

Lighting Up Zinc: Switchable Probes for Biological Imaging

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SUPPLEMENTARY INFORMATION

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MATERIALS AND METHODS.

All the chemicals and reagents used in the synthesis were purchased from Fisher Scientific company. Ethanol and methanol were of analytical grade and used without further purification. Zinc acetate dihydrate was also purchased from Fisher Scientific company. ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker Ascend™ 400MHz spectrometer using $(\text{CD}_3)_2\text{SO}$ as a solvent and TMS as internal standard. The following abbreviations were used to designate chemical shift multiplicities: s = singlet, d= doublet, t = triplet, q = quartet, m = multiplet (denotes complex pattern), dd = doublet of doublets, and dt = doublet of triplets. The electronic spectra were recorded at room temperature on a Horiba-Duetta fluorescence and UV-vis spectrophotometer (OLIS) using a 2mm dual path spectroil quartz cuvette. Fluorescent photographs of the samples were taken under short and long wavelength UV. Mass spectra (MS) of synthesized compounds were measured on a Sciex QTRAP 4500 LC-MS.

GENERAL PROCEDURE FOR PREPARATION OF IMINE PRODUCTS.

Compound 1: Synthesis of 5-[(E)-[(2-hydroxyphenyl)methylidene]amino]pyridine-2-carboxylic acid: A solution of salicylaldehyde (122 mg, 1 mmol) in ethanol (2 mL) was added to a stirred solution of 5-aminopyridine-2-carboxylic acid (138 mg, 1mmol) in ethanol (2mL) and two drops of trifluoroacetic acid. The reaction mixture was refluxed for 6 h after which the resulting yellow precipitate was filtered and allowed to dry then subjected to elemental microanalysis. Yield 65 % and melting point 186-188 °C. ^1H NMR (400 MHz, DMSO) δ 13.12 (s, 1H), 12.29 (s, 1H), 9.06 (s, 1H), 8.72 (dd, J = 0.7, 2.5 Hz, 1H), 8.13 (dd, J = 0.4, 8.4 Hz, 1H), 7.97 (dd, J = 2.5, 8.4 Hz, 1H), 7.74 (dd, J = 1.7, 8.2 Hz, 1H), 7.48 (m, J = 2.9 Hz, 1H), 7.02 (q, J = 5.2 Hz, 2H). ^{13}C NMR: δ = 192.19, 166.56, 143.91, 135.69, 134.71, 132.85, 129.20, 126.59, 125.98, 119.92, 118.92, 117.69, 117.26. MS-ESI: m/z calculated for $\text{C}_{13}\text{H}_{10}\text{N}_2\text{O}_3$ $[\text{M}+\text{H}]^+$ 242.23, found 243.0

Compound 2: Synthesis of 2-[(E)-{[3-chloro-5-(trifluoromethyl)pyridin-2-yl]imino}methyl]phenol: A solution of salicylaldehyde (122 mg, 1 mmol) in ethanol (2 mL) was added to a stirred solution of 2-amino-3-chloro-5-trifluoromethyl pyridine (197 mg, 1mmol) in ethanol (2mL) and two drops of trifluoroacetic acid. The reaction mixture was refluxed for 6 h after which the resulting yellow precipitate was filtered and allowed to dry then subjected to elemental microanalysis. Yield 48 % and melting point 142-144 °C. ^1H NMR (400 MHz, DMSO) δ 12.79 (s, 1H), 9.58 (s, 1H), 8.89 (dd, J = 0.9, 2.1 Hz, 1H), 8.62 (dd, J = 0.5, 2.1 Hz, 1H), 7.91 (dd, J = 1.7, 8.0 Hz, 1H), 7.55 (m, J = 2.9 Hz, 1H), 7.04 (dd, J = 1.0, 8.3 Hz, 1H), 7.04 (m, J = 4.1 Hz, 1H). ^{13}C NMR: 167.70, 161.88, 157.57, 144.84, 137.09, 136.10, 134.09, 126.84, 125.10, 122.04, 120.26, 119.46, 117.50. MS-ESI: m/z calculated for $\text{C}_{13}\text{H}_8\text{ClF}_3\text{N}_2\text{O}$ $[\text{M}+\text{H}]^+$ 300.67, found 301.0

