75µm
Hmpye75µm+ Co2+75µm
Hmpye75µm+ Zn2+75µm
Hmpye100 µm
Co2+100µm
Zn2+ 100µm
Hmpye100µm+
Cells in DMEM no
DMSO

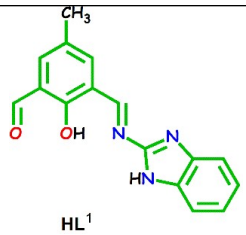
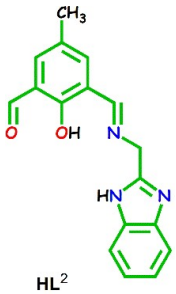
Figure S19. Percentage (%) cell viability of Hela cells treated with different concentrations of **Hmpye** for 24 hours determined by MTT assay.

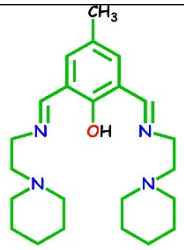
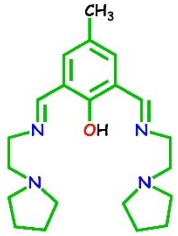
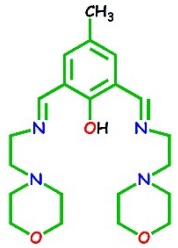
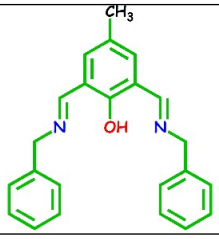
Table S5. Comparison with recently reported probes for selective detection of Zn^{2+} and Co^{2+} ion.

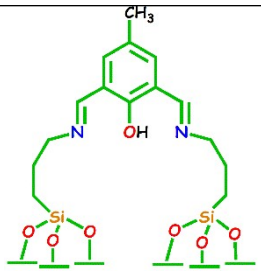
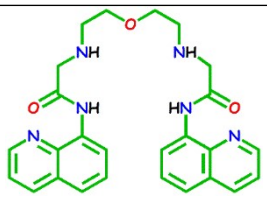
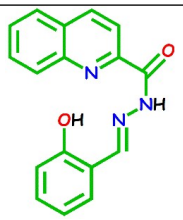
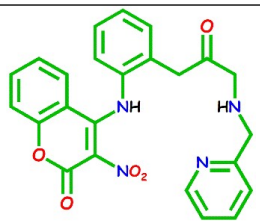
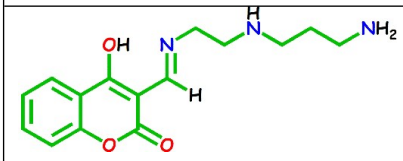
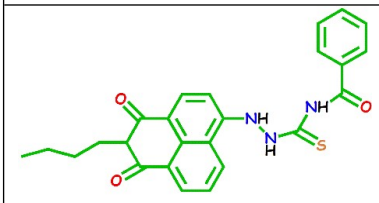
Probes	Solvent media	Detection limit	Binding constant	Ref.
	HEPES buffer (pH = 7.4) [both Zn^{2+} and Co^{2+}]	Zn^{2+} = 4.683×10^{-9} (M) [From the fluorescence titration data] Co^{2+} = 7.591×10^{-9} (M) [From the fluorescence titration data]	Zn^{2+} = 5.203×10^{12} (M^{-2}) [From the fluorescence titration data] Co^{2+} = 9.783×10^{11} (M^{-2}) [From the fluorescence titration data]	Present work
	acetonitrile solution [both Zn^{2+} and Co^{2+}]	Zn^{2+} = 7.93×10^{-7} (M) [From the fluorescence titration data] Co^{2+} = 2.20×10^{-6} (M) [From the UV–vis titration data]	$Zn^{2+} = 7.0 \times 10^{11}$ (M^{-2}) [From the fluorescence titration data] $Co^{2+} = 4.0 \times 10^{11}$ (M^{-2}) [From the UV–vis titration data]	23 (a)
	acetonitrile solution [both Zn^{2+} and Co^{2+}]	Zn^{2+} = 0.28×10^{-6} (M) [From the UV–vis titration data] Co^{2+} = 0.82×10^{-6} (M)	Zn^{2+} = 0.11×10^{6} (M^{-1}) [From the UV–vis titration data] Co^{2+} = 0.24×10^{6} (M^{-1})	23 (b)

