Tetrahedron Letters 55 (2014) 873–876

Contents lists available at ScienceDirect

Tetrahedron Letters

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

A coumarinylaldoxime as a specific sensor for Cu^{2+} and its biological application

Olimpo García-Beltrán [a,b,*], Bruce K. Cassels [a], Natalia Mena [c], Marco T. Nuñez [c], Osvaldo Yañez [d], Julio Caballero [d]

[a] Department of Chemistry, Faculty of Sciences, University of Chile, Santiago, Chile
[b] Facultad de Ciencias Naturales y Matemáticas, Universidad de Ibagué, Carrera 22 Calle 67, Ibagué, Colombia
[c] Department of Biology, Faculty of Sciences, University of Chile, Santiago, Chile
[d] Centro de Bioinformática y Simulación Molecular, Facultad de Ingeniería, Universidad de Talca, 2 Norte 685, Casilla 721, Talca, Chile

ARTICLE INFO

Article history:
Received 4 November 2013
Revised 6 December 2013
Accepted 9 December 2013
Available online 21 December 2013

Keywords:
Cu^{2+}-selectivity
Fluorescent probe
Coumarin derivative
Molecular dynamics
Lipid bilayer

ABSTRACT

In this Letter we present a new probe, (*E*)-7-(diethylamino)-2-oxo-2*H*-chromene-3-carbaldehyde oxime (**JB**), which can detect Cu^{2+} ions in HEPES buffer under physiological conditions. Benesi–Hildebrand and Job plots demonstrate that the stoichiometry of the Cu^{2+} complex formed is 2:1. Possible interference with other analytes was examined, and the decrease of the fluorescence of **JB** at 510 nm when it reacts with Cu^{2+} was shown to be highly selective. This probe accumulates in the plasmalemma of human neuroblastoma SH-SY5Y cells. Molecular dynamics (MD) simulations revealed that **JB** interacts with the lipid bilayer at the level of the glycerol moieties.

Novel colorimetric and fluorescent probes for metal ions have found actual and potential applications in different areas of science.[1] Copper is the third most abundant trace element in many living organisms including humans, and it is involved in redox processes,[2] besides catalyzing the formation of reactive oxygen species (ROS) that are capable of damaging biomolecules leading to the connection of the cellular toxicity of copper ions with serious diseases.[3–6] Cu^{2+} complexes have also attracted considerable interest in nucleic acid chemistry due to their various applications following the discovery of their chemical nuclease activity.[7,8]

In this work, the novel fluorescence probe **JB** was synthetized in three steps as shown in Scheme 1. 4-(Diethylamino)-2-hydroxybenzaldehyde (**1**) was condensed with diethyl malonate in a Knoevenagel reaction, cyclized and decarboxylated in one step to afford 3-amino-7-hydroxycoumarin (**2**), the coumarin was formylated (Vilsmeier-Haack) to obtain compound **3**, which was condensed with hydroxylamine hydrochloride to afford **JB**, characterized by ^{1}H NMR and ^{13}C NMR spectroscopy (Fig. S3A and B; Supporting information).

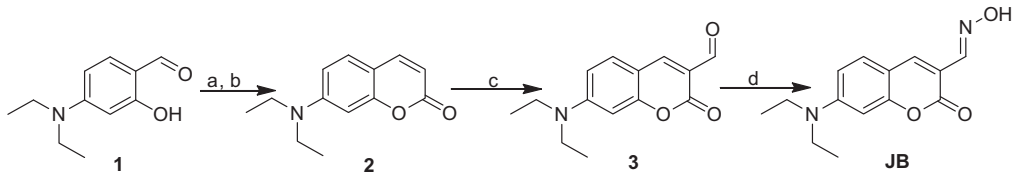
All absorption and emission spectral studies were performed in 20 mM HEPES buffer, pH 7.4, at room temperature, while the corresponding metal chlorides were used as the source of the metal cations. The absorption spectrum of **JB** shows a maximum at 430 nm (Fig. S4; Supporting information), a molar absorptivity (ε) of 23,058 L mol^{-1} cm^{-1} and an emission band at 510 nm (Fig. S5; Supporting information). The quantum yield of this probe is 0.043, with a lifetime (τ) of 2.78 ns and a Stokes shift of 3648 cm^{-1}.

