Tetrahedron Letters 52 (2011) 4333–4336

Contents lists available at ScienceDirect

Tetrahedron Letters

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

FRET-induced nanomolar detection of Fe^{2+} based on cinnamaldehyde-rhodamine derivative

Manoj Kumar *, Naresh Kumar, Vandana Bhalla

Department of Chemistry, UGC center for Advance Studies-1, Guru Nanak Dev University, Amritsar 143005, Punjab, India

ARTICLE INFO

Article history:
Received 6 May 2011
Revised 8 June 2011
Accepted 10 June 2011
Available online 17 June 2011

Keywords:
Rhodamine
Cinnamaldehyde
FRET
Fe^{2+}

ABSTRACT

A new dimethylaminocinnamaldehyde linked rhodamine based fluorescence receptor **3** is synthesized which shows fluorescence resonance energy transfer in the presence of Fe^{2+} ions, thus enhancing rational partition in between donor and acceptor emissions and permitting separated measurement of emissions of both fluorophores.

The development of fluorescent chemosensors for the recognition of environmentally and biologically important cations has been a research area of particular interest. Among different metal ions, iron is a primary metal ion in numerous physiological processes involving electron transfer and oxidation.[1] Iron, being both useful and cytotoxic[2], deficiency throughout the developmental phases may lead to permanent loss of motor skills.[3] Accretion of iron in the central nervous system has been involved in a number of diseases, such as Parkinson's, Huntington's, and Alzheimer's disease, associated with an increased quantity of iron.[2a] Keeping in view the roles played by iron in day to day life the development of techniques for selective determination of iron is in immense demand. Fluorescence spectroscopy has been a very useful technique for selective and sensitive recognition of metal ions. In most of the fluorescent sensors the cation binding involves photophysical changes like photoinduced electron transfer (PET),[4] photoinduced charge transfer (PCT),[5] formation of monomer/excimer,[6] energy transfer[7], and more recently fluorescence resonance energy transfer (FRET) where the excitation energy from one fluorophore (donor) is transferred to another fluorophore (acceptor) without the emission of a photon.[8] FRET is an active field in supramolecular chemistry due to its potential practical benefits in cell physiology, optical therapy, as well as selective and sensitive sensing toward targeted molecular or ionic species.[9] FRET induced by guest binding is a proficient approach to design ratiometric fluorescence probes, as they can emit at two different wavelengths with a single excitation source.[10]

Our research work involves the design, synthesis, and evaluation of fluorogenic receptors for selective sensing of soft metal ions, anions and evaluation of their switching behavior.[11] Recently, we reported a naphthalimide appended rhodamine based fluorescent chemosensor which showed thorough bond energy transfer in the presence of Hg^{2+} ions.[12] In continuation of this work, we have now designed and synthesized a dimethylaminocinnamaldehyde linked rhodamine based chemosensor **3** which undergoes fluorescence resonance energy transfer in the presence of Fe^{2+} ions. Rhodamine and its derivatives which absorb in the range of 450–600 nm (Fig. 1) are well known for their desirable properties, including good photostability, high extinction coefficient, and high fluorescent quantum yield.[13] On the other hand, dialkylaminocinnamaldehyde derivatives emit in the range of 400–550 nm (Fig. 1). Hence, the spectral overlap between the emission spectra of dimethylaminocinnamaldehyde and absorption spectra of the ring-opened form of rhodamine is significant (Fig. 1). On the basis of this, we envisaged that the attachment of dialkylaminocinnamaldehyde moiety with rhodamine would give a system which may exhibit the phenomenon of fluorescence resonance energy transfer. Thus, chemosensor **3** was synthesized in which rhodamine is linked to dimethylaminocinnamaldehyde moiety. Compound **3** undergoes fluorescence resonance energy transfer in the presence of Fe^{2+} ions enhancing rational partition in between donor and acceptor emissions permitting separated measurement of the emissions of both fluorophores. To the best of our knowledge, this is the first report where fluorescence resonance energy transfer phenomenon is observed between dimethylaminocinnamaldehyde and rhodamine moieties on the addition of Fe^{2+} ions.

