SUPPORTING INFORMATION

Rare Crystal Structure of Open Spirolactam Ring along with the Closed Ring Form of a Rhodamine Derivative: Sensing of Cu\textsuperscript{2+} Ions from Spinach

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1. Table S1. Performance Comparison of Existing Methods and Present Method based on Rhodamine based fluorescent probes for Determination of Copper (II) over the last decade.

| Analytes | Sensor type | Detection limit (µM) | Detection method | Detection state | Sensitivity | Response Time | Estimation | Reference |
|----------|-------------|----------------------|------------------|-----------------|-------------|---------------|------------|-----------|
| **Cu²⁺** | Salicylaldehyde-rhodamine B hydra zone | 0.25 | Naked eye UV-Vis Fluorescence | Liquid | high | 27 min | No | ORGANIC LETTERS 2006, 8, 2863-2866 |
| **Cu²⁺** | ethylenediamine-N,N-diacetic acid | n.d. | Naked eye UV-Vis Fluorescence | Liquid | high | >60 min | No | ORGANIC LETTERS 2007, 9, 5039-5042 |
| **Cu²⁺** | Tren/Dansyl-Appended Rhodamine B | n.d. | UV-Vis Fluorescence | Liquid | High | 40 min | No | ORGANIC LETTERS 2008, 10, 213-216 |
| **Cu²⁺** | binaphthyl-Rhodamine B | n.d. | Naked eye UV-Vis Fluorescence | Liquid | Moderate | n.d. | No | Sensors and Actuators B, 2009, 137, 597-602 |
| **Cu²⁺** | Pyrene-rhodamine B conjugate | n.d. | Naked eye UV-Vis Fluorescence | Liquid | Moderate | n.d. | No | ORGANIC LETTERS 2009, 11, 4442-4445 |
| **Cu²⁺** | 1-(rhodamine B) lactam-thiosemicarbazide | 0.04-0.08 | UV-Vis Fluorescence | Liquid | High | 5 min | No | Sensors and Actuators B, 2009, 135, 625-631 |
| **Cu²⁺** | Rhodamine 6G-pyridine conjugate | 0.3 | Naked eye UV-Vis Fluorescence | Liquid | High | ~2 min | yes | Anal. Chem. 2009, 81, 7022-7030 |
| **Cu²⁺**, **Zn²⁺** | N-butyl-1.8-naphthalimide | n.d. | Naked eye UV-Vis Fluorescence | Liquid | Moderate | n.d. | No | ORGANIC LETTERS, 2010, 12, 3852-3855 |
| **Cu²⁺** | 2-amino benzimidazole | 0.28 | Naked eye UV-Vis Fluorescence | Liquid | High | 2 min | No | Bull. Korean Chem. Soc. 2010, 31, 3212-3216. |
| **Cu²⁺** | 2-hydroxy Phenyl-rhodamine B | n.d. | Naked eye UV-Vis Fluorescence | Liquid | Moderate | 5 min | No | J. Fluoresc, 2011, 21, 141-148. |
| **Cu²⁺** | 2,4-dihydroxy benzaldehyde | 3.4 | Naked eye UV-Vis Fluorescence | Liquid | High | <30 sec | No | Sensors and Actuators B, 2011, 156, 546-552. |
| Cu²⁺ | Compound | Detection Limit | Detection Method | Medium | Sensitivity | Reaction Time | Reaction | Source |
|------|----------|-----------------|------------------|--------|-------------|--------------|----------|--------|
| Cu²⁺ | Rhodamine B – benzoyl hydrazine | 0.007 | Naked eye UV-Vis Fluorescence | Liquid | Moderate | n.d. | No | Microchim. Acta. 2011, 174, 247–255. |
| Cu²⁺ | rhodamine-hydrazone-ether | 0.15 | Naked eye UV-Vis | Liquid | Moderate | 6 min | No | Sensors and Actuators B 2013, 182, 530–537. |
| Cu²⁺ | Rhodamine-hydrazone-cinnamaldehyde | 3.0 | Naked eye UV-Vis Fluorescence | Liquid | High | n.d. | No | Sensors and Actuators B, 2013, 176, 482–487. |
| Cu²⁺ | Sugar–Rhodamine B | 0.15 | Naked eye UV-Vis | Liquid | High | 2 min | No | Sensors and Actuators B 2013, 177, 213–217 |
| Cu²⁺ | Rhodamine B–pyrrole imine | 0.28 | Naked eye UV-Vis Fluorescence | Liquid | High | 2 min | yes | Sensors and Actuators B 193 (2014) 679–686. |
| Cu²⁺ | Thiophene-rhodamine B hydrazone | 0.024 | Naked eye UV-Vis Fluorescence | Liquid | High | 40 min | No | Dalton Trans., 2014, 43, 7747–7751. |
| Cu²⁺ | cyclometalated iridium(III)-Rhodamine B | 0.0045-0.005 | Naked eye UV-Vis Fluorescence | Liquid | High | 5 min | No | SCIENTIFIC REPORTS, 2014, 4 : 6794, DOI: 10.1038/srep06794. |
| Cu²⁺, Hg²⁺ | 3,5-dichloro salicylaldehyde and rhodamine B hydrazide | n. d. | Naked eye UV-Vis | Liquid | moderate | 20 min | No | Sensors and Actuators B 199 (2014) 121–126. |
| Cu²⁺ | Rhodamine 6G–Ferrocene Conjugate | 0.7 | Naked eye UV-Vis Fluorescence | Liquid | moderate | 2 min | No | Organometallics 2015, 34, 2962–2970 |
| Cu²⁺ | Ethyl-2-(4 (acryloyloxy)-3-formylphenyl)-4-methyl thiazole-5-carboxylate | 0.3 | Naked eye UV-Vis Fluorescence | Liquid | moderate | 30 min | No | Designed Monomers and Polymers, 2016, 19, 669-678. |
| Cu²⁺ | rhodamine-B carbonyl morpholine | 0.21 | Naked eye UV-Vis Fluorescence | Liquid | high | 120 min | No | Chemosensors 2017, 5, 26; doi:10.3390/chemosensors5030026 |
| Cu²⁺ | 3-methoxy Salicylaldehyde-Rhodamine B | 0.028 | Naked eye UV-Vis Fluorescence | Liquid | high | 5 min | No | Bioorganic & Medicinal Chemistry, 2018, 26, 1448–1452. |
| Cu²⁺ | Quinoline-rhodamine acetamide (QRA) | 2.2 | Naked eye UV-Vis Fluorescence | Liquid / Solid | high | 2 min | yes | This work |
2. Synthesis:

**Compound 1** (2-(2-aminoethyl)-3',6'-bis (diethylamino) spiro [isoindoline-1,9'-xanthen]-3-one): Rhodamine B (2.40 g, 5 mmol) was dissolved in MeOH (20 mL). Ethylenediamine (5 mL, excess) was added drop-wise to the solution and refluxed overnight (15 h) until the solution loses its red color. The solvent was removed by evaporation. Water (20 mL) was added to the resultant and extracted with CH$_2$Cl$_2$ (20 mL × 2). The combined organic phase was washed twice with water and dried over Na$_2$SO$_4$. The solvent was removed by evaporation and dried in vacuo, affording a pale-pink solid of compound 1 (1.9 g, yield, 79%). $^1$H NMR (400 MHz, CDCl$_3$): δ (ppm) = 7.86-7.81 (m, 1H), 7.48-7.40 (m, 2H), 7.09-7.03 (m, 1H), 6.43-6.25 (m, 6H), 3.33 (q, $J$ = 6.7 Hz, 8H), 3.19 (t, $J$ = 6.7 Hz, 2H), 2.88 (t, $J$ = 4.1 Hz, 2H ), 1.16 (t, $J$ = 6.7 Hz, 12H). $^{13}$C NMR (400 MHz, CDCl$_3$): δ (ppm) = 170.07, 153.56, 153.14, 148.95, 132.96, 129.89, 128.19, 123.82, 122.98, 108.53, 103.81, 97.78, 66.50, 44.42, 41.20, 40.19, 12.72. HRMS: anal. calcd for C$_{30}$H$_{36}$N$_4$O$_2$: 484.28; found: 485.3 (M + H$^+$, 100%).

**Compound 2** (N-(2-(3',6'-bis (diethylamino)–3-oxospiro [isoindoline-1,9'-xanthen]-2-yl) ethyl)-2-bromoacetamide): Compound 1 (1.5 g, 3 mmol) mixed with K$_2$CO$_3$ (4.27 g,10 mmol) is suspended into a mixture of ethyl acetate (25 mL) and water (25 mL) and stirred for 30 minutes. Then, bromoacetyl chloride (1.22g, 2.5 mmol) in ethyl acetate (5 mL) is added dropwise into the solution. After 4 h stirring at room temperature, the organic layer is isolated and dried by MgSO$_4$. The ethyl acetate solvent is removed by rotary evaporation to give the crude product that is purified by column chromatography (silica, 220–400 mesh, EtOAc/MeOH = 10:1 v/v). The product is isolated as a pale-pink powder 2 (1.26 g, 84%). $^1$H NMR (400 MHz,
CDCl₃): δ (ppm) = 7.90-7.94 (m, 1H), 7.45-7.48 (m, 3H), 7.05-7.09 (m, 1H), 6.37-6.47 (m, 5H), 3.71 (s, 2H), 3.33 (q, J= 6.7 Hz, 10H), 3.02 (t, J = 6.7 Hz, 2H), 1.18 (t, J = 6.7 Hz, 12H); ¹³C (400 MHz, CDCl₃) δ: 172.07, 157.63, 156.02, 155.56, 151.19, 136.63, 135.04, 131.60, 130.30, 130.24, 129.51, 128.82, 128.44, 126.00, 125.85, 124.51, 120.59, 110.39, 109.06, 106.63, 99.83, 68.70, 49.21, 46.15, 40.96, 40.78, 13.69. ESI/MS: m/z calcd. for [M+H]+ 605.57, found 606.60 (M+H⁺, 100%). Anal. calcd. for C₃₂H₃₇BrN₄O₃: C, 63.47; H, 6.16; N, 9.25; O, 7.93; Br, 13.19. Found: C, 63.41; H, 6.15; N, 9.32; O, 7.92; Br, 13.20.