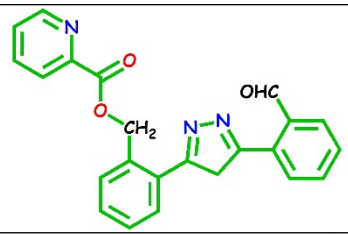
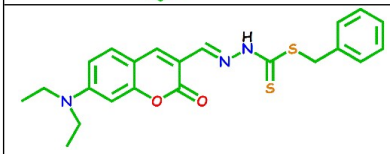
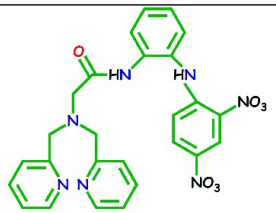
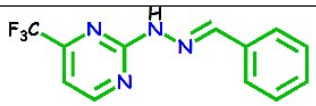
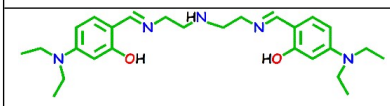
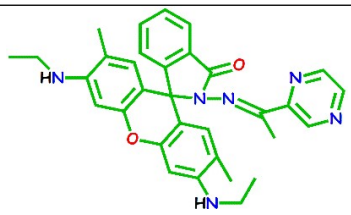
		[From the UV–vis titration data]	[From the UV–vis titration data]	
	bis-tris buffer solution (10 mM, pH 7.0) [both Zn^{2+} and Co^{2+}]	Zn^{2+} = 0.01 × 10^{-6} (M) [From the UV–vis titration data] Co^{2+} = 6.89 × 10^{-6} (M) [From the UV–vis titration data]	Zn^{2+} = 1.30 × 10^{5} (M^{-1}) [From the UV–vis titration data] Co^{2+} = 4.0 × 10^{4} (M^{-1}) [From the UV–vis titration data]	23 (c)
	Zn^{2+} = DMF-buffer solution (95:5, v/v) Co^{2+} = bis-tris buffer solution (10 mM, pH 7.0)	Zn^{2+} = 0.8 (μM) [From the fluorescence titration data] Co^{2+} = 0.34 (μM) [From the UV–vis titration data]	Zn^{2+} = 8.0 × 10^{3} (M^{-1}) [From the fluorescence titration data] Co^{2+} = 1.1 × 10^{4} (M^{-1}) [From the UV–vis titration data]	23 (d)
	Zn^{2+} = HEPES buffer (H_2O: CH_3OH, 1/4, v/v, pH 7.2)	Zn^{2+} = 0.59 (nM) [From the fluorescence titration data]	Zn^{2+} = 2.5 × 10^{6} (M^{-1}) [From the fluorescence titration data]	23 (e)
	Zn^{2+} = HEPES buffer (DMSO: H_2O, 1: 9 (v/v), pH 7.2)	Zn^{2+} = 3.5 × 10^{-8} (M) [From the fluorescence titration data]	Zn^{2+} = 3.1 (±0.1) × 10^{7} (M^{-1}) [From the fluorescence titration data]	23 (f)

	Zn^{2+} = DMSO:H_2O (1:9)	Zn^{2+} = 4.48 × 10^{-7} (M) [From the fluorescence titration data]	Zn^{2+} = 1.69 ×10^{4} (M^{-1}) [From the fluorescence titration data]	23 (g)
	Zn^{2+} = DMSO: CH_3OH (3:7 v/v)	Zn^{2+} = 2.31 ×10^{-8} (M) [From the fluorescence titration data]	Zn^{2+} = 5.22 ×10^{3} (M^{-1}) [From the UV–vis titration data]	23 (h)
	Zn^{2+} = DMSO	Zn^{2+} = 27.80 (nM) [From the fluorescence titration data]	–	23 (i)
	Zn^{2+} = HEPES buffer [50 µM, DMSO:water = 1:9 (v/v), pH=7.2]	Zn^{2+} = 9.727×10^{-7} (M) [From the fluorescence titration data]	Zn^{2+} = 4.812 × 10^{5} (M^{-1}) [From the fluorescence titration data]	23 (j)
	Zn^{2+} = HEPES buffer [50 mM, DMSO:water = 1:9 (v/v), pH = 7.2]	–	Zn^{2+} = 3.2 × 10^{7} (M^{-1}) [From the fluorescence titration data]	23 (k)
	Zn^{2+} =H_2O/THF buffer (HEPES, 4:6, v/v, pH 7.0)	Zn^{2+} = 2.88 (ng L^{-1}) [From the fluorescence titration data]	Zn^{2+} = 1.18 × 10^{12} (M^{-1}) [From the fluorescence titration data]	23 (l)

