Tetrahedron 65 (2009) 4235–4238

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Quinolinecarboxylic acid based fluorescent molecules: ratiometric response to Zn^{2+}

Hisanaka Ito *, Mai Matsuoka, Yohei Ueda, Madoka Takuma, Yoshihisa Kudo, Kazuo Iguchi

School of Life Sciences, Tokyo University of Pharmacy and Life Sciences, 1432-1 Horinouchi, Hachioji, Tokyo 192-0392, Japan

ARTICLE INFO

Article history:
Received 10 November 2008
Received in revised form 10 March 2009
Accepted 11 March 2009
Available online 25 March 2009

ABSTRACT

Novel fluorescent compounds based on quinolinecarboxylic acid were developed. These compounds were easily prepared in two or three steps from commercially available compounds. Compound **2** showed a ratiometric response to zinc(II) salt in aprotic solvent.

1. Introduction

Development of novel fluorescent molecules as chemosensors for the detection of chemically, biologically, and environmentally important functional molecules has received much attention in recent years.[1] Although a large number of fluorescent chemosensors have been prepared based on some basic fluorophores, development of novel poly-functionalized fluorophores is still an important theme for creation of chemosensors. PRODAN is one of the most fundamental fluorophores having a relatively large molar extinction coefficient and a high fluorescence quantum yield (Fig. 1).[2] This molecule is sensitive to environmental changes, and with an increase in dielectric constant of the solvent, the maxima of emission spectra of the molecule shifts to longer wavelength regions.

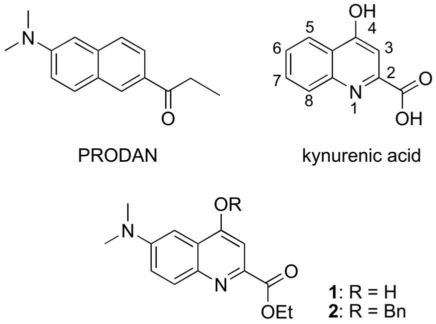


Figure 1. Structures of PRODAN, kynurenic acid and fluorescent compounds **1** and **2**.

Based on the PRODAN structure, compounds **1** and **2** were newly designed (Fig. 1). Compound **1** is a 6-dimethylamino-derivative of kynurenic acid ethyl ester. Kynurenic acid and related quinolinecarboxylic acid derivatives have been well known not only as important compounds for neurobiology[3] but also as receptors for metal ions.[4] Kynurenic acid derivatives can be prepared through short steps including Conrad–Limpach cyclization[5] and can contain extendable functional groups such as hydroxyl and ethoxycarbonyl groups. Moreover, the sp^2 nitrogen atom serves as a Lewis base on the fluorophore, and together with the ethoxycarbonyl group, could be expected to behave as a coordination site for an external acidic functional group.

Zinc ion is the second most abundant heavy metal ion and is known to play important roles in cellular events including structural co-factors, regulators, and catalytic centers of enzymes, DNA binding, and neuronal signal transmission.[6] In relation to these biological roles, many ratiometric fluorescent probes for zinc ion have been already developed.[7] In this paper, we report the development of novel fluorescent compounds and their ratiometric response to zinc(II) salt.

2. Results and discussions

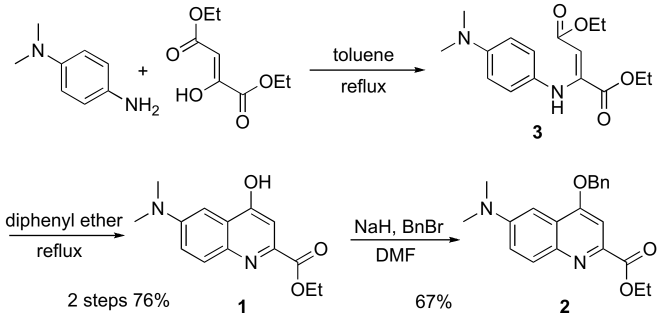
2.1. Synthesis

Compounds **1** and **2** were prepared in a similar manner as the preparation of kynurenic acid derivatives[8] in two or three steps sequence from commercially available compounds and results are shown in Scheme 1. Condensation of *N*,*N*-dimethyl-*p*-phenylenediamine with oxalacetic acid diethyl ester gave intermediate **3** and subsequent cyclization under reflux conditions in diphenyl ether gave fluorescent compound **1** in 76% yield. Compound **2** was obtained by benzyl etherification of **1** in 67% yield.