3. NMR Studies:

![Figure S1. ¹H NMR of QRA in CDCl₃ (400 MHz).](image)
4. Mass spectrum of QRA:

**Figure S3.** ESI-MS of QRA
5. Crystallography

Crystal data for **QRA closed**: CCDC-1861404, C41H43N5O4, M = 669.80, colourless crystals, 0.12 x 0.12 x 0.20 mm³, triclinic, space group P-1, a = 11.256(3) Å, b = 11.921(3) Å, c = 15.208(4) Å, α = 69.23(1)°, β = 71.02(1)°, γ = 73.10(1)°, V = 1768.4(8)Å³, Z = 2, Dc = 1.258 g/cm³, F000 = 712, µ= 0.082 mm⁻¹, T = 296(2) K, θmax = 23.1°, 4987 total reflections, 3225 with Io > 2σ(Io), Rint = 0.1078, 4464 data, 443 parameters, 6 restraints, GooF = 1.150, R = 0.1276 and wR= 0.3169 [Io > 2σ(Io)], R = 0.1571 and wR= 0.3579 (all reflections), 0.617 < Δρ < -0.538 e/Å³, extinction coefficient 0.083(12).

Crystal data for **QRA open**: CCDC-1861403, C41H43N5O4, M = 905.74, violet crystals, 0.10 x 0.18 x 0.26 mm³, monoclinic, space group P21/n, a = 14.698(6) Å, b = 19.777(8) Å, c = 15.179(6) Å, α = 90°, β = 96.467(4)°, γ = 90°, V = 4384(3) Å³, Z = 4, Dc = 1.372 g/cm³, F000 = 1900, µ= 0.220 mm⁻¹, T = 296(2) K, θmax = 25.00°, 7706 total reflections, 4402 with Io > 2σ(Io), Rint = 0.1014, 7706 data, 587 parameters, 6 restraints, GooF = 1.053, R = 0.0786 and wR= 0.2088 [Io > 2σ(Io)], R = 0.1428 and wR= 0.2764 (all reflections), 0.789 < Δρ < -0.748 e/Å³.

![Figure S4](image.png)

**Figure S4.** The CPK model of **QRA-closed** (a) and the angle between the xanthene and quinoline plane and the distance between the centre of the xanthene and quinoline ring (b).
Figure S5. The CPK model of QRA-open (a), the xanthene and quinoline rings are nearly co-planar and the distance between the centre of the xanthene and quinoline rings (b).

6. UV-Vis and fluorescence titration studies:

A stock solution of QRA (10 µM) was prepared in acetonitrile-water (1:8, v/v). Copper perchlorate solutions of different concentration were prepared in Millipore water. All experiments were carried out in aqueous medium (pH 7.2, Tris-HCl buffer). During titration, each time a 10 µM solution of QRA was filled in a quartz optical cell of 1 cm optical path length and Cu²⁺ stock solution of a particular concentration was added into the quartz optical cell by using a micropipette until saturation. Spectral data were recorded at 2-3 min after the addition of Cu²⁺ solution.

For all fluorescence measurements, excitations were provided at 530 nm, and emissions were collected between 550 to 680 nm.
Absorbance and fluorescence comparative studies of QRA with various cationic analytes:

Figure S6. Comparative (a) absorption and (b) fluorescence emission spectra of QRA (10 µM) in CH$_3$CN/H$_2$O, 1:8 (v/v), (pH 7.2, 10 mM Tris-HCl buffer) after addition of various comparative cations up to 3 equiv. ($\lambda_{ex}$= 530 nm).