* Corresponding author. Tel.: +91 183 2258802 9; fax: +91 183 2258820.
E-mail address: mksharmaa@yahoo.co.in (M. Kumar).

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doi:10.1016/j.tetlet.2011.06.044

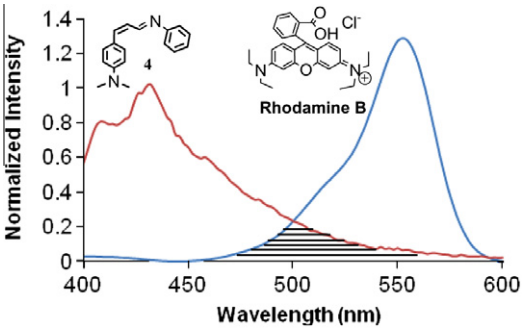


Figure 1. Spectral overlap between donor (**4**) emission (red) and ring opened rhodamine B absorption (blue).

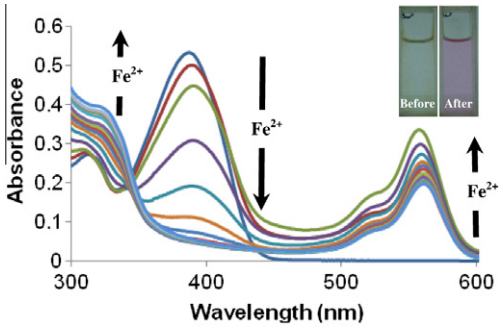


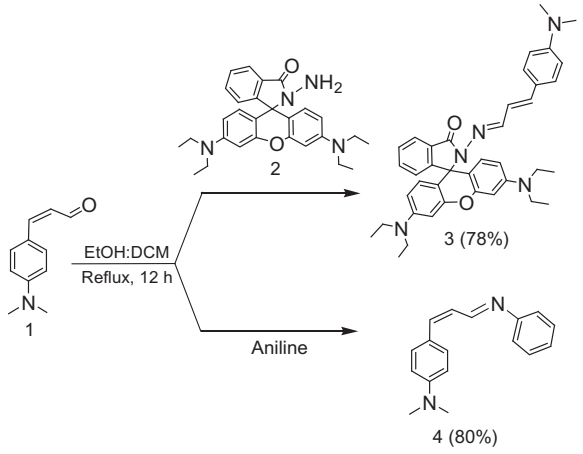
Figure 2. UV–vis spectra of **3** (5 μM) in the presence of Fe^{2+} ions (20 equiv) in THF; Inset showing the color change before and after the addition of Fe^{2+} ions.

The condensation of rhodamine hydrazide **2**[14] with *N,N*-dimethylaminocinnamaldehyde **1** furnished compound **3** in 78% yield (Scheme 1).[15] We also synthesized the model compound **4** by the condensation of **1** with aniline.[16] The structure of compound **3** was confirmed from its spectroscopic and analytical data. The IR spectrum of compound **3** showed stretching band at 1677 cm^{-1} corresponding to the C=N group. There is no absorption band corresponding to free aldehyde and amino groups, which indicates that condensation has taken place. The ^1H NMR spectrum (Supplementary data S6) of compound **3** showed a triplet (12H) at 1.15 ppm corresponding to the methyl protons, a singlet (6H) at 2.94 ppm corresponding to the methyl protons, a quartet (8H) at 3.28–3.58 ppm corresponding to the methylene protons, five multiplets (2, 9, 1, 2 and 1H respectively) at 6.23–6.27, 6.42–6.67, 7.03–7.04, 7.39–7.43 and 7.95–7.96 ppm corresponding to aromatic and CH protons, two doublets (1H each) at 7.23 and 8.09 ppm corresponding to aromatic and HC=N protons respectively. A parent ion peak at *m/z* 614 $[M+H]^+$ corresponding to the condensation product **3** was observed in TOF MS ES+ spectrum (Supplementary data S8). These spectroscopic data corroborate the structure **3** for this compound.