---

* Corresponding author. Tel.: +81 42 676 5473; fax: +81 42 676 7282.
E-mail address: itohisa@ls.toyaku.ac.jp (H. Ito).

0040-4020/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2009.03.037



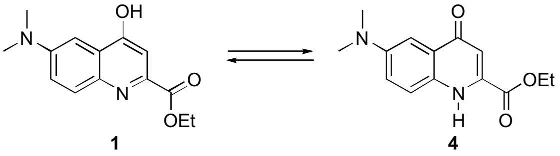
Scheme 1. Synthesis of compounds **1** and **2** through Conrad–Limpach cyclization.

2.2. Fluorescent characteristics

The fluorescent characteristics of compounds **1** and **2** are shown in Table 1. Excitation and emission spectra for both compounds were strongly influenced by solvent polarity. Compound **1** having a free phenolic hydroxyl group showed maxima of emission spectra at 501 nm (toluene) and 552 nm (MeOH). Compound **2**, in which the hydroxyl group is protected by a benzyl group, showed maxima of emission spectra at 422 nm (toluene) and 484 nm (MeOH). These results indicated that in the case of compound **1**, the contribution of tautomeric isomer **4** significantly affects both excitation and emission spectra (Scheme 2).

Table 1
Fluorescent properties of **1** and **2**

Compound	Solvent	ε (M^{-1} cm^{-1})	Q	Abs (nm)	Em (nm)
1	Toluene	8.06×10^3	0.35	426	501
1	MeOH	4.94×10^3	0.06	434	552
2	Toluene	15.42×10^3	0.77	372	422
2	MeOH	17.17×10^3	0.38	387	484



Scheme 2. Tautomerization of **1**.

2.3. Recognition of metal salts

As mentioned above, the present fluorescent molecules have a nitrogen atom on the quinoline ring and a carbonyl group in the ester moiety, and both functional groups could serve as coordination sites for a metal ion. Therefore, the behavior of compounds **1** and **2** in the presence of metal salt was examined. Addition of a solution of copper(II) salt to a solution of compound **1** or **2** in toluene, resulted in a quenching of the fluorescence. However, no significant effects during the addition of a solution of zinc(II) salt to a solution of compound **1** in toluene were observed. This result also supported the presence of tautomer **4**, which does not have a quinoline ring. On the other hand, zinc(II) salt gave a ratiometric response in both excitation and emission spectra with compound **2** in toluene (Fig. 2). Thus, upon addition of a solution of zinc bromide in toluene, the absorption intensities of **2** at 305 and 455 nm gradually increased, accompanied by a decrease in absorption intensities at 343 and 373 nm (Fig. 2a). In the emission spectra, the characteristic strong emission band at 426 nm decreased, and a new band centered at 503 nm arose, corresponding to a λ_{max} (emission) red-shift of 77 nm and an isosbestic point at 472 nm (Fig. 2b).