Compound 3: Synthesis of 2-[(E)-{[6-(trifluoromethyl)pyridin-3-yl]imino}methyl]phenol: A solution of salicylaldehyde (122 mg, 1 mmol) in ethanol (2 mL) was added to a stirred solution of 5-amino-2-trifluoromethylpyridine (162 mg, 1mmol) in ethanol (2mL) and two drops of trifluoroacetic acid. The reaction mixture was refluxed for 6 h after which the resulting yellow precipitate was filtered and allowed to dry then subjected to elemental microanalysis. Yield 52 % and melting point 127-128 °C. ^1H NMR (400 MHz, DMSO) δ 12.14 (s, 1H), 9.06 (s, 1H), 8.79 (d, J = 2.5 Hz, 1H), 8.08 (dd, J = 2.2, 8.4 Hz, 1H), 8.00 (d, J = 8.7 Hz, 1H), 7.75 (dd, J = 8.1, 1.7 Hz, 1H), 7.49 (m, J = 2.9 Hz, 1H), 7.03 (q, J = 5.2 Hz, 2H). ^{13}C NMR: 167.05, 160.61, 148.31, 144.56, 144.11, 134.84, 132.75, 130.14, 123.60, 121.98, 120.88, 119.96, 117.29. MS-ESI: m/z calculated for $\text{C}_{13}\text{H}_9\text{F}_3\text{N}_2\text{O}$ $[\text{M}+\text{H}]^+$ 266.22, found 267.1

Compound 4: Synthesis of 2-[(E)-[(2-hydroxyphenyl)methylidene]amino]pyridin-3-ol: A solution of salicylaldehyde (122 mg, 1 mmol) in ethanol (2 mL) was added to a stirred solution of 2-amino-3-hydroxypyridine (110 mg, 1mmol) in ethanol (2mL) and two drops of trifluoroacetic acid. The reaction mixture was refluxed for 6 h after which the resulting yellow precipitate was filtered and allowed to dry then subjected to elemental microanalysis. Yield 55 % and melting point 158-160 °C. ^1H NMR (400 MHz, DMSO) δ 13.77 (s, 1H), 10.20 (s, 1H), 9.48 (s, 1H), 8.00

(dd, J = 1.5, 4.5 Hz, 1H), 7.77 (dd, J = 1.7, 7.9 Hz, 1H), 7.44 (ddd, J = 1.7, 7.1, 8.5 Hz, 1H), 7.38 (dd, J = 1.6, 8.1 Hz, 1H), 7.24 (dd, J = 4.5, 8.1 Hz, 1H), 6.97 (m, J = 4.0 Hz, 1H), 6.97 (dd, J = 0.9, 8.3 Hz, 1H). ¹³CNMR: 162.26, 161.80, 148.13, 145.87, 139.32, 134.11, 133.69, 125.02, 124.67, 119.71, 119.44, 117.31. MS-ESI: m/z calculated for C₁₂H₁₀N₂O₂ [M+H]⁺ 214.22, found 216.0.

ABSORBANCE AND EMISSION SPECTRA OF SYNTHESIZED PROBES (2-4)

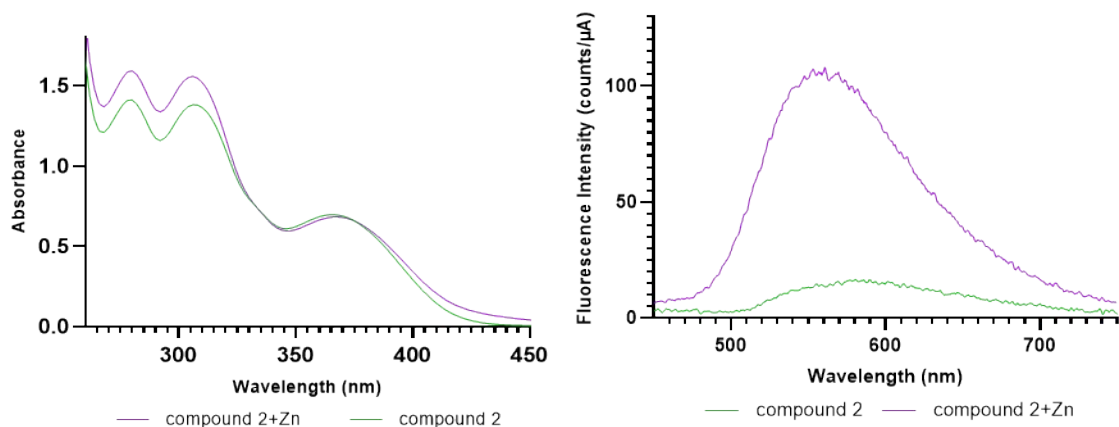


Figure S1: Absorbance and Emission spectra of compound **2** and its Zn^{+2} complex in methanol.