In order to evaluate the selectivity of **JB**, its fluorescence spectra were recorded with different metal ions added (Hg^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}, Cu^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Ni^{2+}, Pb^{2+} or Cd^{2+}). Among these ions, only Cu^{2+} significantly affected the fluorescence intensity of **JB** (Fig. 1). Fig. 2 shows the Cu^{2+} concentration-dependent emission fluorescence spectrum of **JB** (20 μM). When excited at 430 nm, the emission fluorescence intensity at 510 nm decreases about 8-fold upon increasing the concentration of Cu^{2+} from 0 to 100 μM (Fig. 2).

A Benesi–Hildebrand graph of the fluorescence data (Fig. S6; Supporting information) was non-linear, indicating that the stoichiometry of the Cu^{+2} complex formed is different from 1:1. The binding stoichiometry of the **JB**-Cu^{2+} complex was determined from the Job plot (Fig. S7; Supporting information). Maximum emission intensity was observed when the mole fraction of Cu^{2+} was close to 0.34, which indicates the formation of a 2:1 complex between **JB** and Cu^{2+} at a total concentration of 100 μM. This was further confirmed by the appearance of a peak at *m*/*z* 292.9497 assignable to $[2MX+Cu]^{+2}$ in the ESI spectrum (Fig. S8; Supporting

* Corresponding author.
E-mail address: ojgarciab@ug.uchile.cl (O. García-Beltrán).

0040-4039/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2013.12.033



Scheme 1. Synthetic route to **JB**. Reagents and conditions: (a) Diethyl malonate, piperidine, AcOH, EtOH, reflux, 6 h; (b) HCl, AcOH, reflux, 24 h; (c) $POCl_3$, DMF, 60 °C, 24 h; (d) hydroxylamine hydrochloride, Et_3N, rt 1 h.

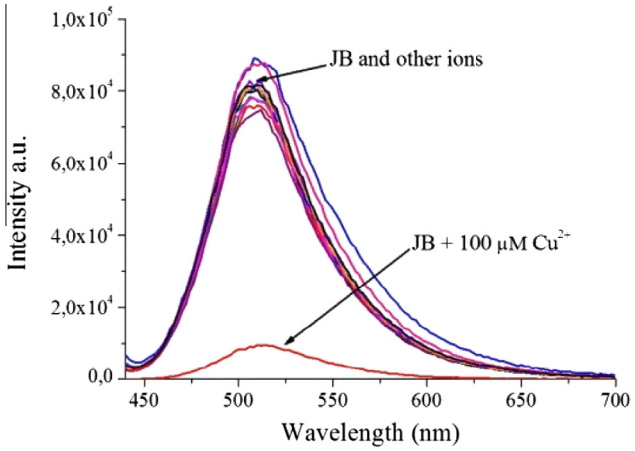


Figure 1. Fluorescence spectra of **JB** (20 μM) alone and in the presence of several different metal salts (Hg^{2+}, Fe^{3+}, Fe^{2+}, Co^{2+}, Cu^{2+}, Ca^{2+}, Zn^{2+}, Mn^{2+}, Mg^{2+}, Ni^{2+}, Pb^{2+} or Cd^{2+}; μM) in 20 mM HEPES buffer, pH 7.4.

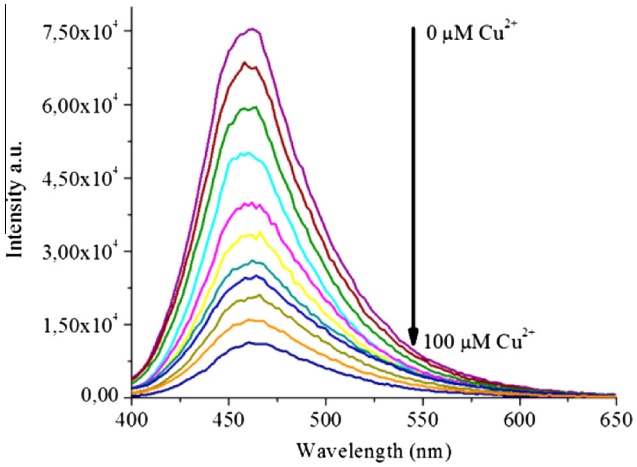
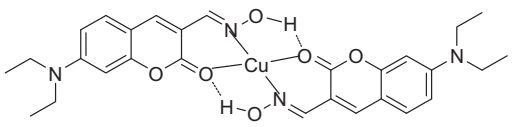


Figure 2. Fluorescence emission spectra (λ_{ex} = 460 nm) of **JB** (10 μM) upon addition of $CuCl_2$ (0–100 equiv) in 20 mM HEPES buffer, pH 7.4.



