Supplementary Information

Differentially selective sensor with fluorescence turn-on response to Zn^{2+} and dual-mode ratiometric response to Al^{3+} in aqueous media

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1. General experimental procedure

All the solvents and reagents were obtained from Sigma-Aldrich and used as received unless otherwise mentioned. The solutions of metal ions were prepared from Al(ClO₄)₃·9H₂O, LiClO₄·3H₂O, NaClO₄, Mg(ClO₄)₂, Ca(ClO₄)₂·4H₂O, Sr(NO₃)₂, Ba(ClO₄)₂, Mn(ClO₄)₂·6H₂O, Fe(ClO₄)₂·H₂O, Co(ClO₄)₂·6H₂O, Cd(ClO₄)₂·H₂O, Ag(ClO₄)₂, Hg(ClO₄)₂, Pb(ClO₄)₂, Ni(ClO₄)₂·6H₂O, Cu(ClO₄)₂·6H₂O and Zn(ClO₄)₂·6H₂O respectively in CH₃CN. ¹H and ¹³C NMR were recorded on a Bruker AV-400 spectrometer with chemical shifts reported as ppm (in DMSO-*d*₆, tetramethylsilane as internal standard). UV-Vis spectra were recorded on a Perkin Elmer Lambda 900 spectrophotometer and fluorescence spectra were recorded on a Perkin Elmer LS 55 spectrophotometer. Mass spectra were recorded on Shimadzu LC-MS 2020 spectrometer.

2. Synthesis of quinoline-triazolyl-pyrrolidinyl-coumarin (QC)

Synthesis of 8-(propargyloxy)quinoline (1). To a solution of 8-hydroxyquinoline (5.0 g, 34 mmol) in dry acetone (200 mL) excess anhydrous K₂CO₃ (28.15 g, 204 mmol) was added and the mixture was refluxed for 30 min. To the mixture propargyl bromide (4.09 g, 34 mmol) was added over a period of 5 h using a pressure equalizing funnel. The resulting mixture was refluxed over a period of 17 h. The reaction mixture was then cooled, filtered and filtrate was evaporated. The brown oily residue was dissolved in CHCl₃ and then solution was washed twice with water (50 mL) followed by saturated brine solution (50 mL). Organic layer was dried over anhydrous Na₂SO₄ and the solvent was evaporated under vacuo. The crude product was purified using column chromatography on silica gel using EtOAc /Pet Ether (4:6) as eluent to afford 5.16 g brown solid product (yield 88%).¹H NMR (400 MHz, CDCl₃) δ 2.54 (1H, t, J = 2.4), 5.05 (2H, d, J = 2.4), 7.27 (1H, dd, J = 5.6 Hz, 1.6 Hz), 7.42-7.51 (3H, m), 8.14 (1H, dd, J = 6.8 Hz, 1.6 Hz), 8.94 (1H, dd, J = 2.4 Hz, 1.6 Hz). LCMS (EI): m/z = 184 (M+H⁺).

Synthesis of Boc-pyrrolidinyl-triazolyl-quinoline (3). To a mixture of N-Boc-2-azidomethyl pyrrolidine (2)¹ (1.5 g, 6.63 mmol) and 8-(propargyloxy)quinoline (1.21 g, 6.63 mmol) in water/ ethanol (160 mL, v/v = 1:1), sodium ascorbate (1.3 mL, 1.32 mmol) of freshly prepared 1 M solution in water was added, followed by the addition of copper(II) sulfate 7.5 % in water (0.705 mL, 0.332 mmol). The heterogeneous mixture was stirred vigorously overnight in the dark at

room temperature. The ethanol was removed under vacuo and the residue was diluted with water, cooled in ice, and then the precipitate was collected by filtration. After washing the precipitate with cold water (10 mL), it was dried under vacuum and the crude product was purified using column chromatography on silica gel with CHCl₃/MeOH (9:1) as eluent to afford brown oily product (2.26 g, yield 96%).¹H NMR (400 MHz, CDCl₃) δ 1.3 (11H, m), 1.82 (2H, m), 2.77-3.23 (2H, m), 4 (1H, m), 4.46 (2H, m), 5.54 (2H, s), 7.19 (1H, m), 7.33 (3H, m), 7.57 (1H, m), 8.07 (1H, d, *J* = 8 Hz), 8.88 (1H, d, *J* = 2.8 Hz). LCMS (EI): m/z = 410 (M+H⁺).