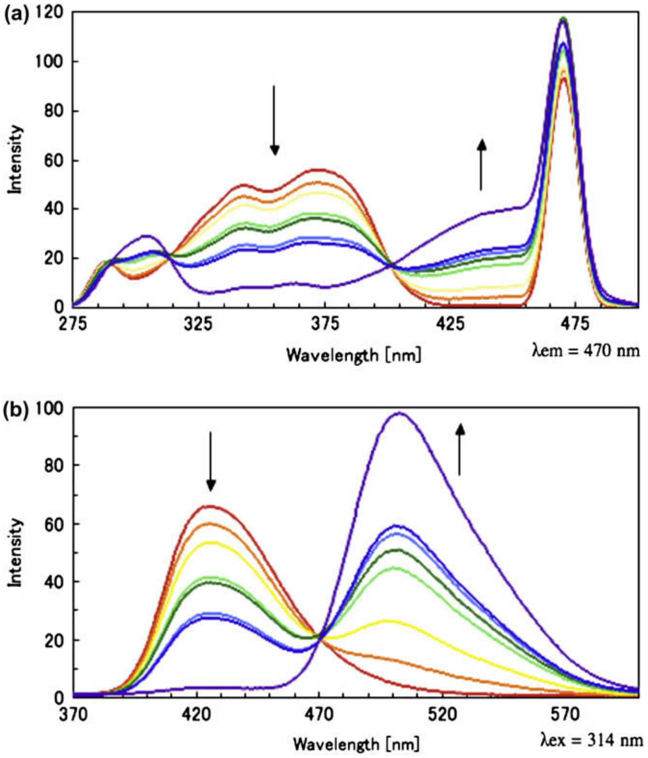


Figure 2. Changes in excitation (a) and emission (b) spectra of **2** in toluene with various amounts of Zn^{2+} ions ([**2**]=0.29 μM, [Zn^{2+}]=0, 0.10, 0.20, 0.30, 0.40, 0.50, 0.60, and 0.90 μM) in methanol/toluene.

The ratiometric fluorescent response of compound **2** in the presence of other metal salts is shown in Figure 3. As mentioned above, compound **2** showed a quenching response to copper(II) salt,[9] and the ratiometric response of **2** is shown only with the zinc(II) salt.

To elucidate the stoichiometry of complex formation of **2** with zinc(II) salt, Job's plot analysis was examined and the result indicated that the 1:2 complex of **2** and zinc(II) salt was formed in toluene under the presented conditions (Fig. 4).

A single crystal of the complex of compound **2** with zinc bromide was obtained by recrystallization in toluene. The X-ray crystal structure demonstrated that the zinc(II) salt adopts a tetrahedral

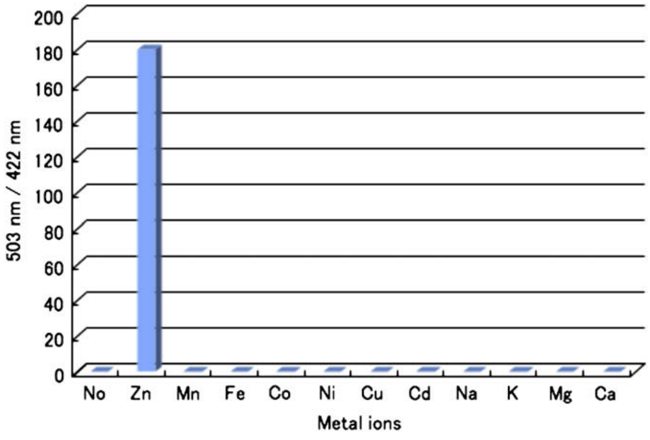


Figure 3. Ratiometric fluorescent response of **2** with various metal salts in toluene (ex 314 nm).

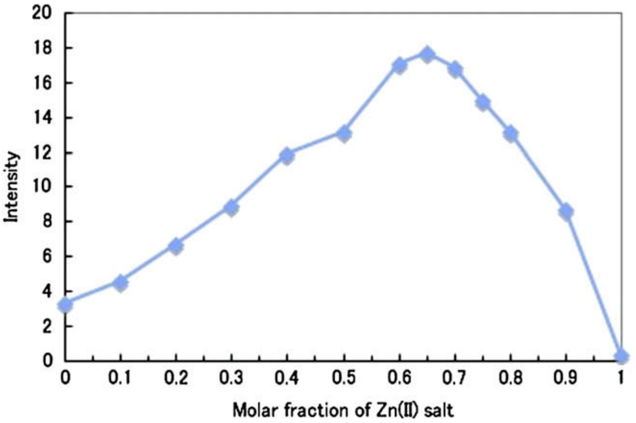


Figure 4. Job's plot for the complexation equilibrium of **2** with $ZnBr_2$ in toluene (ex 314 nm, em 505 nm).