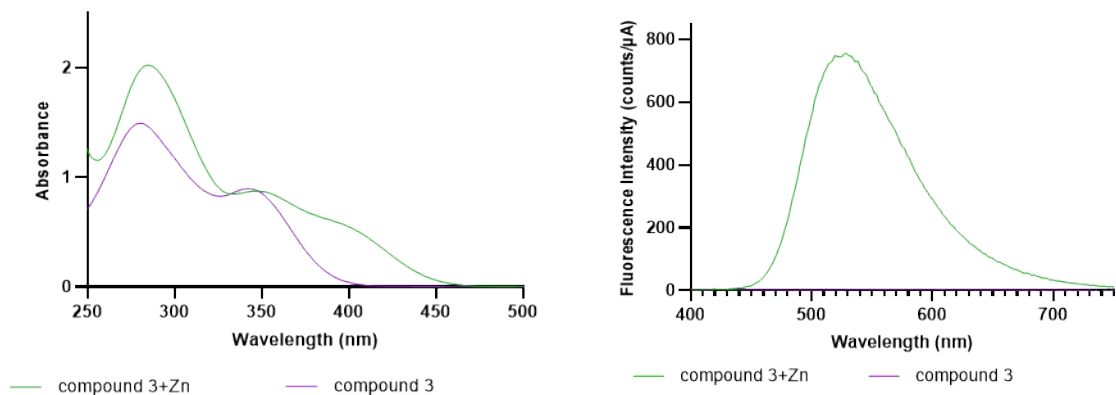


Figure S2: Absorbance and Emission spectra of compound **3** and its Zn^{+2} complex in methanol.

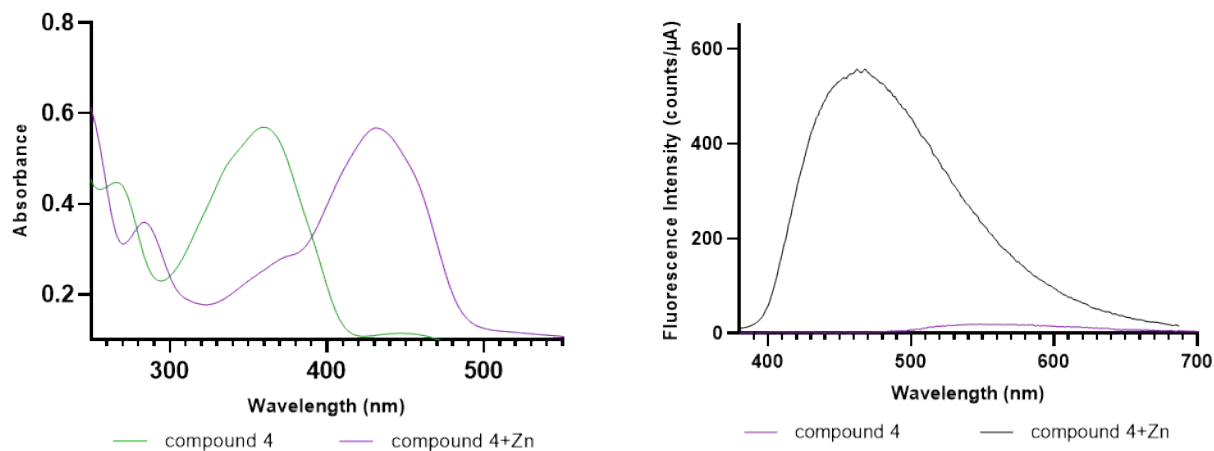


Figure S3: Absorbance and Emission spectra of compound **4** and its Zn^{+2} complex in methanol.

MASS SPECTRA OF COMPOUNDS.