Synthesis of quinoline-triazolyl-pyrrolidinyl-coumarin (QC). To Boc-protected product 3 (0.5 g, 1.22 mmol), CH₂Cl₂-TFA (30 mL, 50:50) was added slowly at ice temperature. The mixture was warmed to room temperature and stirred for 2 h. After evaporating reaction mixture to dryness under vacuo, the residue was dissolved in CH₂Cl₂ (15 mL) and then treated with a saturated NaHCO₃ solution (30 mL) for 1 h at room temperature. The aqueous layer was extracted with CHCl₃ (10 mL x 3) and combined extracts were dried over anhydrous Na₂SO₄. After filtration the CH₂Cl₂ was evaporated to obtain dark brown oily Boc-deprotected amine (0.35 g, Yield 92 %). This amine (0.35 g, 85.6 mmol) was dissolved in dry DMF (15 mL) and coumarin-carboxylic acid (0.163 g, 85.6 mmol) was added. Then HOBt (0.14 g, 1.027 mmol) and EDC.HCl (0.197 g, 1.027 mmol) were added to this solution followed by dropwise addition of DIPEA (0.5 mL, 2.56 mmol) at ice temperature. The reaction mixture was stirred at room temperature for 10 h. After removal of solvent in vacuo, the residue was dissolve in 15 mL CH₂Cl₂. This organic layer was washed with 10 mL of water (10 mL x 3). The organic phase was dried over Na₂SO₄ and solvent was evaporated under vacuo. The crude product was purified by column chromatography on silica gel with methanol/chloroform (1:10) as an eluent to afford crystalline light pink product quinoline-triazolyl-pyrrolidinyl-coumarin (QC) (0.52 g, 93 % yield). ¹H NMR (400 MHz, CDCl₃) 1.25 (1H, m), 1.62 (1H, m), 1.92 (1H, m), 2.05 (1H, m), 3.02 (1H, m), 3.25 (1H, m), 4.52 (1H, m), 4.63 (1H, m), 4.91 (1H, dd, J = 9.2 Hz, 4.8 Hz), 5.59 (2H, s), 7.39 (6H, m), 7.53 (1H, dd, J = 6.4 Hz, 1.2 Hz), 7.60 (1H, m), 7.95 (1H, s), 8.09 (2H, m), 8.88 (1H, dd, J = 2.8 Hz, 1.6 Hz).¹³C NMR (400 MHz, CDCl₃) δ 23.9, 27.5, 48.3, 50.7, 57.4, 62.7, 110.3, 116.8, 118.1, 120.1, 121.5, 124.9, 125.0, 125.5, 126.6, 128.8, 129.4, 133.0, 135.9, 140.3, 143.1, 144.2, 149.2, 153.8, 154.1, 157.7, 164.2. LCMS (EI): $m/z = 482 (M+H^+)$.

3. General method for measurements of photophysical properties

UV-Vis spectra were recorded on Perkin Elmer Lambda 900 spectrophotometer and fluorescence spectra were recorded on a Perkin Elmer model LS 55 spectrophotometer. 1 cm cells were used for absorption and emission titration. For UV-Vis and fluorescence titrations stock solution of ligands **QC** were prepared ($c = 2000 \mu$ M) in CH₃CN. The solutions of guest cations were prepared in CH₃CN in the order of 10⁻³ M. Working solutions of **QC** and metal ions were prepared from the stock solutions. Excitation was carried out at 340 nm for **QC** with 10 nm excitation and 10 nm emissions slit widths.



Figure S1. UV-Vis absorption spectra of **QC** [20 μ M] in the presence of 25 eqv. of different metal ions in mixed media (acetonitrile/water = 9/1).



Figure S2. Fluorescence spectra of **QC** (20 μ M) upon addition of Zn²⁺ (0, 10, 20, 50, 60, 75, 100, 150, 175, 200, 250, 275, 300, 350, 400, 450 and 500 μ M in mixed media (acetonitrile/water = 9/1). Inset: Fluorescence intensity as a function of [Zn²⁺].

4. Job plot

A series of solutions containing **QC** and Zn^{2+} / Al^{3+} were prepared such that the sum of the total concentration of **QC** and Zn^{2+} / Al^{3+} remained constant (100 µM). The mole fraction (X) of Zn^{2+} / Al^{3+} was varied from 0.1 to 1.0.



Figure S3. Job plot for the determination of the stoichiometry of QC and Zn^{2+} in the complex.



Figure S4. Job plot for the determination of the stoichiometry of \mathbf{QC} and Al^{3+} in the complex.

5. Response Parameter and determination of binding constant

The response parameter α is defined as the ratio of the free ligand concentration to the initial concentration of the ligand. α defined as the ratio between the free ligand concentration ([L]) and the total concentration of ligand [L_T]:

$$\alpha = \frac{[L]}{[L_T]}$$

 α can be determined from the emission changes in the presence of different concentrations of M^{n+} :

$$\alpha = \frac{[I - I_0]}{[I_1 - I_0]}$$

where I_1 and I_0 are the limiting emission values for $\alpha = 1$ (in the absence of M^{n+}) and $\alpha = 0$ (probe is completely complexes with M^{n+}), respectively.