The binding behavior of compound **3** was studied toward different metal cations (Hg^{2+}, Fe^{2+}, Fe^{3+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Ag^+, Co^{2+}, Mn^{2+}, Mg^{2+}, Ba^{2+}, Li^+, Na^+, and K^+) as their perchlorate salts in THF by UV–vis and fluorescence spectroscopy. The absorption spectrum of compound **3** (5.0 μM) shows two absorption bands (Fig. 2) at 308 and 380 nm due to the dimethylaminocinnamaldehyde moiety, assigned to the transition from S_0 to S_2 and S_1 states, respectively.[17] Low energy absorption at 380 nm indicates that this is a π–π* type of transition. On the addition of Fe^{2+} ions the band at 380 nm decreases while a new absorption band appears at 554 nm corresponding to the ring opened form of the rhodamine (Fig. 2). The addition of other metal ions like Hg^{2+}, Fe^{3+}, Cu^{2+}, Ni^{2+}, and Ag^+ also results in the appearance of the characteristic absorption band of rhodamine moiety (Supplementary data S2). This indicates that compound **3** is interacting with different metal ions in the ground state.

The UV–vis behavior of compound **3** in the presence of different metal ions is attributed to the alteration in electronic properties of **3** that is, increased internal charge transfer (ICT) on metal ion complexation, while characteristic absorbance of rhodamine is attributed to the opening of spirolactam ring to its amide form along with a color change from colorless to pink. The fluorescence spectrum of compound **3** (1.0 μM) exhibits a strong emission at 470 nm attributed to dimethylaminocinnamaldehyde moiety when excited at 380 nm (Fig. 3). The rhodamine moiety in **3** remains in a closed, non-fluorescent spirolactam form indicating weak spectral overlap between dimethylaminocinnamaldehyde (energy donor) emission and rhodamine (energy acceptor) absorption. As a result the fluorescence resonance energy transfer (FRET) is suppressed and the emission due to the dimethylaminocinnamaldehyde moiety is observed at 470 nm. In the presence of Fe^{2+} ions compound **3** shows a ratiometric response, the fluorescence spectrum shifted to 582 nm, the region of rhodamine emission with the gradual decrease in donor emission at 470 nm (Fig. 3). This is attributed to the binding of Fe^{2+} ions with spirolactam ring of rhodamine resulting in its opening to amide form. Efficient overlap between energy donor (dimethylaminocinnamaldehyde moiety) and energy acceptor (rhodamine moiety) is thus possible as the energy gap between energy donor and energy acceptor is greatly reduced enhancing the intramolecular FRET.

Under the same conditions as used above for compound **3**, we also carried out fluorescence studies of model compound **4** which



Scheme 1.

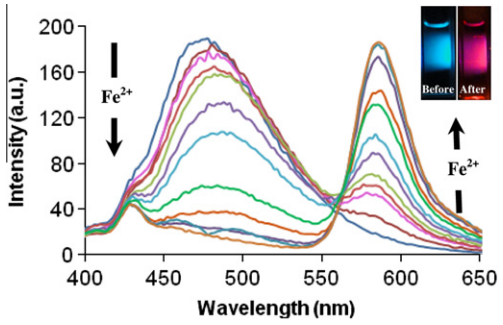


Figure 3. Fluorescence spectra of **3** (1.0 μM) in response to the presence of Fe^{2+} ions (20 μM) in THF; λ_{ex} = 380 nm Inset showing the fluorescence change before and after the addition of Fe^{2+} ions.