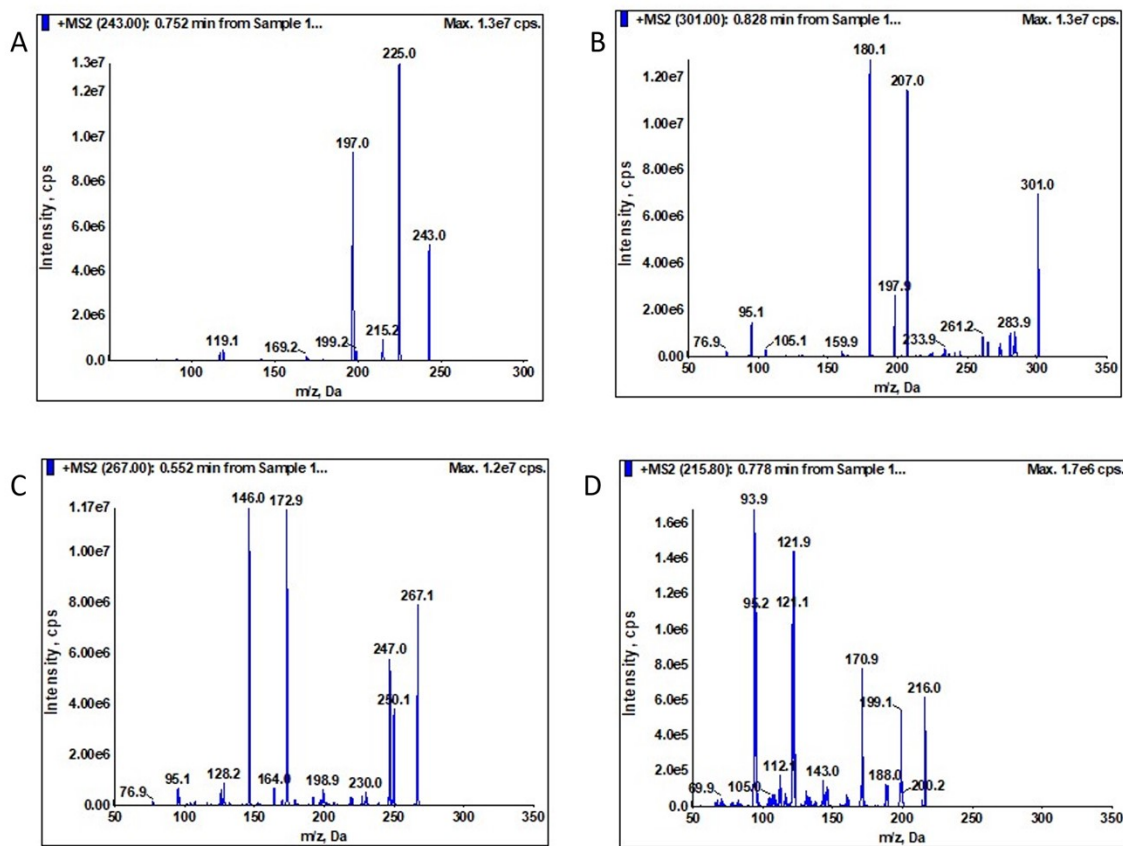


Fig S4: **(A)** MSMS result of compound 1 at 243 m/z **(B)** MSMS result of compound 2 at 301 m/z **(C)** MSMS result of compound 3 at 267 m/z **(D)** MSMS result of compound 4 at 216 m/z