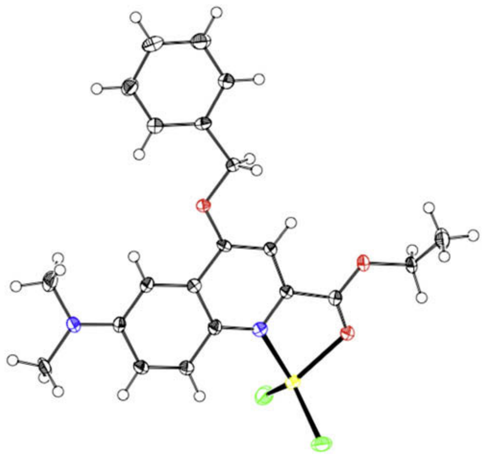


Figure 5. ORTEP view of the complex of **2** with $ZnBr_2$.

geometry (Fig. 5). Although the formation of 1:2 complex of **2** and zinc(II) salt was indicated by the result of Job's plot analysis, the 1:1 complex was obtained as a single crystal. The nitrogen atom on the quinoline ring and carbonyl group of the ester moiety coordinate the zinc(II) salt to form a 1:1 complex.

3. Conclusion

Novel fluorescent molecules having a coordination site for metal ions were developed. Compound **2** acted as a chemosensor for zinc(II) salt and showed a ratiometric response in both excitation and emission spectra in aprotic solvent. Compounds **1** and **2** contain an ester moiety that can be easily modified to other functional groups, enabling the introduction of the present fluorescent compounds into biologically functionalized molecules. Moreover, the present compounds are analogs of kynurenic acid, which is an important molecule for neurobiology. Development of fluorescent probes by the introduction of compounds **1** and **2** to biologically active functional molecules and an application for the visualization of kynurenic acid-dynamics in neurobiology is now underway.

4. Experimental

4.1. General

All reactions were carried out under an argon atmosphere. Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. ^1H and ^{13}C NMR spectra were measured on a Bruker AV-300 and chemical shifts are given in parts per million using CH_3OH (3.34 ppm) in CD_3OD for ^1H NMR and CD_3OD (49.0 ppm) for ^{13}C NMR as internal standards, respectively. IR spectra were taken with a Perkin–Elmer PARAGON 1000 FT-IR and only noteworthy absorptions are listed. Mass spectra were measured on a Micromass LCT. Fluorescence spectroscopic studies were performed with a RF-5300PC (Shimadzu Corp.). Quantum yield was measured on an absolute PL quantum yield measurement system (C9920-02, Hamamatsu Photonics K.K.). All solvents for measurement of photochemical properties were of analytic grade. The solutions of **1** and **2** in toluene were prepared before use. The solutions of metal salts were prepared from $ZnBr_2$, $MnCl_2 \cdot 4H_2O$, $FeCl_2 \cdot 4H_2O$, $CoCl_2$, $NiCl_2 \cdot 6H_2O$, $CuCl_2$, $CdCl_2 \cdot 5/2H_2O$, NaCl, KCl, $MgCl_2 \cdot 6H_2O$, $CaCl_2$ in methanol (10 mM). The solution was diluted with toluene (100 μM for NaCl, KCl, $MgCl_2$, $CaCl_2 \cdot H_2O$, and 1 μM for other metal salts) before use.