Tsein equation² to the following equations that can be used in any stoichiometric ratio between the ligand and analyte.

$$[\mathbf{M}^{\mathbf{n}^+}]^{\mathbf{m}} = \frac{1}{n \cdot K} \bullet \frac{1}{[L]_T^{n-1}} \bullet \frac{1-\alpha}{\alpha^n}$$

Where K is complex equilibrium constant, M_mL_n is metal-ligand, L is ligand, [L], [Mⁿ⁺], and $[M_mL_n]$ are the concentrations of respective species.

The stoichiometric ratio of the Zn^{2+} : fluoroionophore is 1:1. So, this equation can be written as

$$[Zn^{2+}] = \frac{1}{2KL} \cdot \frac{1-\alpha}{\alpha^2}$$

The stoichiometric ratio of the Al³⁺: fluoroionophore is 1:1. So, this equation can be written as

$$[\mathrm{Al}^{3+}] = \frac{1}{3KL^2} \bullet \frac{1-\alpha}{\alpha^2}$$



Figure S5. Response parameter values (α) of **QC** as a function of the logarithm of $[Zn^{2+}]$. α is defined as the ratio between the free ligand concentration and the initial concentration of ligand.



Figure S6. Response parameter values (α) of **QC** as a function of the logarithm of [Al³⁺]. α is defined as the ratio between the free ligand concentration and the initial concentration of ligand.

6. Competitive binding studies



Figure S7. Relative fluorescence intensities of **QC** and its complexation with Zn^{2+} in the presence of various other metal ions. Dark grey bar: **QC** (20 µM) with 20 eqv. of metal ion stated. Light grey bar: 20 µM of **QC** and 20 eqv. of Zn^{2+} with 20 eqv. of metal ions stated (for Zn^{2+} effect 40 eqv. of Zn^{2+}). The responses of the **QC** to Zn^{2+} , in the absence of competing ions, is included as controls. Extreme left dark bar: 20 µM of **QC** with no metal ion; light bar, 20 µM of **QC** with 20 eqv. of Zn^{2+} . Sovent: mixed media (acetonitrile/water = 9/1).



Figure S8. Relative fluorescence intensities of **QC** and its complexation with Al^{3+} in the presence of various other metal ions. Dark grey bar: **QC** (20 µM) with 20 eqv. of metal ion stated. Light grey bar: 20 µM of **QC** and 20 eqv. of Al^{3+} with 20 eqv. of metal ions stated (for Al^{3+} effect 40 eqv. of Al^{3+}). The responses of the **QC** to Al^{3+} , in the absence of competing ions, is included as controls. Extreme left dark bar: 20 µM of **QC** with no metal ion; light bar, 20 µM of **QC** with 20 eqv. of Al^{3+} . Sovent: mixed media (acetonitrile/water = 9/1).

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7. Competitive studies for Al³⁺ in water



Figure S9. Relative fluorescence intensities of **QC** and its complexation with Al^{3+} in the presence of various other metal ions in water. **QC** (20 μ M) is mixed with 20 eqv. of Al^{3+} in presence of 20 eqv. of all other metal ions.

8. NMR Spectra



¹H NMR spectrum of 8-(propargyloxy)quinolone (1)



¹H NMR spectrum of Boc-pyrrolidinyl-triazolyl-quinoline (3)

¹H NMR spectrum of quinoline-triazolyl-pyrrolidinyl-coumarin (QC)









Figure S10. ¹H NMR spectra of **QC** with Zn^{2+} in CD₃CN. (i) **QC**, (ii) **QC** with 0.5 eqv. of Zn^{2+} , (iii) **QC** with 1.0 eqv. of Zn^{2+} and (iv) **QC** with 1.5 eqv. of Zn^{2+} .



Figure S11. ¹H NMR spectra of **QC** with Al^{3+} in CD₃CN. (i) **QC**, (ii) **QC** with 0.5 eqv. of Al^{3+} , (iii) **QC** with 1.0 eqv. of Al^{3+} and (iv) **QC** with 1.5 eqv. of Al^{3+} .

9. ESI-MS spectra



ESI-MS spectrum of 8-(propargyloxy)quinolone (1)



ESI-MS spectrum of Boc-pyrrolidinyl-triazolyl-quinoline (3)



ESI-MS spectrum of quinoline-triazolyl-pyrrolidinyl-coumarin (QC)

10. pH dependent fluorescence study



Figure S12. Dependence of the fluorescence intensity at 482 nm of **QC** on pH. Black trace: **QC** (20 μ M) and red trace: **QC** with 20.0 equivalents of Al³⁺ in aqueous medium.

10. References

- 1. T. Govindaraju and V. A. Kumar, Chem. Commun., 2005, 495.
- 2. G. Grynkiewcz, M. Poenie and R. Y. Tsein, J. Biol. Chem., 1985, 260, 3440.