Scheme 2. Possible binding mode of **JB** with Cu^{2+}.

information). A possible binding mode between **JB** and Cu^{2+} is proposed in Scheme 2.

To further check the practical applicability of **JB** as a Cu^{2+}-selective fluorescent sensor, we carried out competitive experiments in

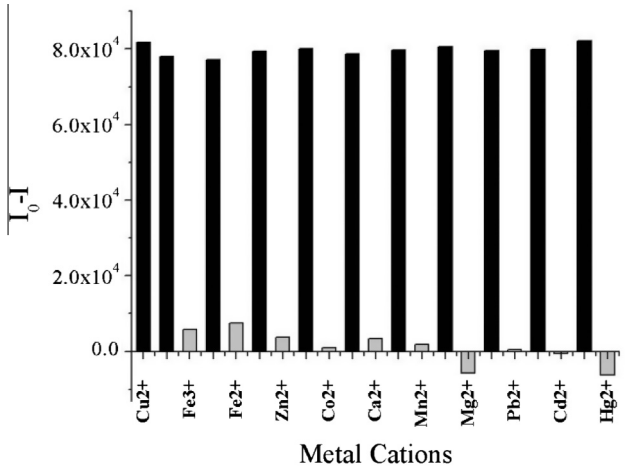


Figure 3. Selectivity of the fluorescence quenching of **JB** by Cu^{2+} in HEPES buffer pH 7.4. Gray bars indicate the fluorometric responses of **JB** with 10 equiv of Fe^{3+}, Zn^{2+}, Cu^{2+}, Co^{2+}, Ca^{2+}, Mn^{2+}, Mg^{2+}, Pb^{2+}, Cd^{2+}, and Hg^{2+}. Black bars represent the florescence response after addition to the same solutions of 10 equiv of Cu^{2+}.

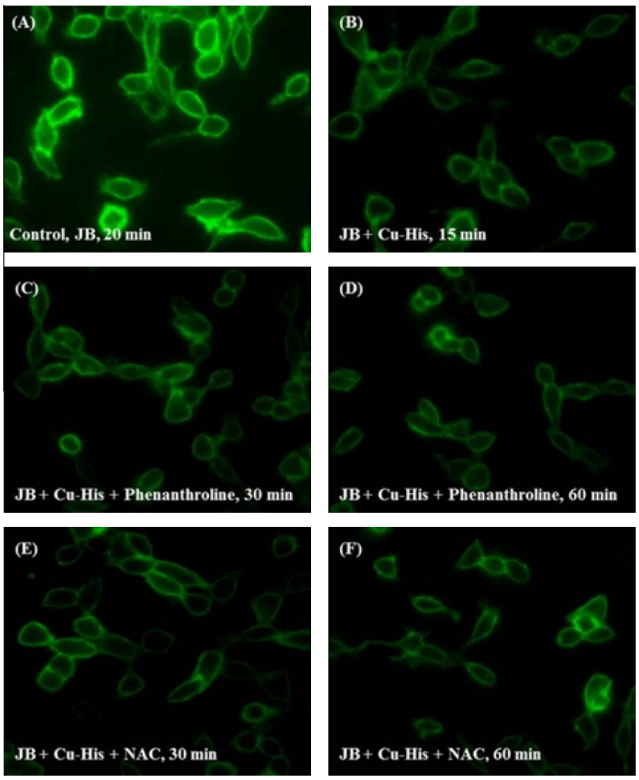


Figure 4. In vitro tests of the potential of **JB** as a cell membrane probe for Cu^{2+}. (A) SH-SY5Y cells were incubated with **JB** (10 μM, 20 min), washed, and the basal fluorescence measured. (B) The cells were then incubated with Cu-His (200 μM, 15 min). (C) And (D) fluorescence after adding 1,10-phenanthroline (20 μM, 30 and 60 min). (E) And (F) fluorescence after adding NAC (15 mM, 30 and 60 min). The fluorescence was recorded by epifluorescence microscopy, 63× objective.