4.2. Synthesis

*4.2.1. 6-Dimethylamino-4-hydroxy-2-quinolinecarboxylic acid ethyl ester (**1**)*

A solution of oxalacetic acid diethyl ester (1.6 g, 8.8 mmol) and *N*,*N*-dimethyl-*p*-phenylenediamine (1.2 g, 8.8 mmol) in toluene (20 mL) was stirred at reflux for 18 h. The mixture was concentrated under vacuum. The resulting residue was dissolved in diphenyl ether (10 mL) and the solution was stirred at reflux for 10 min. The mixture was concentrated under vacuum and the residue was purified by silica gel column chromatography (hexane/isopropanol, 3:1, followed by AcOEt only) to give compound **1** (1.74 g, 6.7 mmol) as yellow crystals in 76% yield. Mp 220–222 °C. IR (KBr) ν cm^{-1}: 3066, 1727, 1508, 1382, 1272. ^1H NMR (300 MHz, CD_3OD) δ; 1.47 (3H, t, J=7.1 Hz), 3.11 (6H, s), 4.52 (2H, q, J=7.1 Hz), 6.93 (1H, s), 7.37 (1H, d, J=2.8 Hz), 7.48 (1H, dd, J=2.8, 9.3 Hz), 7.82 (1H, d, J=9.3 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ; 14.4 (CH_3), 40.6 ($CH_3 \times 2$), 63.9 (CH_2), 103.7 (CH), 108.7 (CH), 121.5 (CH), 122.3 (CH), 128.4 (C), 133.5 (C), 137.7 (C), 149.6 (C), 163.3 (C), 179.8 (C). HRESIMS calcd for $C_{14}H_{17}N_2O_3$: 261.1239 $(M+H)^+$, found: 261.1248. Anal. Calcd for $C_{14}H_{16}N_2O_3$: C, 64.60; H, 6.20; N, 10.76. Found: C, 64.64; H, 6.35; N, 10.37.

*4.2.2. 4-Benzyloxy-6-dimethylamino-2-quinolinecarboxylic acid ethyl ester (**2**)*

A solution of **1** (2.0 g, 7.69 mmol) in DMF (8 mL) was added to a suspension of NaH (60%, 369 mg, 9.23 mmol) in DMF (8 mL) at ambient temperature. After being stirred for 30 min at the same temperature, benzyl bromide (1.71 g, 1.19 mL, 10 mmol) was added to the mixture at ambient temperature and the resulting mixture was stirred at the same temperature for 2.5 h. Water was added to the mixture and the resulting mixture was extracted with AcOEt three times. The combined organic layer was washed with brine, dried over $MgSO_4$, and concentrated under vacuum. The residue was purified by silica gel column chromatography (hexane/AcOEt, 1:1) to give compound **2** (1.8 g, 5.14 mmol) as yellow crystals in 67% yield. Mp 130–134 °C. IR (KBr) ν cm^{-1}: 1702, 1618, 1345, 1241, 1091. ^1H NMR (300 MHz, CD_3OD) δ; 1.48 (3H, t, J=7.1 Hz), 3.12 (6H, s), 4.48 (2H, q, J=7.1 Hz), 5.42 (2H, s), 7.16 (1H, d, J=2.8 Hz), 7.35–7.54 (4H, m), 7.56–7.62 (3H, m), 8.00 (1H, d, J=9.4 Hz). ^{13}C NMR (75 MHz, CD_3OD) δ; 14.6 (CH_3), 40.5 ($CH_3 \times 2$), 62.8 (CH_2), 71.5 (CH_2), 99.2 (CH), 102.2 (CH), 121.0 (CH), 125.2 (C), 128.7 (CH), 129.3 (CH), 129.8 (CH), 130.8 (CH), 137.5 (C), 142.7 (C), 145.0 (C), 150.9 (C), 161.6 (C), 166.7 (C). HRESIMS calcd for $C_{21}H_{23}N_2O_3$: 351.1709 $(M+H)^+$, found: 351.1712. Anal. Calcd for $C_{21}H_{22}N_2O_3$: C, 71.98; H, 6.33; N, 7.99. Found: C, 71.92; H, 6.21; N, 7.84.

4.3. Job's plot

A series of solutions containing **2** and $ZnBr_2$ were prepared such that the sum of the total metal and total fluorophore concentration remained constant at 0.29 μM in toluene. The molar fraction of the fluorophore was varied between 0.1 and 1.0.