 HL^1  HL^2	Zn^{2+} = HEPES buffer (H_2O: CH_3OH, 1: 9, v/v, pH 7.2)	HL^1 Probe: Zn^{2+} = 0.832 (nM) HL^2 Probe: Zn^{2+} = 0.474 (nM)	HL^1 Probe: Zn^{2+} = 4.38 × (±0.09) × 10^4 (M^{-1}) [From the fluorescence titration data] HL^2 Probe: –	1(e)

 HL[1]  HL[2]  HL[3]	Zn^{2+} = CH_3CN	HL[1] Probe: – HL[2] Probe: – HL[3] Probe: –	HL[1] Probe: Zn^{2+} = 2.5 × 10^4 (M^{-1}) HL[2] Probe: Zn^{2+} = 2.35 × 10^4 (M^{-1}) HL[3] Probe: Zn^{2+} = 2.2 × 10^4 (M^{-1}) [From the fluorescence titration data] Zn^{2+} = 1.5 × 10^4 (M^{-1}) [From the fluorescence titration data]	1(d)
	Zn^{2+} = HEPES buffer, pH 7.4	–		23 (m)

	Zn^{2+} = HEPES buffer, pH 7.4	Zn^{2+} = 6.54 (µg L^{-1}) [From the fluorescence titration data]	Zn^{2+} = 8.7 × 10^3 (M^{-1}) [From the fluorescence titration data]	23 (n)
	Zn^{2+} = near perfect aqueous solution (bis-tris buffer:DMSO = 999:1)	Zn^{2+} = 0.6 (µM) [From the fluorescence titration data]	Zn^{2+} = 8.6 (±0.1) × 10^3 (M^{-1}) [From the fluorescence titration data]	23 (o)
	Zn^{2+} = MeOH–HEPES buffer (3/7, v/v, pH 7.4)	Zn^{2+} = 2.1 ×10^{-8} (M) [From the fluorescence titration data]	Zn^{2+} = 1.68 × 10^5 (M^{-1}) [From the fluorescence titration data]	23 (p)
	Co^{2+} = near perfect aqueous solution	Co^{2+} = 1.8 (µM) [From the UV–vis titration data]	Co^{2+} = 1.1 × 10^4 (M^{-1}) [From the UV–vis titration data]	23 (q)
	Co^{2+} = Ethanol: water (1:9)	Co^{2+} = 7.09 (μM) [From the UV–vis titration data]	Co^{2+} = 2.9 × 10^4 (M^{-1}) [From the UV–vis titration data]	23 (r)
	Co^{2+} = CH_3CN/HEPES (4/1, *v*/*v*, pH = 7.4)	Co^{2+} = 0.26 (µM) [From the fluorescence titration data]	Co^{2+} = 1.2 × 10^4 (M^{-1}) [From the UV–vis titration data]	23 (s)

	Co^{2+} = CH_3CN	Co^{2+} = 3.92 (μM) [From the UV–vis titration data]	Co^{2+} = 5.29 × 10^2 (M^{-1}) [From the UV–vis titration data]	23 (t)
	Co^{2+} = H_2O	Co^{2+} = 0.31 (μM) [From the UV–vis titration data]	Co^{2+} = 6.91 (M^{-2}) [From the UV–vis titration data]	23 (u)
	Co^{2+} = bis-tris buffer solution containing 0.1% DMSO (10 mM, pH 7)	Co^{2+} = 0.99 (μM) [From the UV–vis titration data]	Co^{2+} = 8.9 × 10^5 (M^{-1}) [From the UV–vis titration data]	23 (v)
	Co^{2+} = Bis-tris Buffer	Co^{2+} = 0.11 (μM) [From the UV–vis titration data]	Co^{2+} = 5.0 × 10^9 (M^{-1}) [From the UV–vis titration data]	23 (w)
	Co^{2+} = bis–tris buffer solution (10 mM, pH 7)	Co^{2+} = 0.65 (μM) [From the UV–vis titration data]	Co^{2+} = 1.9 × 10^5 (M^{-1}) [From the UV–vis titration data]	23 (x)
	Co^{2+} = THF/Tris-HCl buffer-solution (4/6, V/V)	Co^{2+} = 0.31 (μM) [From the fluorescence titration data]	–	23 (y)