lacks acceptor unit in the presence of Fe^{2+} ions (Supplementary data S5). It was observed that no energy transfer takes place in the case of compound **4** in the presence of Fe^{2+} ions. Thus, the advantage of the FRET system for energy transfer is obvious in compound **3** where two moieties are linked together. Thus, in case of compound **3** the addition of Fe^{2+} ions 'trigger' the FRET process from the energy donor to energy acceptor upon excitation at donor absorption wavelength. The energy transfer efficiency[18] from donor to acceptor was calculated to be 89.7% (Supplementary data S4). This type of ratiometric fluorescence behavior is not induced by the addition of any other metal ions investigated except Fe^{3+} which also induces ratiometric behavior but to small extent (Fig. 5A). Other metal ions like Hg^{2+}, Cu^{2+}, Ag^{+} show unusual fluorescence behavior (Fig. 4). The addition of Hg^{2+} ions to the solution of **3** results in the 100% linear increase in emission at 470 nm. The reason for increase in the fluorescence emission at 470 nm on the addition of Hg^{2+} ions is due to the coordination of Hg^{2+} ions with nitrogen atom of imino moiety of receptor **3** which decreases the electron density on nitrogen and suppresses the electron transfer from nitrogen to dialkylaminocinnamaldehyde moiety. The addition of Cu^{2+} ions leads to small decrease (22%) in emission which may be attributed to the photo-induced electron transfer from the dialkylaminocinn-amaldehyde moiety to the Cu^{2+} ions. While in the presence of Ag^{+} ions there is red shift to 518 nm with enhancement in emission which is due to the photo-induced charge transfer (ICT) process operating in the presence of Ag^{+} ions.

Further, by considering the ratio of the fluorescence intensity of energy acceptor at 582 nm (I_{582}) to that of energy donor at 470 nm (I_{470}), we observed 9.5-fold fluorescence increase in the case of **3**-Fe^{2+} complex. To check the practical ability of compound **3** as a Fe^{2+} selective ratiometric fluorescent sensor, we carried out competitive experiments in the presence of Fe^{2+} at 20 μM mixed with Hg^{2+}, Fe^{3+}, Pb^{2+}, Cd^{2+}, Cu^{2+}, Zn^{2+}, Ni^{2+}, Ag^{+}, Co^{2+}, Mn^{2+}, Mg^{2+}, Ba^{2+}, Li^{+}, Na^{+}, and K^{+} at 20 μM. As shown in Figure 5B no significant variation in ratiometric emission was observed by comparison with or without the other metal ions. It was found that **3** has a detection limit of 60×10^{-9} mol L^{-1} for Fe^{2+} which is sufficiently low for the detection of submillimolar concentration range of Fe^{2+} ions found in many chemical systems. Fitting the changes in the fluorescence spectra of compound **1** with Fe^{2+} ions using the nonlinear regression analysis program SPECFIT[19] gave a good fit and demonstrated that 1:1 stoichiometry (host:guest) was the most stable species in the solution with a binding constant (log β) = 5.45 with ±0.32 error. The method of continuous variation (Job's plot) was also used to prove the 1:1 stoichiometry (Supplementary data S4).[20]

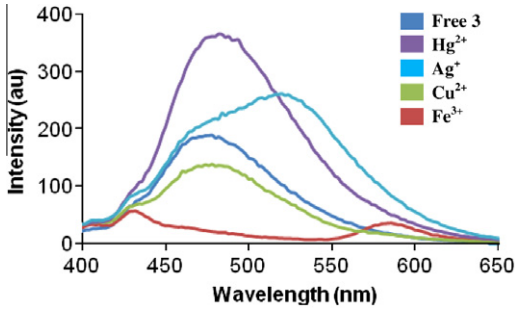


Figure 4. Fluorescence spectra of **3** (1.0 μM) in the presence of different metal ions (20 μM each) in THF; λ_{ex} = 380 nm.

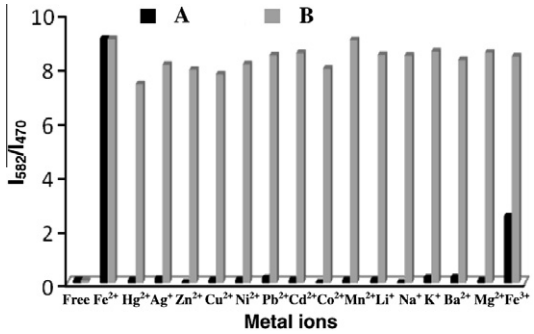


Figure 5. Fluorescence response of **3** (1.0 μM) to various cations (20 μM each) in THF; λ_{ex} = 380 nm. Black bars represent selectivity (I_{582}/I_{470}) of **3** upon addition of different metal ions; gray bars represent competitive selectivity of receptor **3** toward Fe^{2+} ions (20 μM) in the presence of other metal ions (20 μM).