NMR SPECTRA OF COMPOUNDS

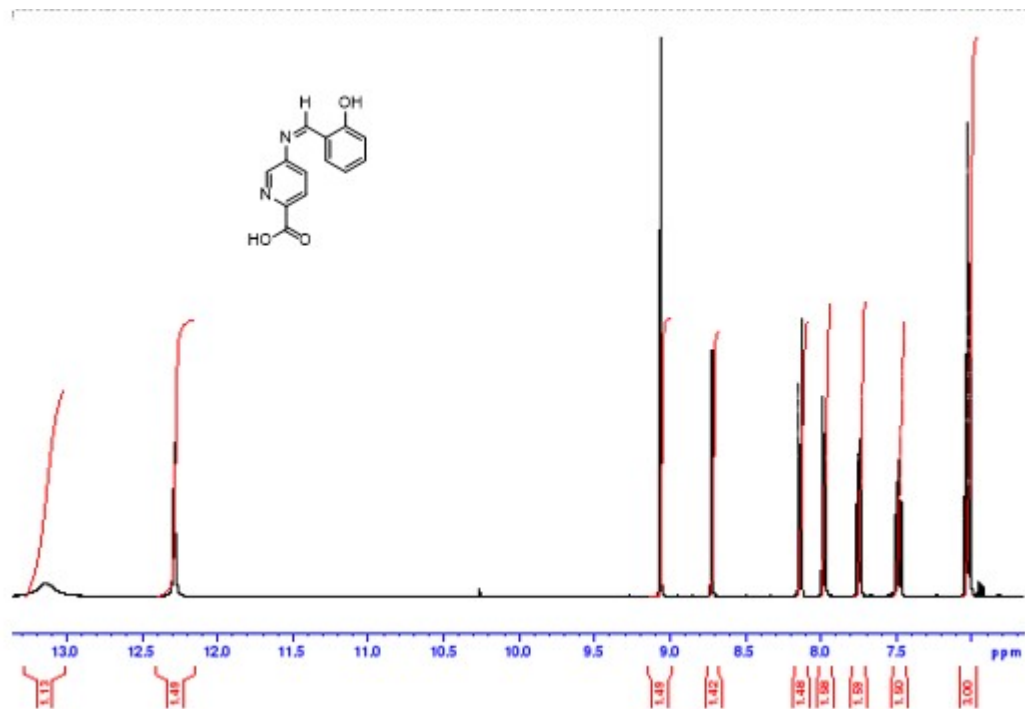


Figure S5: Compound 1 ¹H NMR

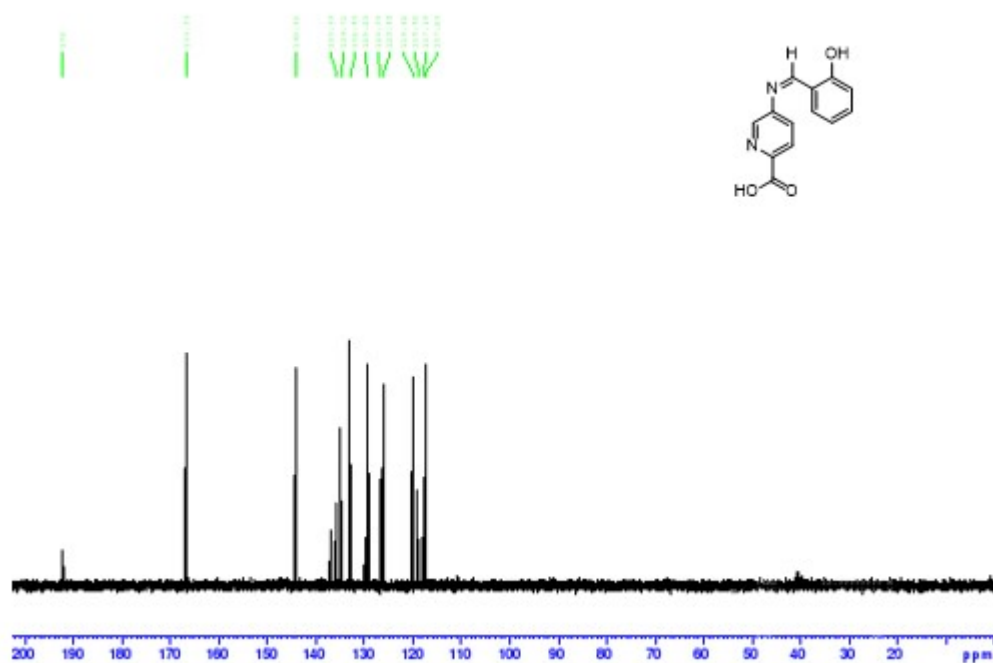


Figure S6: Compound 1 ¹³C NMR

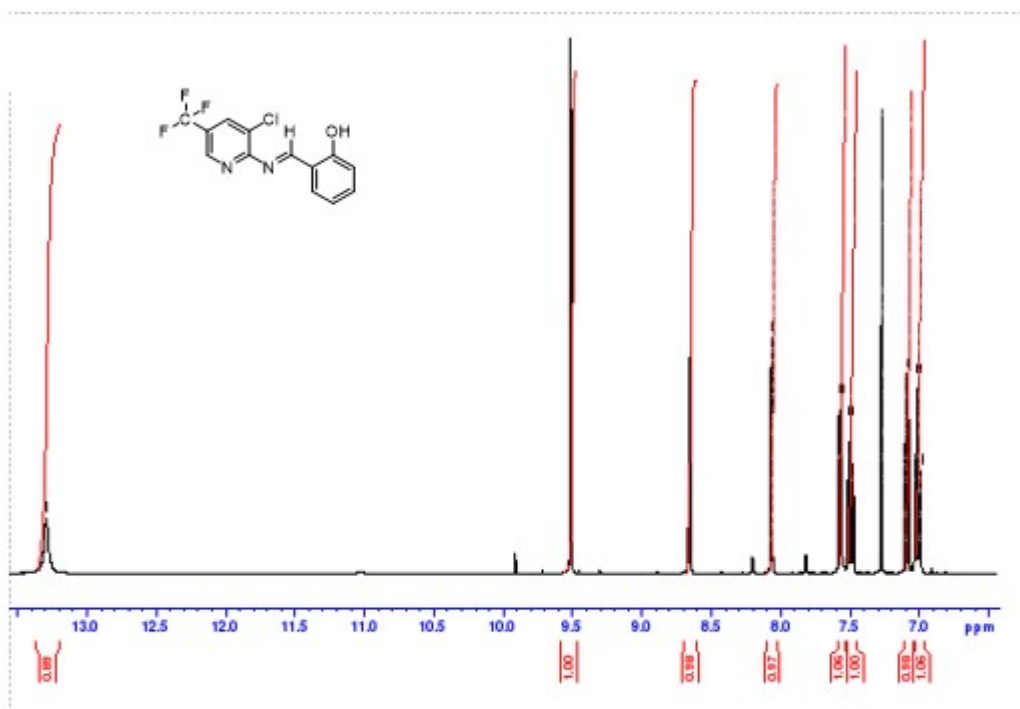


Figure S7: Compound 2 ¹H NMR

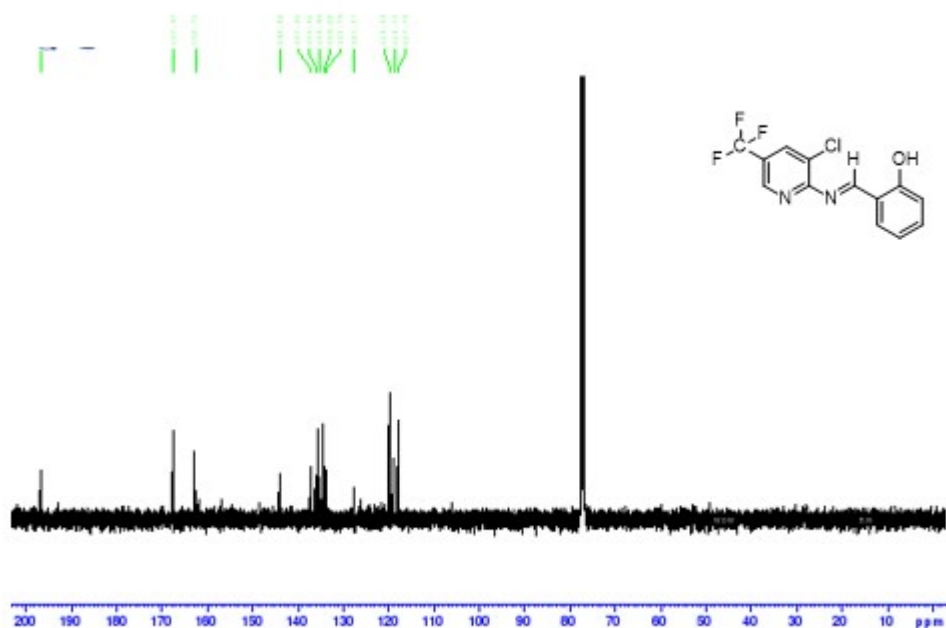


Figure S8: Compound 2 ¹³C NMR

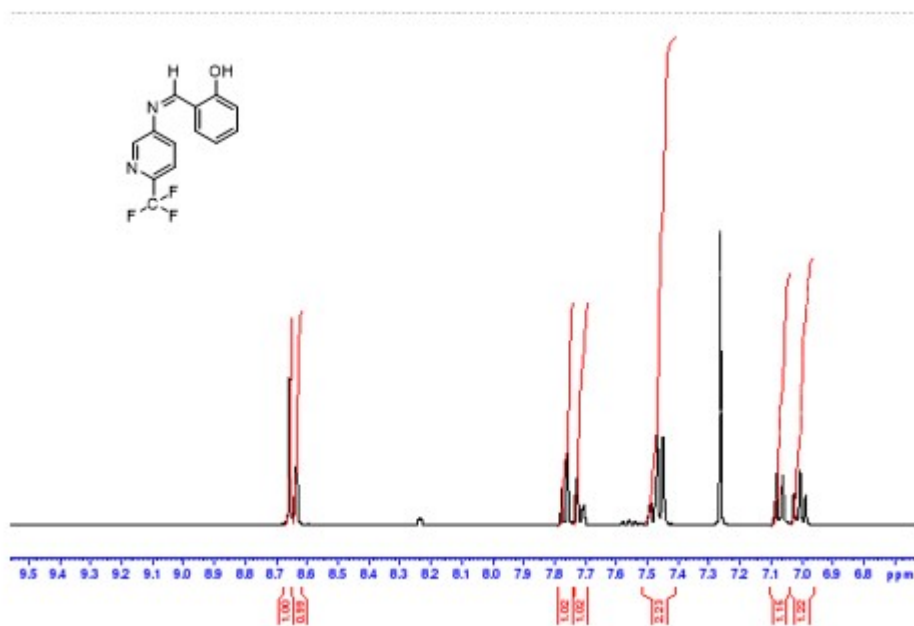


Figure S9: Compound 3 ¹H NMR

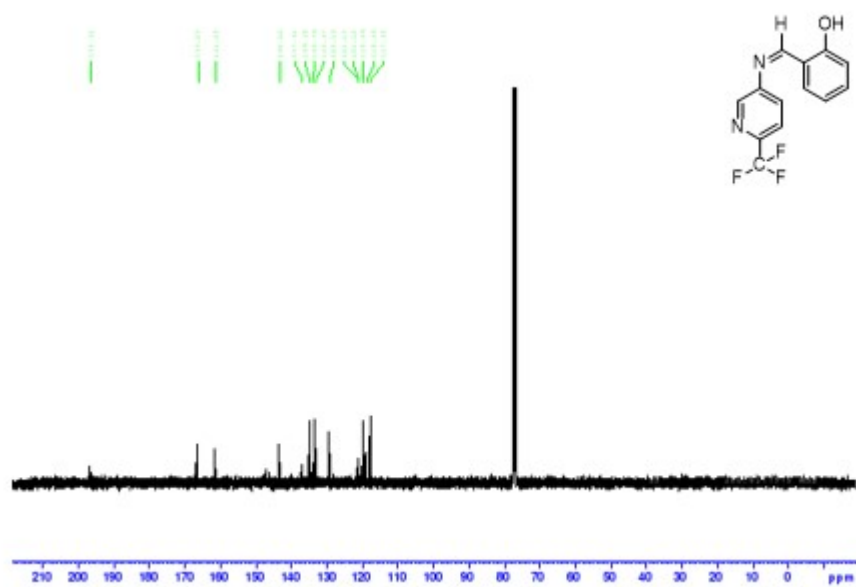


Figure S10: Compound 3 ¹³C NMR

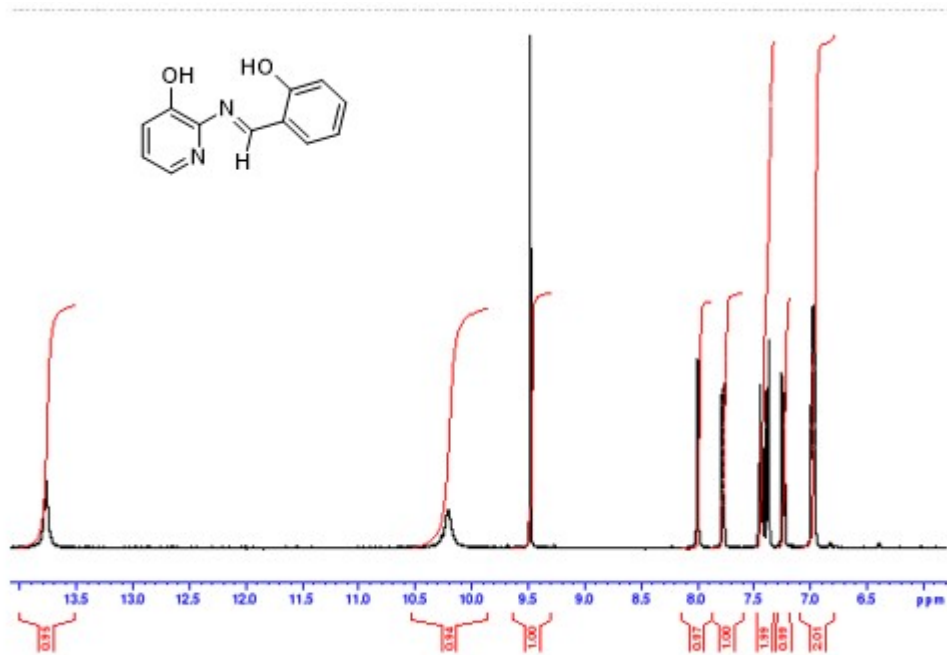


Figure S11: Compound 4 ¹H NMR

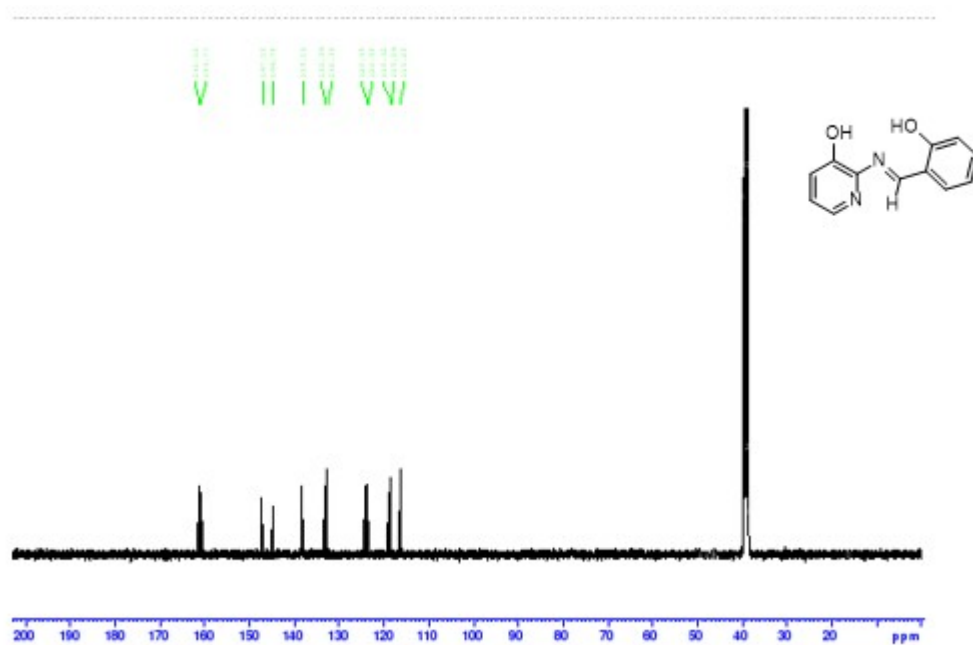


Figure S12: Compound 4 ¹³C NMR

Computational methodology

The optimal molecular electronic structure of the synthesized probes were calculated using the Gaussian 16 package.^[1] Geometry optimizations and electronic structure calculations, including excitation energies and molecular orbital distributions, were performed employing the Coulomb-Attenuating Method (CAM-B3LYP) functional.^[2] For the basis sets, a combination of 6-311++G** for C, H, N, and O atoms, and the Def2SVP basis set with effective core potentials for Zn, was utilized. Time-Dependent Density Functional Theory (TD-DFT) was employed to investigate the spectroscopic profiles of the probe in the UV-Vis range. Solvation effects were considered using the Polarizable Continuum Model (PCM), with a relative dielectric constant of 32.70 to represent methanol as the solvent.^[3] To ensure the most energetically stable geometrical structures were obtained for the studied systems, geometry optimizations were initiated from the lowest possible spin states. These initial structures were iteratively refined to achieve convergence criteria for energy and forces, ensuring reliable results.