7. Fluorescence response of QRA in presence of different biologically relevant analytes in neutral medium:

Figure S7. Comparative fluorescence response of QRA with different analytes i.e. 1. Blank, 2. Cu$^{2+}$, 3. Zn$^{2+}$, 4. Mg$^{2+}$, 5. Fe$^{2+}$, 6. CO, 7. NO, 8. H$_2$O$_2$, 9. Cl$^-$, 10. AcO$^-$, 11. NO$_2^-$, 12. H$_2$S, 13.
ClO\(^-\) and 14. SO\(_3\)\(^-\) in neutral aqueous medium (\(\lambda_{\text{ex}}=530\) nm). Standard deviations are represented by error bars (n=3).

8. Dependence of the fluorescence intensity of QRA as a function of time with increasing concentration of Cu\(^{2+}\):

![Graph](image)

**Figure S8.** Fluorescence intensity enhancement of QRA (10 µM) upon gradual addition of Cu\(^{2+}\) up to 3 equiv. with respect of time in CH\(_3\)CN/H\(_2\)O (1:8, v/v) (\(\lambda_{\text{ex}}=530\) nm).

9. Fluorescence response of QRA in solid phase:

![Image](image)

**Figure S9.** Fluorescence color changes visualized on filter paper strips after 10 minutes of incubation of QRA and QRA-Cu\(^{2+}\).
10. Job’s plot for determining the stoichiometry of interaction by fluorescence method:

![Job's plot](image)

**Figure S10.** Job’s plot of interaction of QRA with Cu\(^{2+}\) in acetonitrile-water (1:8, v/v), neutral pH, ([QRA] = [Cu\(^{2+}\)] = 10 μM) by fluorescence method (λ\(_{ex}\) = 530 nm). Standard deviation are represented by error bars (n=3).

11. Calculation of limit of detection (LOD) of QRA towards Cu\(^{2+}\):

![Linear fit curve](image)

**Figure S11.** Linear fit curve of QRA with respect to Cu\(^{2+}\) concentration. Standard deviation are represented by error bars (n=3).

From the linear fit graph of Cu (II), we determined the Limit of Detection = 2.2 μM. Therefore QRA can detect copper (II) up to this very lower concentration by fluorescence technique.
12. pH titration curve of QRA upon gradual addition of Cu\textsuperscript{2+}:

![pH titration curve](image)

**Figure S12.** Fluorescence responses of probe QRA (red) and QRA-Cu\textsuperscript{2+} complex (black) in different pH conditions in CH\textsubscript{3}CN/H\textsubscript{2}O (1:8, v/v) (\(\lambda_{ex}=530\) nm). Standard deviations are represented by error bars (n=3).

13. \(^1\)H NMR titration spectrum of QRA with Cu\textsuperscript{2+}:

![\(^1\)H NMR titration spectrum](image)

**Figure S13.** \(^1\)H NMR titration spectra [400 MHz] of QRA in CD\textsubscript{3}CN at 25\textdegree C and the corresponding changes after addition of 1 equiv. Cu\textsuperscript{2+} in D\textsubscript{2}O.
14. $^{13}$C NMR titration spectrum of QRA with Cu$^{2+}$:

![Figure S14](image)

**Figure S14.** $^{13}$C NMR titration spectra [400 MHz] of QRA in CD$_3$CN at 25$^0$C and the corresponding changes after addition of 1 equiv. Cu$^{2+}$ in D$_2$O.

15. Details of energy calculations using Density Functional Theory (DFT):

| Details                  | QRA closed      | QRA-Cu$^{2+}$ complex | QRA open       |
|--------------------------|-----------------|------------------------|----------------|
| Calculation method       | CAM-B3LYP       | CAM-B3LYP              | CAM-B3LYP      |
| Basis set                | 6-31G**/def2TZVP| 6-31G**/LANL2DZ/def2TZVP| 6-31G**/def2TZVP|
| E(CAM-B3LYP) (a.u.)      | -2163.506       | -2512.119              | -2163.512      |
| Charge, Multiplicity     | 0, 1            | 2, 1                   | 0, 1           |
| Solvent (CPCM)           | Water           | Water                  | Water          |

**Table S2.** Details of the geometry optimization in Gaussian 09 program.
Figure S15. Energy optimized geometries of QRA, QRA-Cu$^{2+}$ complex and QRA open structure obtained at the CAM-B3LYP/6-31G**/LANL2DZ levels of theory with CPCM solvation (H$_2$O).

16. Estimation of copper in spinach plant:

For estimation of Cu$^{2+}$ ion, 100 g of each of root, stem and leaf from the treated plant were taken. All the samples were grinded separately to a fine paste. Finally they were diluted to 100 mL, filtered and fluorescence titrations of the filtrate with the probe QRA were performed with the help of standard fluorescence curve.