In conclusion, we have synthesized a new fluorescence resonance energy transfer '*turn-on*' dimethylamino-cinnamaldehyde linked rhodamine based ratiometric fluorescent chemosensor **3** which is used for the selective recognition of Fe^{2+} ions over other chemically close metal ions with a detection limit up to the nanomolar range. The design of such systems which involve irradiation of different fluorescent labels with single excitation source has importance that the dye which has to emit at a longer wavelength absorbs at the excitation source more effectively and hence results in no net loss in fluorescence intensity which is an important issue in those cases where detection of low levels of fluorescence is involved.

Acknowledgments

We are thankful to the DST (New Delhi) (Ref. No. SR/FTP/CS-10/2006), CSIR (New Delhi) (Ref. No. 01 (2326)/09/EMR-II) for financial support and Guru Nanak Dev University for laboratory facilities.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.06.044.

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15. *Synthesis of* **3**. A mixture of compound **1** (0.10 g, 0.21 mmol) and *N,N*-dimethylcinnamaldehyde (0.04 g, 0.26 mmol) in a 1:1 mixture of dry dichloromethane and absolute ethanol was refluxed for 12 h. After the completion of the reaction solvent was evaporated and the residue left was crystallized from $CHCl_3$/CH_3OH to give compound **3** in 78% yield; mp 212 °C. IR (KBr) ν_{max} = 1677 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ = 1.15 (t, *J* = 6 Hz, 12H, CH_3), 2.94 (s, 6H, CH_3), 3.28–3.58 (q, 8H, CH_2), 6.23–6.27 (m, 2H, Ar-H), 6.42–6.67 (m, 9H, Ar-H and CH), 7.03–7.04 (m, 1H, CH), 7.23 (d, 1H, CH), 7.39–7.43 (m, 2H, Ar-H), 7.95–7.96 (m, 1H, Ar-H), 8.09 (d, *J* = 9H, HC=N) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ = 12.76, 40.26, 44.29, 65.47, 98.07, 105.79, 108.17, 11.95, 122.70, 122.87, 123.45, 124.59, 127.88, 128.29, 133.21, 139.71, 148.98, 150.30, 150.54, 152.68 ppm. MS ES+, *m*/*z*: = 614 $[M+H]^+$. $C_{39}H_{43}N_5O_2$: calcd C 76.32, H 7.06, N 11.41. Found C 76.09, H 7.28, N 11.17.
16. *Synthesis of* **4.** A mixture of *N,N*-dimethylcinnamaldehyde (0.50 g, 2.85 mmol) and aniline (0.39 g, 4.28 mmol) in a 1:1 mixture of dry dichloromethane and absolute ethanol was refluxed for 12 h. After the completion of the reaction solvent was evaporated and the residue left was crystallized from $CHCl_3$/CH_3OH to give compound **3** in 80% yield; mp 136 °C. ^1H NMR ($CDCl_3$, 300 MHz): δ = 3.02 (s, *J* = 6H, CH_3), 6.69 (d, *J* = 6H, 2H, Ar-H), 6.89–6.97 (m, 1H, CH), 7.05 (s, 1H, Ar-H), 7.14–7.20 (m, 3H, Ar-H and CH), 7.33–7.44 (m, 4H, Ar-H), 8.21 (d, *J* = 9H, HC=N) ppm. ^{13}C NMR ($CDCl_3$, 300 MHz): δ = 40.20, 112.01, 120.92, 123.50, 123.92, 125.50, 128.99, 145.05, 151.29, 152.13, 162.36 ppm. $C_{17}H_{18}N_2$: calcd C 81.56, H 7.25, N 11.19. Found C 81.30, H 7.50, N 11.56.
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