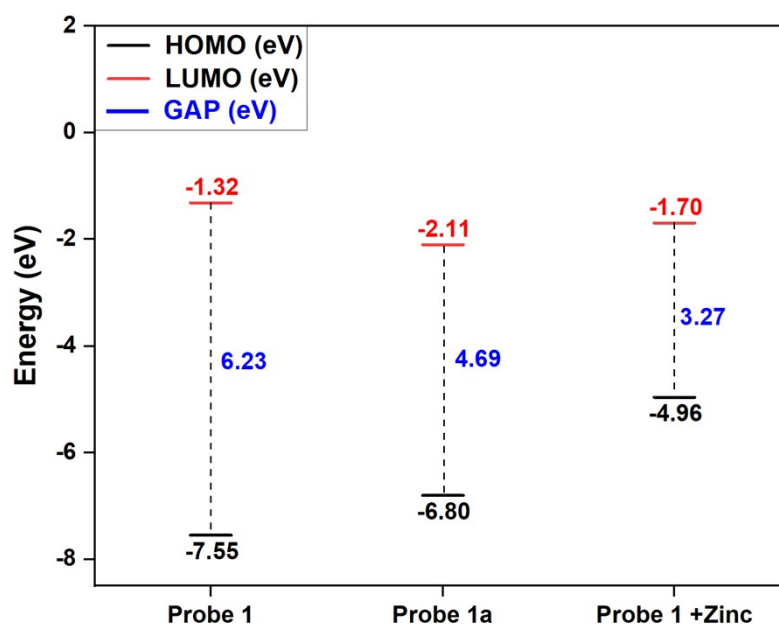


Figure S13: The HOMO and LUMO energies of probe 1, probe 1a and probe 1 + zinc.

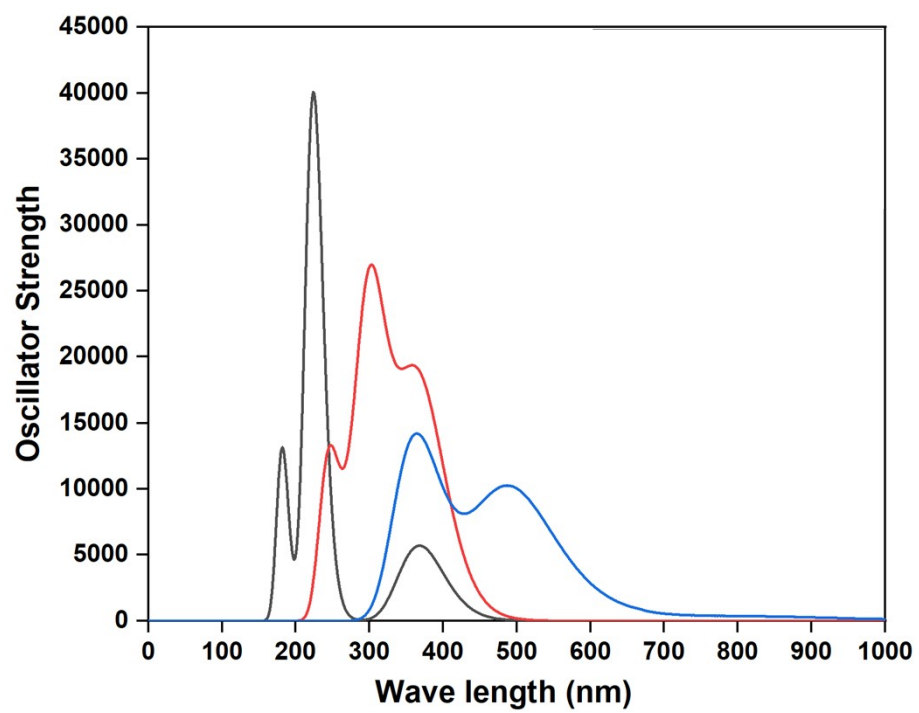


Figure S14: UV-vis spectra of starting material amine (black), probe-1, and probe 1 + zinc, respectively.

References

1. Frisch, M. J., et al., Gaussian 16, Revision C.01, Gaussian, Inc., Wallingford, CT, (2016).
2. Yanai, T., Tew, D. P., and Handy, N. C. A new hybrid exchange–correlation functional using the Coulomb-attenuating method (CAM-B3LYP). *Chem. Phys. Lett.* **393**, 51-57 (2004).
3. Barone, V., and Cossi, M. Quantum calculation of molecular energies and energy gradients in solution by a conductor solvent model. *J. Phys. Chem. A* **102**, 1995-2001 (1998).