4.4. X-ray structure determination

Single crystals of compound **2**–$ZnBr_2$ complex were grown from a toluene solution at ambient temperature. Crystallographic data for the structural analysis of **2**–$ZnBr_2$ have been deposited with the Cambridge Crystallographic Data Centre, CCDC 707602.

Acknowledgements

This work was supported by Dynamic Biology Project of New Energy and Industrial Technology Development Organization. We thank Dr. Kengo Suzuki (Hamamatsu Photonics K.K.) for measurement of the quantum yields of the present compounds.

References and notes

1. (a) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515; (b) Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3; (c) Callan, J. F.; de Silva, A. P.; Magri, D. C. *Tetrahedron* **2005**, *61*, 8551.
2. Weber, G.; Farris, F. J. *Biochemistry* **1979**, *18*, 3075.
3. (a) Stone, T. W. *Pharmacol. Rev.* **1993**, *45*, 309; (b) Stone, T. W. *Trends Pharmacol. Sci.* **2000**, *21*, 149.
4. For examples, see: (a) Murakami, K.; Haneda, M.; Yoshino, M. *BioMetals* **2006**, *19*, 429; (b) Li, W.; Olmstead, M. M.; Miggins, D.; Fish, R. H. *Inorg. Chem.* **1996**, *35*, 51.
5. Conrad, M.; Limpach, L. *Chem. Ber.* **1887**, *20*, 944.
6. Berg, J. M.; Shi, Y. *Science* **1996**, *271*, 1081.
7. Recent examples for ratiometric fluorescent probes for zinc ion: (a) Taki, M.; Wolford, J. L.; O'Halloran, T. V. *J. Am. Chem. Soc.* **2004**, *126*, 712; (b) Woodroofe, C. C.; Won, A. C.; Lippard, S. J. *Inorg. Chem.* **2005**, *44*, 3112; (c) Kiyose, K.; Kojima, H.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2006**, *128*, 6548; (d) Zhang, L.; Dong, S.; Zhu, L. *Chem. Commun.* **2007**, 1891; (e) Sumalekshmy, S.; Henary, M. M.; Siegel, N.; Lawson, P. V.; Wu, Y.; Schmidt, K.; Bredas, J. L.; Perry, J. W.; Fahrni, C. J. *J. Am. Chem. Soc.* **2007**, *129*, 11888; (f) Komatsu, K.; Urano, Y.; Kojima, H.; Nagano, T. *J. Am. Chem. Soc.* **2007**, *129*, 13447; (g) Wang, J.; Qian, X.; Cui, J. *J. Org. Chem.* **2006**, *71*, 4308; (h) Coskun, A.; Akkaya, E. U. *J. Am. Chem. Soc.* **2006**, *128*, 14474; (i) Kim, J. S.; Choi, M. G.; Song, K. C.; No, K. T.; Ahn, S.; Chang, S. K. *Org. Lett.* **2007**, *9*, 1129; (j) Wegner, S. V.; Okesli, A.; Chen, P.; He, C. *J. Am. Chem. Soc.* **2007**, *129*, 5910; (k) Nolan, E. M.; Lippard, S. J. *J. Am. Chem. Soc.* **2007**, *129*, 5910; (l) Royzen, M.; Dai, Z.; Canary, J. W. *J. Am. Chem. Soc.* **2005**, *127*, 1612; (m) Xu, Z.; Xiao, Y.; Qian, X.; Cui, J.; Cui, D. *Org. Lett.* **2005**, *7*, 889; (n) Xu, Z.; Qian, X.; Cui, J. *Org. Lett.* **2005**, *7*, 3029; (o) Yang, H.; Liu, Z.; Zhou, Z.; Shi, E.; Li, F.; Du, Y.; Yi, T.; Huang, C. *Tetrahedron Lett.* **2006**, *47*, 2911.
8. Riegel, B.; Albisetti, C. J., Jr.; Lappin, G. R.; Baker, R. H. *J. Am. Chem. Soc.* **1946**, *68*, 2685.
9. Iron(II) salt also showed quenching response.